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ORIGINAL PAPER

OsMND1 regulates early meiosis and improves the seed set rate in polyploid rice

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Abstract

The meiotic processes of most polyploid rice (*Oryza sativa*) are genetically abnormal, leading to low pollen fertility, which results in low seed set rates. Some polyploid meiosis stability (PMeS) lines with high seed set rates have been bred but their meiotic mechanisms remain unknown. In this study, we investigated the function of *OsMND1* regulated polyploid rice meiosis. *OsMND1* localized in the nucleus, and its expression level in panicles of PMeS line HN2026-4x was higher than in HN2026-2x and the other lines without the PMeS background. *OsMND1*'s overexpression improved pollen fertility and viability, early normal embryo development and the seed set rate in Balilla-4x. However, *OsMND1* RNAi in PMeS line HN2026-4x impeded pollen and embryo development significantly. The results of chromosome behavior analyses indicated that *OsMND1* participates in stabilizing meiosis by maintaining the balance of pairing, synapsis and recombination during early meiosis. Many univalent, trivalent, and even multivalent systems appeared in the *OsMND1* RNAi line, resulting in the presence of many lagging chromosomes. The outcome indicated that *OsMND1* plays a critical role in stabilizing meiosis, improving pollen fertility and reducing early embryo abortions, ultimately increasing the seed set rate. Additionally, *OsMND1* affected some key meiosis-related gene expression levels. These results raise interesting issues in polyploid breeding theory and application, which require integrated solutions in the future.

Keywords Polyploid rice · Meiosis · *OsMND1* · Pollen fertility · Seed set rate.

Abbreviations

PMeS Polyploid meiosis stability
MND1 Meiotic nuclear divisions 1

Introduction

Meiosis, a crucial process in the life cycle, encompasses a single round of DNA replication and two successive nuclear divisions. These events allow the requisite segregation of homologous chromosomes, and generate both genetic conservation and diversity in the progeny, which are crucial to gamete formation and sexual reproduction (Nishizawa-Yokoi et al. 2012). Plants, especially *Arabidopsis thaliana*, provide an outstanding system for the characterization of molecular mechanisms of major events that occur in meiosis (Mercier and Grelon 2008; Osman et al. 2011). Rice (*Oryza sativa*) is a significant crop with a 430 Mb genome size, which is smaller than those of other cereal crops (Eckardt 2000; Luo et al. 2014). The small chromosomal sizes and their moderate number are advantageous for making pachytene chromosome preparations in rice. Thus, rice is also considered a good model organism for investigating the molecular mechanism of meiosis in higher plants (Cheng 2013). Some rice meiotic genes have been characterized using different technologies (Nonomura et al. 2004; Cheng 2013). All of these genes were identified in diploid rice, and *PAIR1, PAIR2, PAIR3, OsDMC1, OsREC8, OsCOM1, OsRAD51* and MEICA 1 were especially carefully probed (Nonomura et al. 2004; Tian et al. 2010; Cheng 2013; Hu et al. 2017). Polyploidy is an important manifestation of evolution in

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species. However, meiotic irregularities in polyploid rice include multivalent associations and unbalanced chromosome segregation, leading to aneuploid gametes. Little is known about the molecular mechanism maintaining meiotic stability in polyploid rice. The evidence suggests that whole genome duplication events and abiotic environments are two especially potent challenges to meiotic chromosomal segregation and probably necessitate adaptive evolutionary responses (Storme and Geelen 2013; Bomblies et al. 2015). The seed set rate of most polyploid rice lines is less than 40% and yields are low. There have been no breakthroughs for a long time in polyploid rice breeding because of abnormal meiosis (He et al. 2010; Li et al. 2016a). *Ph* was discovered 50 years ago in wheat (*Triticum aestivum*); it has been characterized at the molecular level and is considered the main regulator of recombination in allopolyploid species (Griffiths et al. 2006). The polyploid meiosis stability (PMeS) line HN2026-4x, was selected through hybridization because its especially stable meiotic behavior is similar to its diploid. It is sub-autopolyploid and may represent a *Ph*-like genetic material (Cai et al. 2007; He et al. 2010). Balilla-4x is autopolyploid, has disorganized meiotic behaviors and a low seed set rate, without the PMeS background. However, the regulation of meiosis in polyploid rice is very complicated and ambiguous. Some PMeS lines, which have stable meiotic behaviors and high seed set rates, have been bred successfully. Therefore, materials for meiotic mechanism research are available.

The molecular basis of meiotic recombination has been well studied in yeast (*Saccharomyces cerevisiae*) and other organisms. It is initiated by programmed double-strand breaks (DSBs), catalyzed by a topoisomerase-like protein known as Spo11 (Keeney 2001; Yu et al. 2010). The immediate and proper repair of DSBs is crucial for maintaining the integrity and function of the genome (Li and Heyer 2008). Homologous recombination (HR) plays a critical role in repairing programmed DNA DSBs during meiosis. HOP2 (homologous-pairing protein 2) and MND1 (meiotic nuclear division protein 1) were determined to take part in homologous chromosome pairing and meiotic DSB repair. MND1 and HOP2 formed a complex, which may be an essential member in meiotic HR (Tsubouchi and Roeder 2002; Zhao and Sung 2015). The initiating event of meiotic recombination is the formation of the DSB. The location and numbers of DSBs formed are controlled by some factors and define the locations of recombination events. These DSBs must subsequently be repaired to form either crossovers or noncrossovers (Gray and Cohen 2016). Two recombinases, the RecA homologs Rad51 and Dmc1, form presynaptic nucleoprotein filaments with single-stranded DNA during meiosis, and DMC1 and RAD51 cooperate with MND1 (Ogawa et al. 1993; Tsubouchi and Roeder 2002; Schommer et al. 2003; Cloud et al. 2012). The MND1 protein localizes to chromatin throughout meiotic prophase in wild type, and Mnd1 localization requires Hop2 (Tsubouchi and Roeder 2002). The HOP2/MND1 heterodimeric complex, which acted during the formation of meiotic DSBs, was especially important for meiotic recombination in yeast (Pezza et al. 2007; Uanschou et al. 2013). HOP2/MND1 cooperated to form DMC1-mediated D-loops during DNA repair in *Arabidopsis* meiosis. The loss of the HOP2/MND1 complex function results in the suboptimal support of DMC1, and failure to repair meiotic DSBs using homologous chromosomes, but it allows *RAD51*-mediated IS-repair activation (Schommer et al. 2003; Uanschou et al. 2013). The major interaction site of HOP2–MND1 was identified in the central coiled-coil domains, and an open collinear parallel arrangement of HOP2 and MND1 within the complex has been predicted (Rampler et al. 2015). The identification of HOP2 and MND1 homologs in other organisms suggests that the functions of this complex are conserved among eukaryotes (Tsubouchi and Roeder 2002; Petukhova et al. 2005; Chi et al. 2007; Pezza et al. 2007; Bugreev et al. 2014; Zhao and Sung 2015).

MND1 plays a critical role in meiosis in yeast and *Arabidopsis*, but in rice, no research has reported on the function of *MND1*. Additionally, the regulatory mechanism of *MND1* in meiosis was important in polyploid rice. We isolated a novel meiotic gene, *MND1*, in polyploid rice (4x), encoding a putative coiled-coil protein that is similar to *PAIR1* in rice. In this research, the function of *MND1* in the regulation of polyploid rice meiosis was carefully investigated. We showed that *OsMND1* played a critical role in improving the seed set rate in PMeS rice by reducing the unsuccessful fertilization and the early embryo abortion rates caused by abnormal pollen development. Furthermore, these observations confirmed that *OsMND1* does participate in regulating meiosis in polyploid rice, suggesting that the effects of *OsMND1* might be involved in the processes of pairing, synapsis and recombination during early meiosis. Additionally, the expression levels of some important meiosis-related genes as determined by RT-qPCR indicated that *OsMND1* is a crucial player in establishing homologous chromosome pairing and in repairing meiotic DSBs through homologous chromosomes, and is related to *HOP2, DMC1* and *ZEP1*, but independent of *RAD51*. A stable meiosis is an important precondition of normal pollen development and a high seed set rate in polyploid rice. The unique regulating mechanisms of meiosis and pollen development in PMeS rice presents a very interesting problem for polyploidy breeding theory and application, which requires a solution.

Materials and methods

Plant materials and growth conditions

After several years of crosses between indica and japonica rice lines followed by backcrosses, the PMeS line

HN2026-4x, was selected and bred by Cai et al. (2007). It is a sub-autopolyploid, and it may be a *Ph*-like genetic material because of its especially stable meiotic behavior, which is similar to the diploid line HN2026-2x (Cai et al. 2007; He et al. 2010). Balilla-4x is autopolyploid with disorganized meiotic behaviors and a low seed set rate, without the PMeS background. It is the direct doubling of the diploid line Balilla-2x.

All of the rice plants used were grown in a standard greenhouse under a 16-h-light at 30 °C and 8-h-dark at 25 °C cycle in Wuhan, China.

Isolation of *OsMND1*

MND1 plays a critical role in meiosis in *Arabidopsis*. The *MND1* family protein (NP_001329788.1) of *A. thaliana* was sequenced by BLASTP in NCBI, There is a high homology sequence on the ninth chromosome in the rice genome. Total RNA was isolated from the panicles of two polyploid rice lines, HN2026-4x and Balilla-4x undergoing meiosis with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and used to synthesize the first-strand cDNA. Then, *OsMND1* was cloned by PCR amplification with the specific primers *MND1*-F (5′-atgtcgaagaagaggggtct-3′) and *MND1*-R (5′-ttactgcagatattcgaaat-3′). The amplified cDNA fragments were gel-purified, sub-cloned into the simple 19-T vector and then sequenced.

Construction of overexpression and RNAi vectors

A full-length 624-bp coding sequence of *OsMND1* was cloned into the transgenic vector pCU3 (Chen et al. 2007), which contains the strong promoter Ubi. The primers used were *OsMND1*-F1 (5′-ataggatccatggatatcagaccatctggaagtg-3′, with the *BamHI* site underlined) and *OsMND1*-R1 (5′-ataggatccctacagtgacaagtcgccaccca-3′, with the *BamHI* site underlined). Then, the recombinants were selected and sequenced. Two 624-bp fragments specific to *OsMND1* cDNA were amplified by PCR with primers *OsMND1*-xf (5′- atactcgaggggagaggatcatgtcgaagaag-3′, with the *XhoI* site underlined) and *OsMND1*-er (5′-atagaattcttgctgagttgggttacatacgg-3′, with the *EcoRI* site underlined), and primers *OsMND1*-xr (5′-atatctagatttgctgagttgggttacatacg-3′, with the *XbaI* site underlined) and *OsMND1*-bf (5′-ataggatccgggagaggatcatgt-3′, with the *BamHI* site underlined). After digestion with corresponding restriction enzymes, the two fragments were sub-cloned into vector pHANNIBAL (Biovector Science Lab, Inc., Beijing, China) to generate the recombinant pHANNIBAL-dbmnd1 (Chen et al. 2007). Finally, the fragment in pHANNIBAL-dbmnd1 containing an intron flanked by two 624-bp opposite fragments was digested by *Not*I and subcloned into pART27 (Biovector Science Lab, Inc), resulting in the RNAi vector, pART-dbmnd1.

Both constructs were introduced into *Agrobacterium tumefaciens* strain EHA105 and independently transferred into two polyploid rice lines, HN2026-4x and Balilla-4x. *OsMND1* overexpression plants (B-O) and *OsMND1* RNAi plants (HN-RNAi) were produced.

Subcellular localization analysis

OsMND1 cDNA was cloned by PCR amplification from panicles undergoing meiosis. The amplified PCR product was directly cloned into pPK100. An Agro-infiltration procedure for the transient protein expression in tobacco (*Nicotiana benthamiana*) leaves was performed according to the method described by Li et al. (2005). 4-week-old leaves were infiltrated with the bacterial cultures through abaxial air spaces. Simultaneously, onion (*Allium cepa* L.) epidermises were infected by microinjection. The GFP fluorescence was observed at 12 h intervals with a BX51 microscope (Olympus, Tokyo, Japan).The protocol of nuclear location was performed according to He et al. (2007).

Phenotypic analysis

Anthers were stained using 1% I_2–KI for 2–3 min for fertility observations. For viability investigations, anthers were treated with 100 μg/mL fluorescein diacetate (FDA; Sigma-Aldrich, St. Louis, MO, USA) for 5 min. Five independent experiments were designed and each experiment had five replicates. All of the images were taken with a BX51 microscope (Olympus).

RNA extraction and RT-PCR

Total RNA was isolated from rice panicles with TRIzol reagent (Invitrogen) and used to synthesize the first-strand cDNA. RT-PCR was carried out to amplify the *OsMND1* transcripts with 40 PCR cycles, using first-strand cDNA as a template. Two housekeeping genes (*ACTIN*1 and *GAPDH*) were also amplified as the controls. All of the primer information is presented in Supple. Table 1.

The 25-μL RT-PCR reactions contained 2.5 μL of diluted cDNA, 2 μL of 7.5 μM primers, 12.5 μL of 2 × qPCR mix and 8 μL SDW. RT-qPCR was performed on a Rotor Gene 3000 system (Corbett Research) using a SYBR green detection protocol according to the manufacturer's instructions (SYBR Premix Ex Taq System; Toyobo, Osaka, Japan). A linear standard curve and threshold cycle number versus log (designated transcript level) were constructed using a series of dilutions of each PCR product; and the transcript levels in all of the unknown samples were determined according to the standard curve. Five biological repeats were performed for each sample.

Semi-thin analysis of the anther structure

The anther semi-thin analysis method of He et al. (2010) was used. At every stage, half-thin sections (100 nm) were tested. All of the images were taken with a BX51 microscope (Olympus).

Meiotic analysis

For meiotic behavior studies, we used the previously published methods of Yan et al. (1998) with some modifications. The young panicles (40–60 mm), including flowers in meiosis, were fixed for 1–2 d with 3:1 ethanol:acetic acid and stored with 95% ethanol at 4 °C until observation. The flowers in meiosis were incubated in 1 mol/L cellulase (Onozuka R-10) and 1 mol/L pectinase (SERVA Feinbiochemica, Heidelberg, Germany) at 26 °C for 30 min, and then stained in carbol fuchsin. Chromosomal behaviors could be observed with a BX51 microscope (Olympus). All samples were tested in five independent experiments with five replicates each. Thus, $n = 30 \times 5$ independent biological replicates.

Southern blot analysis

Genomic DNA was extracted from leaves of wild type and the putative transgenic lines. Plasmid DNA and genomic DNA were digested with suitable restriction enzymes at 37 °C overnight. Digested DNA was separated on 0.8% (w/v) agarose gel (Sigma-Aldrich) and blotted onto Hybond N^+ nylon membrane (Sigma-Aldrich) for 1–2 d. The membrane was hybridized with a DIG-labelled specific probes RNAi-NPT-F (5′-TGGCTGCTATTGGGCGAAGT-3′) and RNAi-NPT-R (5′-GCCCCTGATGCTCTTCGTCC-3′), and overexpression-HYG-F (5′-ATGCTTTGGGCCGAGGACTG-3′) and overexpression-HYG-R (5′-GCGCGTCTGCTGCTCCATAC-3′) at 42 °C overnight. The hybridized membrane was washed and detected according to the protocol of High Prime DNA Labeling and Detection Starter Kit I (Roche, USA).

Bioinformatics analysis

All of the homologous sequences were obtained from the National Center for Biotechnology Information. Sequence alignments were generated with DNA Star. ExPASy was used to forecast the primary structure of the MND1 protein. Signal peptide and transmembrane domains were analyzed by TMHMM Server2.0. The subcellular localization prediction came from WOLFPSORT.

Statistical analysis

All of the values shown are the averages of the means of five replicates. The results were analyzed for variance using the SAS/STAT statistical analysis package (version 6.12; SAS Institute, Cary, NC, USA) to determine significant differences. Averaged means followed by common letters are not significantly different at $P = 0.05$ using a protected least-significant difference.

Accession code GenBank: AK070961.

Results

Cloning and bio-informatics analysis of *OsMND1*

The *Oryza sativa* gene homologue of the *A. thaliana AtMND1* and yeast gene has been named *OSMND1*. A 624-bp fragment of cDNA from *OsMND1* was amplified with gene-specific primers using a cDNA library of meiotic rice panicles. The information indicated from RACE (Gene ID: 4346611) that *OsMND1* was located on the 9th chromosome, which included 10 exons and 9 introns, and encoded a putative protein of 208 amino acids (Supple. Fig. 1). The putative protein was $C_{1054}H_{1667}N_{285}O_{336}S_6$, with a molecular mass of 23,899.9 kD and an isoelectric point value estimated at 5.03 for the whole protein, which indicated it was an acidic protein. The TMHMM Server 2.0 program predicted that the protein lacked a membrane-spanning domain, and no signal peptide domain was found, indicating that it was not a secreted protein. Thus, it was predicted to be located in the nucleus. The secondary structure provided by Phyre2 indicated that this protein belongs to the *MND1* super family, which included two β-chains and five α-helixes in the crystal structure; the helix structure was discovered at 75–143 amino acids. Finally, 197 residues were modelled with 100.0% confidence by the single highest scoring template, the protein 3D structure of *OSMND1* were established by template-based modeling of Phyre2, based on template c4y66C (Kang et al. 2015) (Supple. Fig. 1b).

A database search using the BLAST 2.0 program indicated that the cDNA sequence possessed significant homology to the meiotic genes in yeast (*Schizosaccharomyces pombe*), mouse (*Mus musculus*) and *Arabidopsis* (Supple. Fig. 2). The neighbor joining (NJ) trees using bootstrap analysis with 1000 replicates were constructed using MEGA 5 (Liu et al. 2018). Comparing the *OsMND1* cDNA with those of yeast, mouse and *Arabidopsis*, the identities were 42%, 42% and 74%, respectively. The OsMND1 protein was also similar to the proteins of these organisms, with amino acid sequence similarities of 42.9%, 42.9% and 74.4%, respectively. This indicated that *MND1* was conserved among

different species, suggesting that it might play similar functions in regulating meiosis.

Expression pattern of *OsMND1* and its protein subcellular localization

The *OsMND1* expression level in panicles of HN2026-4x was higher than in the diploid lines (HN2026-2x and Balilla-2x) or in the other low seed set rate line, Balilla-4x (Supple. Fig. 3a). The high-level expression of *OsMND1* was discovered in the reproductive tissues, such as meiotic panicles and mature seeds, and also almost half expression was found in the roots. Furthermore, the relative expression was very low in stems and leaves. This was consistent with the predicted function of regulating meiosis (Supple. Fig. 3b).

A 35S-GFP-*OsMND1* fusion construct was transformed transiently by microinjection into onion and tobacco epidermal cells, the result revealed that the fusion protein was localized in the nucleus, suggesting that *OsMND1* acts in the nucleus (Supple. Figs. 4a1–a3, b1–b3).

The functional analysis of *OsMND1* by overexpression and RNAi confirmation of the transgenic lines

To test whether transgenic lines were really positive, *OsMND1* overexpressed lines were checked for the antibiotic marker gene *hygromycin* and the Ubi promoter by PCR (data not show). The results suggested that nine lines were positive. Then, the relative gene expression level of *OsMND1* in overexpression lines was investigated further by qRT-PCR to confirm the prior results, and the data demonstrated that the gene expression level improved significantly in the five different lines compared with the wild type (Fig. 1a). To verify the prior PCR and RT-qPCR results, and avoid false positive information, Southern blotting was performed. DNA fragments of the antibiotic *hygromycin* gene were amplified by the s-hyg-F/s-hyg-R primer pair, and then a digoxin-labeled probe was designed using the random primer method. Genomic DNA was isolated and fully digested with *Eco*RI, and the plasmid was digested by *Xho*I. The Southern blot information indicated that line 1 (wild Balilla-4x) had no signal, lines 2 and 3 (putative transgenic plants) had positive signals, and line 4 (the over-expression plasmid) had a positive signal of a different size (Fig. 1b). These results suggested that positive plants had been produced and identified for further investigations.

OsMND1 RNAi plants were checked for the antibiotic marker gene (NPT II) and the Ubi promoter by PCR. The results suggested that 11 lines were positive (data not shown). To confirm this result, the relative gene expression level of *OsMND1* in positive RNAi plants was investigated further by RT-qPCR. After RNAi was introduced, the expression levels in five RNAi-expressing lines were only 60.7% of that in the wild type (Fig. 1c). Southern blotting was performed to verify the PCR and qRT-PCR results and to avoid false positives (Fig. 1d). DNA fragments of G418 from the RNAi vector were amplified by specific primers, and then a digoxin-labeled probe was designed using the random primer method. From these results, we concluded that truly positive RNAi-expressing plants had been produced and identified for further investigations.

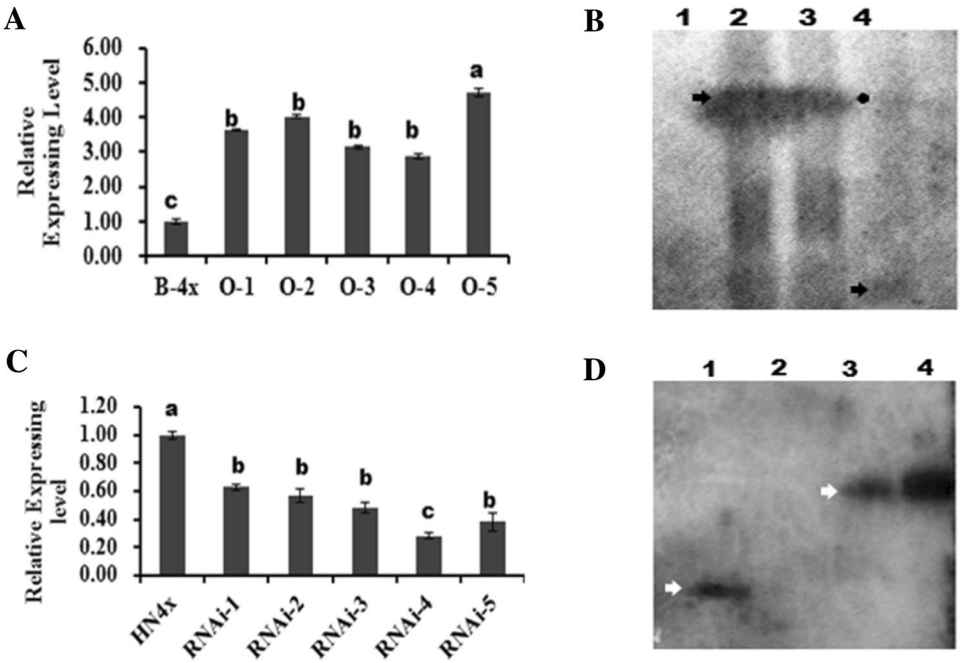

Fig. 1 Identification of the overexpression and RNAi lines in the polyploid rice. **a** Relative expression levels of *OsMND1* in overexpression lines compared with B-4x (Ballina-4x). **b** Southern blots of overexpression lines. From lines 1–4: Ballina-4x, Ballina-4x overexpression line 1, Ballina-4x overexpression line 2, and *OsMND1* overexpression plasmid, respectively. **c** Relative expression levels of *OsMND1* in RNAi lines compared with HN-4x (HN2026-4x). **d** Southern blot results of RNAi lines. From Lines 1–4: RNAi line, HN2026-4x, *OsMND1* RNAi plasmid and *OsMND1* RNAi plasmid, respectively

OsMND1 regulates pollen fertility and viability, the seed set rate, and early embryo development

OsMND1 overexpression improved pollen fertility and viability, normal early embryo development and the seed set rate (Supple. Table 2). The *OsMND1* overexpression line seed set rate ascended significantly, and the average seed set rate of 10 independent lines was 50.49%, while it is only 30.58% in the wild type (Supple. Table 2). *OsMND1* RNAi restricted the pollen fertility and viability, and early embryo development. The seed set rate decreased significantly compared with HN2026-4x (Supple. Table 2). The pollen fertility and viability of HN2026-4x-*OsMND1* RNAi dropped significantly (Fig. 2b1–b4, c, d), while the pollen fertility of the Balilla-4x *OsMND1* overexpression line increased (Fig. 2a1–a4, c, d). The pollen fertility and viability of HN2026-4x-OsMND1 RNAi were 44.8% and 20.3%, respectively, but the pollen fertility and viability were still 85.2% and 52.5%, respectively, in HN2026-4x. After *OsMND1* overexpression in Balilla-4x, the pollen fertility was as high as 66.7%, but it was only 52.4% in Balilla-4x. The viability in Balilla-4x was only 33.7%, but it increased in the Balilla-4x *OsMND1* overexpression line to as high as 64.3%, which was almost two times that in Balilla-4x (Fig. 2d). These results implied that *OsMND1* overexpression was directly beneficial to pollen fertility and viability, and significantly improved the pollen viability (Fig. 2a1–a4, b1–b4). In conclusion, we also found that the relative *OsMND1* expression level was related to pollen fertility and viability, and the seed set rate.

In the interest of investigating the detailed reasons for the *OsMND1* expression level causing changes in the seed set rate, the embryo abortion rate was tested at different stages in *OsMND1*-overexpression Balilla-4x lines and HN2026-4x *OsMND1* RNAi-expressing lines. The data indicated that the *OsMND1* expression level was related to the seed set rate and the embryo abortion type (Supple. Fig. 5). Among the abnormal embryos in the Balilla-4x lines, abortion arrested at 3 d after pollination was 16.5%; at 15 d after pollination 38.7% of the abnormal embryos were blocked, and as high as 46.4% of the ovules did not complete fertilization. However, in the aborted embryos of the *OsMND1*-overexpression lines, only 20.4% of the abnormal embryos did not complete fertilization, with 12.2% of the abortions occurring during early embryo development, while most of the aborted embryos appeared in the filling period (Supple. Fig. 5). These results indicated that *OsMND1* overexpression played an important role in reducing the early embryo abortion ratio led by sterile pollen and unsuccessful fertilization. The abnormal embryo development schedule was also analyzed at different stages in HN2026-4x *OsMND1* RNAi-expressing lines. The unsuccessful fertilization of

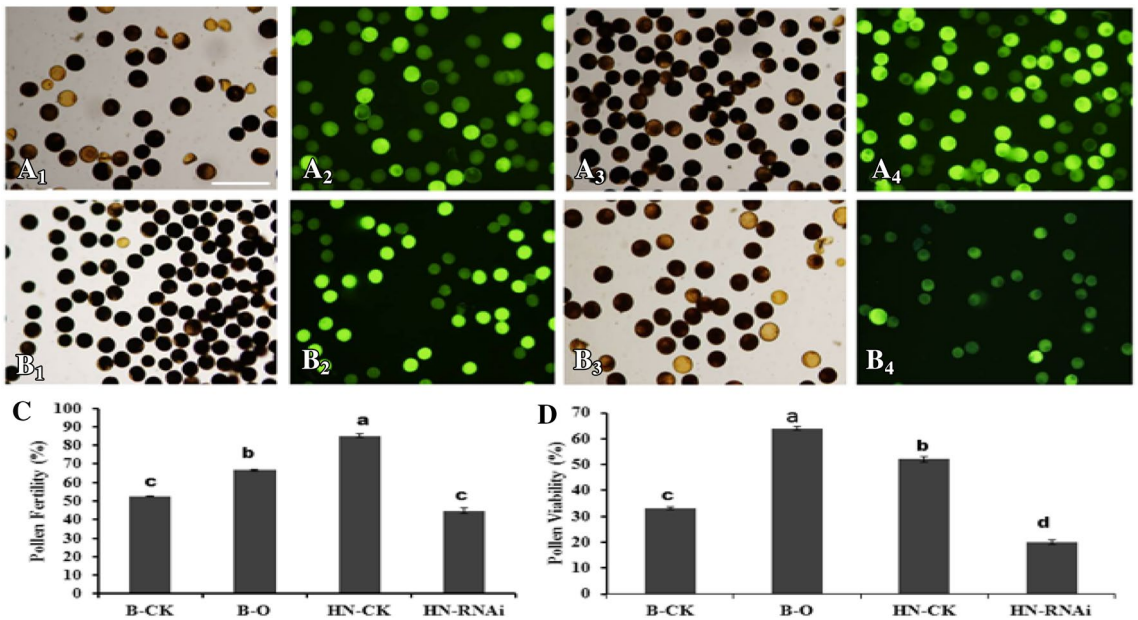

Fig. 2 The *OsMND1*-expression level affected pollen fertility and viability (Bar = 100 μm) (n = 30 × 5 independent biological replicates: all samples were tested in five independent experiments with each included 30 rice plants). **a1** Pollen of Balilla-4x stained by I_2-KI. **a2** Pollen of Balilla-4x stained by FDA. **a3** Pollen of the *OsMND1* over-expression Balilla-4x line stained by I_2-KI. **a4** Pollen of the *OsMND1* over-expression Balilla-4x line stained by FDA. **b1** Pollen of HN2026-4x stained by I_2-KI. **b2** Pollen of HN2026-4x stained by FDA. **b3** Pollen of *OsMND1* RNAi HN2026-4x stained by I_2-KI **b4** Pollen of *OsMND1* RNAi HN2026-4x stained by FDA. **c** The *OsMND1*-expression level affected pollen fertility. **d** The *OsMND1*-expression level affected pollen viability. *B-CK* Balilla-4x; *B-O* Balilla-4x *OsMND1* overexpression; *HN-CK* HN2026-4x; *HN-RNAi* HN2026-4x *OsMND1* RNAi

HN2026-4x *OsMND1* RNAi lines was the main reason for abnormal embryo formation, with a frequency of almost 70.7%, which was markedly higher than that in HN2026-4x. These observations implied that the low seed set rate of HN2026-4x OsMND1 RNAi was affected by *OsMND1*'s functional interference.

OsMND1 functioned in regulating pollen development

To elucidate why *OsMND1* improved the pollen development, the microstructure of pollen of Balilla-4x-*OsMND1* overexpression lines and wild type lines were studied. Pollen fertility was increased significantly because of *OsMND1* overexpression in Balilla-4x lines with low seed set rates. In the wild type line, without the PMeS background, before meiosis (Fig. 3a) and the MMC (megaspore mother cell) stage, the three cell wall layers and the tapetum of the anther formed successfully, but the four MMCs were non-uniform in size (Fig. 3b), and some of these collapsed when they were slightly more vacuolated. The MMC of the wild type shrunk and became a smaller size than those of the overexpression lines. Some of the MMCs began to disintegrate (Fig. 3c), and the tetrad of the wild type was separated from the tapetum (Fig. 3d). The single nucleus pollen shriveled and became abnormal in shape (Fig. 3i). The mature pollen was irregular and could not be stained by I–KI (Fig. 3k). However, in Balilla-4x *OsMND1* overexpression lines, before meiosis (Fig. 3e), the three cell wall layers of the anther and the tapetum formed normally and were similar to those of the wild type. The four MMCs were similar in size with a normal shape (Fig. 3f), and the tetrad was closely linked with the tapetum (Fig. 3g, h). The single nucleus pollen began to accumulate starch and became crescent shaped (Fig. 3j). At the mature stage, pollen cells were round and filled with starch (Fig. 3m). These results suggested that

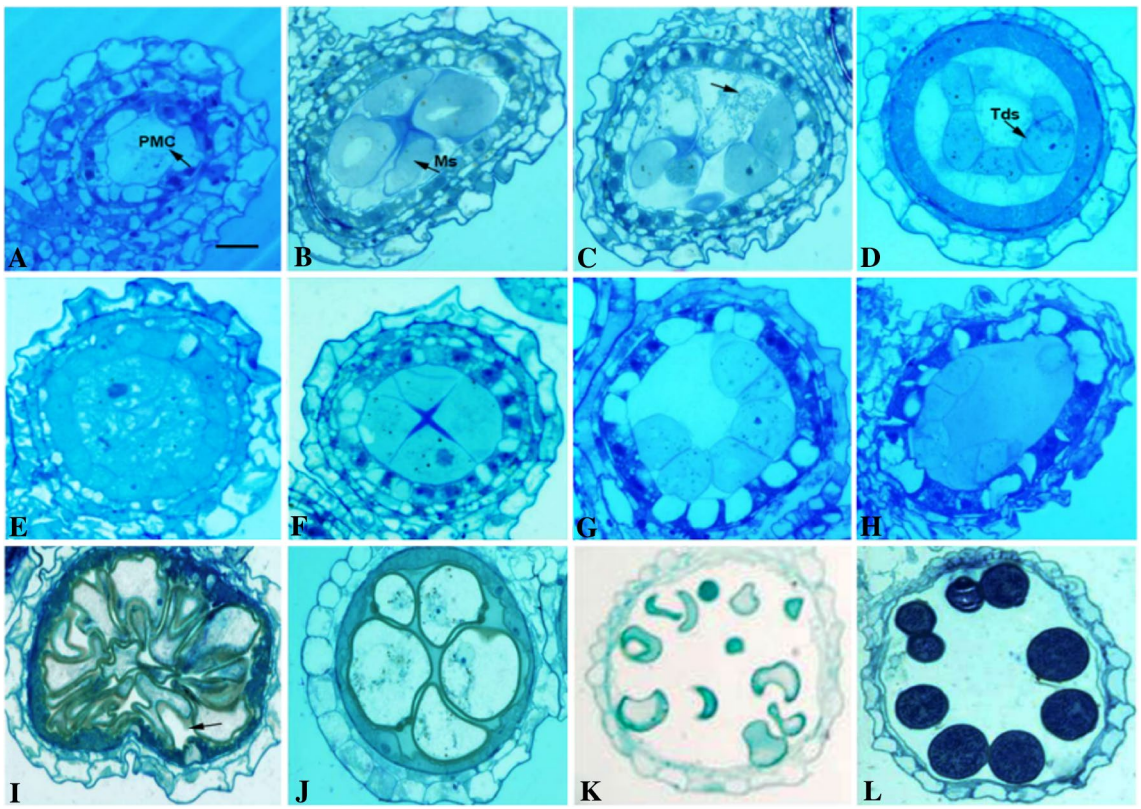

Fig. 3 Transverse sectional analysis of the anther development of the Balilla-4x and the Balilla-4x *OsMND1* overexpression lines (n = 20 × 5 independent biological replicates; all of the samples were tested in 5 independent experiments, each including 30 rice plants) Cross sections of Balilla-4x (a–d, I, and K) and B-O (Balilla-4x *OsMND1* overexpression; e–h, j, l). **a** Three cell wall layers and the tapetum formed at the early pre-meiotic stage in Balilla-4x. **b** Different sized MMCs in Balilla-4x, arrow indicates the small cell, **c** Tetrad beginning to disintegrate (arrow) at the meiotic stage in Balilla-4x. **d** The tetrad separated from the tapetum gathered at the center in Balilla-4x. **e** Three cell wall layers and the tapetum formed at the early pre-meiotic stage in B-O. **f** The four MMCs were of similar size with normal shapes in B-O. **g** The tetrad is closely linked with the thick tapetum in B-O. **h** The tetrad is still closely linked with the tapetum in B-O. **i** Abnormal vacuolated pollen in Balilla-4x. **j** Crescent-shaped normal pollen in B-O. **k** Sterile mature pollen in Balilla-4x. **l** Round pollen filled with starch in B-O. PMC, pollen mother cell; *E* epidermis; *En* endothecium; *ML* middle layer; *T* tapetum; *Ms* microsporocyte; *Tds* tetrads; *Msp* microspore. Bars = 25 μm

OsMND1 overexpression improved the pollen fertility and viability by regulating pollen meiosis. To analyze the pollen development in OsMND1 RNAi lines, the microstructures of pollen in OsMND1 RNAi and wild type plants was studied in detail. *OsMND1* RNAi impeded pollen development significantly. The pollen fertility decreased markedly due to the *OsMND1* RNAi. No detectable difference was discovered between wild type and *OsMND1* RNAi lines before meiosis or at the MMC stage (Fig. 4a, e, b, f), the three cell wall layers of the anther and tapetum formed successfully (Fig. 4b, f), but at the early stage of meiosis, the tapetum of the *OsMND1* RNAi plant was swollen (Fig. 4g), while it was normal in the wild type (Fig. 4c). At the tetrad stage, the size of the tetrad was not uniform, and it was separated from the tapetum in the *OsMND1* RNAi plants (Fig. 4h); however, the tetrad was closely connected to the tapetum in the wild type (Fig. 4d). Single nucleus-containing pollen in the *OsMND1* RNAi plant was empty and irregular in shape (Fig. 4j, l), and the mature pollen as aberrant and wizened, with no abundant starch accumulation. Therefore, the fertility of this pollen was very low (Fig. 4n). The single nucleus-containing and mature pollen of the wild type were normal in shape and contained an abundant amount of starch (Fig. 4i, k, m).

OsMND1 functioned in maintaining meiotic stability in rice

Normal Balilla-4x is a non-PMeS background line, and the meiotic disturbance leads to a drop in the seed set rate. To better understand the mechanism of male sterility in

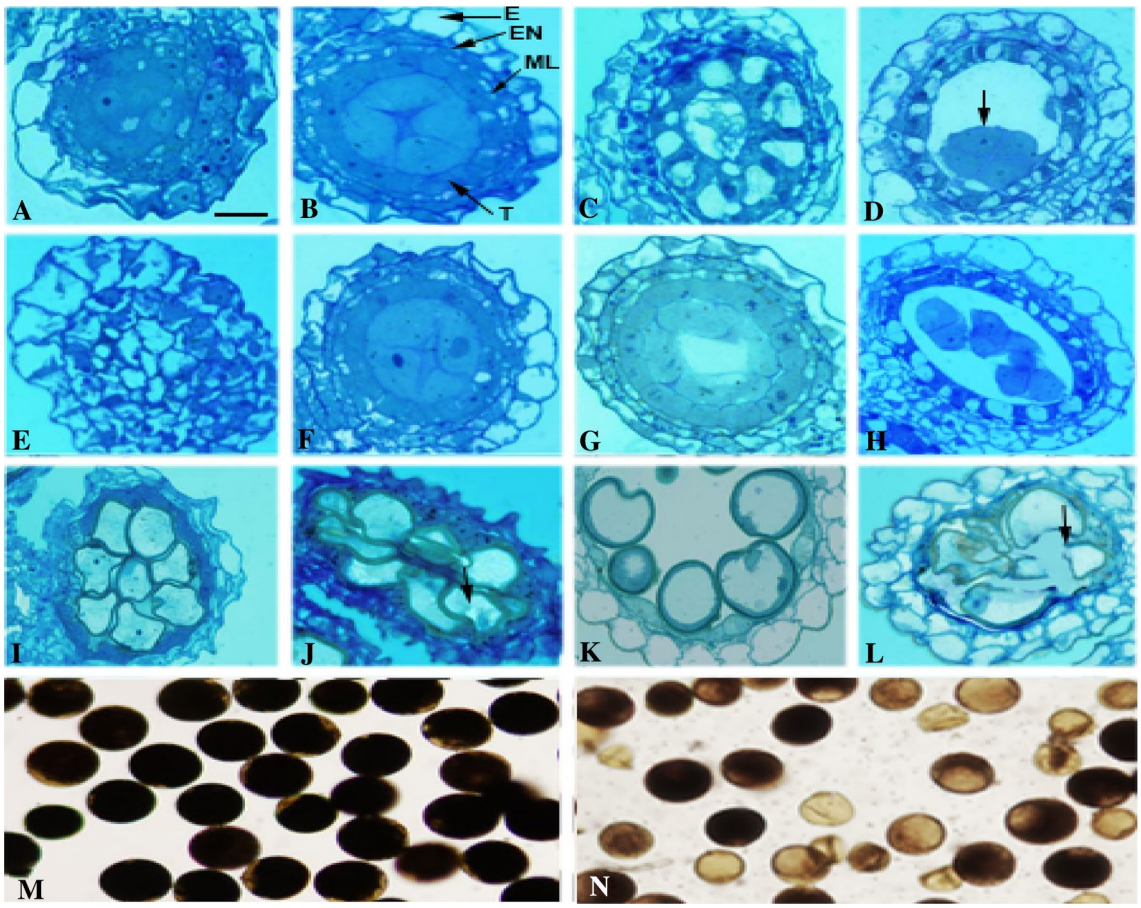

Fig. 4 *OsMND1* RNAi impeded pollen development. (n = 20 × 5 independent biological replicates; all of the samples were tested in 5 independent experiments, each including 30 rice plants) Transverse sections of HN2026-4x (a–d, I, K, and M) and HN2026-4x RNAi (e–h, j, l, n). **a** The three cell wall layers formed at the early pre-meiotic stage in HN2026-4x. **b** The tapetum formed successfully at the MMC stage in HN2026-4x. **c** The tapetum is normal at meiosis in HN2026-4x. **d** The tetrad (arrow head) connected with the tapetum in HN2026-4x. **e** The three cell wall layers formed at the early pre-meiotic stage in HN2026-4x RNAi. **f** The tapetum formed successfully at the MMC stage in HN2026-4x RNAi. **g** At the early stage of meiosis, the tapetum was swollen in HN2026-4x RNAi. **h** The tetrad separated from the tapetum in HN2026-4x RNAi. **i** Normal pollen at the vacuolated stage in HN2026-4x. **j** Irregular shaped pollen (arrow head) in HN2026-4x RNAi at the vacuolated stage. **k** Round and normal pollen in HN2026-4x. **l** Irregular pollen degrading (arrow head) in HN2026-4x RNAi. **m** Round mature pollen with starch in HN2026-4x. **n** Aberrant mature pollen lacking abundant starch accumulation in HN2026-4x RNAi. *E* epidermis; *En* endothecium; *ML* middle layer; *T* tapetum

non-PMeS Balilla-4x, the chromosomal behavior in PMCs of Balilla-4x and B-O (Balilla-4x *OsMND1* overexpression) were compared. In the wild type, the homologous chromosomes underwent pairing and synapsis at zygotene (Fig. 5a2), and at pachytene, the chromosomes continued to condense but showed more lines, leading to an abnormal chromosome state (Fig. 5b2). In the subsequent diakinesis stage (Fig. 5c2), many univalents, trivalents, and even multivalents appeared. Each cell had an average of 1.25 univalents and 0.25 trivalents, resulting in the presence of lagging chromosomes at anaphase I (Figs. 5d2, 7b, 5e2) and anaphase II (Fig. 5h2), and lagging chromosomes were found in almost 46.81% of the whole cells (Supple. Table 3). In an *OsMND1* overexpression Balilla-4x line, chromosomes condensed normally at zygotene (Fig. 5a1) and homologous chromosomes began to pair and synapsis (Fig. 5b1) at pachytene. At diakinesis (Fig. 5c1) normal chromosome segregation occurred and bivalent or quadrivalent structures appeared, with each cell having only an average of 0.06 univalents and 0.02 trivalents, which was beneficial to normal chromosome behavior at metaphase I (Fig. 5d1; Fig. 7b), anaphase I (Fig. 5e1–g1), and anaphase II (Fig. 5h1), and only 23.91% of the checked cells had lagging chromosomes. This was amended significantly compared with the wild type Balilla-4x (Supple. Table 3). However, no visible difference could be found in the second division (Fig. 5i1, i2), and the four sister cells formed (Fig. 5j1, j2).

The aberrant kinetics of homologous chromosome in *OsMND1* RNAi meiocytes was investigated. Meiosis in PMeS lines was relatively normal, and the seed set rate was very high. At zygotene, homologous chromosomes displayed successful pairing and synapsis in PMeS line HN2026-4x (Fig. 6a2). Homologous chromosomes were synapsed fully with the formation of the SC during pachytene (Fig. 6b2).

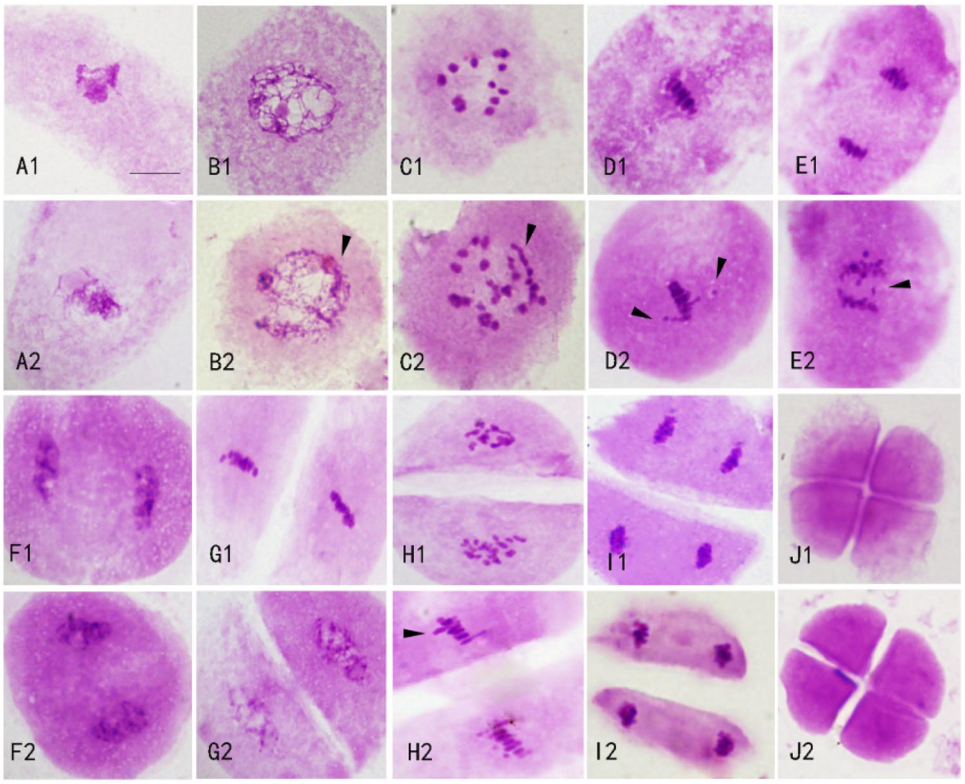

Fig. 5 *OsMND1* overexpression affected the behavior of chromosomes in meiosis. (n = 20 × 5 independent biological replicates; all of the samples were tested in 5 independent experiments, each including 30 rice plants) Male meiocyte meiotic analysis of Balilla-4x (a2–j2) and B-O (Balilla-4x *OsMND1* over-expression: a1–j1). **a1** Chromosomes condensed normally at zygotene. **a2** The homologous chromosomes underwent pairing and synapsis at zygotene in Balilla-4x. **b1** Homologous chromosomes began to pair and synapsis at pachytene in B-O. **b2** At pachytene the tangled chromosomes (arrow) appeared confused in Balilla-4x. **c1** Normal chromosome segregation and bivalent or quadrivalent structures appeared at diakinesis in B-O. **c2** Many univalents, trivalents, and even multivalents (arrow) appeared at diakinesis in Balilla-4x. **d1** Metaphase I, chromosomes arrayed on a line in B-O. **d2** Lagging chromosomes (arrow) discovered in Balilla-4x. **e1** Chromosomes undergo equal division at anaphase I in B-O. **e2** Unequal division with many lagging chromosomes (arrow) at anaphase I in Balilla-4x. **f1** Preparing for the first division at anaphase I in B-O. **f2** Preparing for the first division at anaphase I in Balilla-4x. **g1** Prophase I with two cells in B-O. **g2** Prophase I with two cells in Balilla-4x. **h1** Metaphase II, two sister cells form equally in B-O. **h2** Two sister cells form with lagging chromosomes in Balilla-4x. **i1** Second division in B-O. **i2** Second division in Balilla-4x. **j1** Four sister cells form a tetraspore in B-O. **j2** Four sister cells formed in Balilla-4x. Bars = 10 μm

By diakinesis (Fig. 6c2), chromosomes further condensed to produce very short chromosomal pairs. At metaphase I, chromosomes were in a line (Fig. 6d2) and no lagging chromosomes were discovered at anaphase I in HN2026-4x (Fig. 6e2), with each cell having an average of 1.25 univalents and 0.03 trivalents (Fig. 7a). Additionally, only 13.79% of the checked cells were found to have lagging chromosomes (Supple. Table 3). No visible differences were found during anaphase I and prophase II between HN2026-4x RNAi and HN2026-4x (Fig. 6f1, f2, g1, g2). The normal four sister cells were produced because of stable chromosomal behavior and consistent division in HN2026-4x (Fig. 6f2, g2, h2, i2). In HN2026-4x *OsMND1*-RNAi, signal lines that did not pair were observed at zygotene (Fig. 6a1). At pachytene (Fig. 6b1), chromosomes entwined excessively and showed more signal lines, indicating non-synaptic chromosomes. At diakinesis (Fig. 6c1), chromosomes connected to each other so that they could not spread out, and gathered in the center of the cell, with each cell having an average of 4.58 univalents and 0.62 trivalents (Fig. 7a). Then, at metaphase I (Fig. 6d1), anaphase I (Fig. 6e1, f1) lagging chromosomes appeared, and 51.22% of the checked cells had lagging chromosomes (Supple. Table 3). In addition, the division appeared slowly at anaphase II (Fig. 6i1), indicating that the

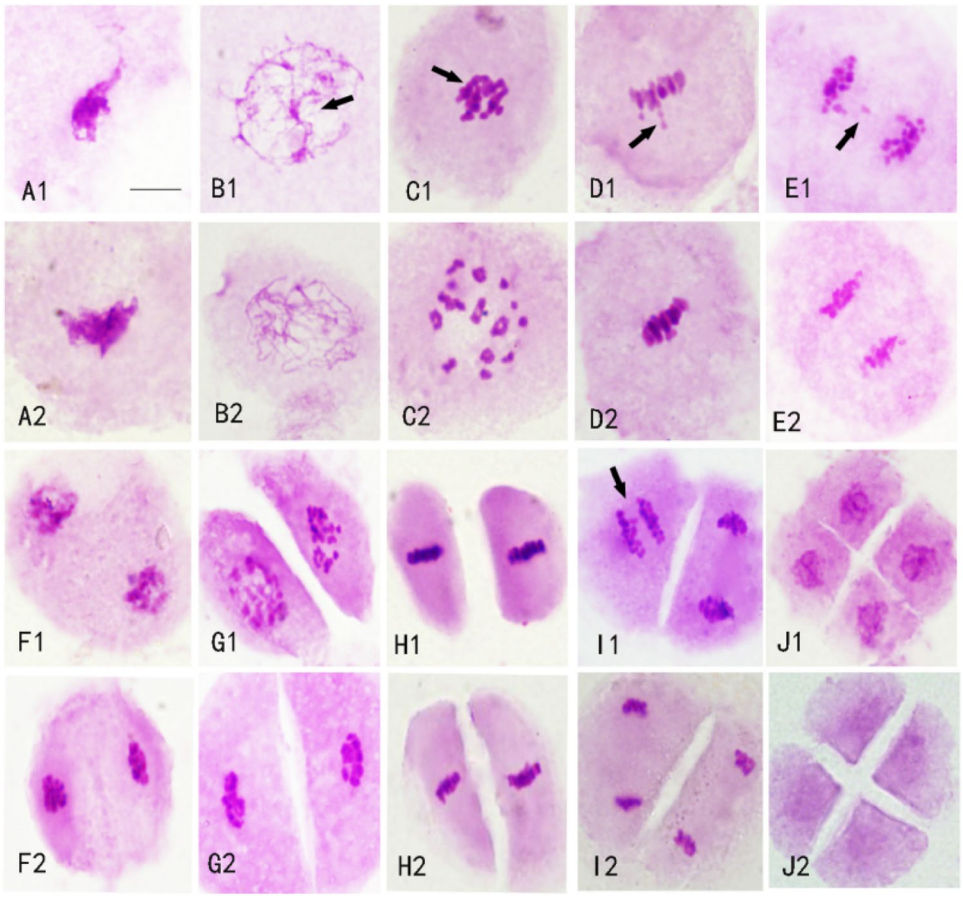

Fig. 6 *OsMND1* RNAi affected the behavior of chromosomes during meiosis (n = 20 × 5 independent biological replicates; all of the samples were tested in five independent experiments, each including 30 rice plants) chromosomal behavior of HN2026-4x RNAi (a1–j1); HN2026-4x (a2–j2) male meiocytes at various stages. **a1** Some signal lines are not pairing at zygotene in HN2026-4x RNAi. **a2** Homologous chromosomes underwent pairing and synapsis at zygotene in HN2026-4x. **b1** At pachytene, chromosomes entwined (arrow head) excessively in HN2026-4x RNAi, arrow indicates tangled area. **b2** Homologous chromosomes are fully synapsed with the completion of the SC in HN2026-4x. **c1** At diakinesis, arrow shows non-homologous chromosomes gathered together in HN2026-4x RNAi. **c2** Chromosomes further condense to produce very short chromosomal pairs at diakinesis in HN2026-4x. **d1** Lagging chromosomes (arrow head) appeared at anaphase I in HN2026-4x RNAi. **d2** No lagging chromosomes at anaphase I in HN2026-4x. **e1** Anaphase I with lagging chromosomes (arrow) in HN2026-4x RNAi. **e2** Anaphase I with no lagging chromosomes in HN2026-4x. **f1** Preparing for the first division at anaphase I in HN2026-4x RNAi. **f2** Preparing for the first division at anaphase I in HN2026-4x. **g1** Prophase II with two cells in HN2026-4x RNAi. **g2** Prophase II with two cells in HN2026-4x. **h1** The first division finished in HN2026-4x RNAi. **h2** Metaphase II, the first division finished in HN2026-4x. **i1** At anaphase II, the division is inconsistent in HN2026-4x RNAi, arrow head indicates late division. **i2** At anaphase II, the division consistent in HN2026-4x. **j1** Four sister cells form a tetraspore in HN2026-4x RNAi. **j2** Four sister cells form a tetraspore in HN2026-4x. Bars = 10 μm

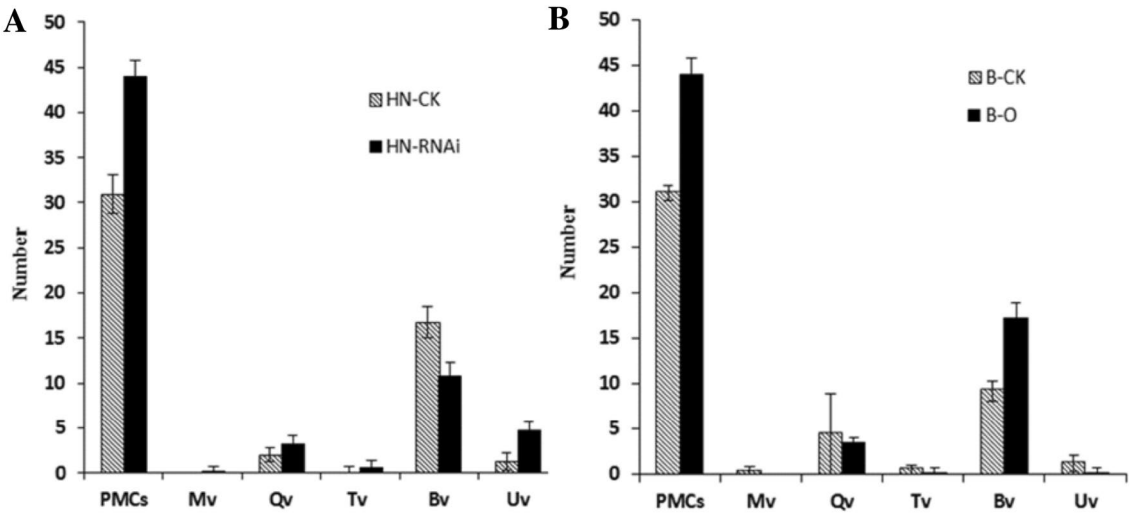

Fig. 7 *OsMND1* affects the chromosomal behavior of pollen mother cells in meiotic prophase I (n = 30 × 5 independent biological replicates; all of the samples were tested in 5 independent experiments, each including 30 rice plants). **a** The chromosomal behavior of pollen mother cells in RNAi lines (HN-RNAi) compared with HN-CK (HN2026-4x). **b** The chromosomal behavior of *OsMND1* in overexpression lines (B-O) compared with B-CK (Ballina-4x) PMCs: pollen mother cells; Mv: multivalent, means the association of more than four chromosomes; *Qv* quadrivalent IV; *Tv* trivalent; *Bv* bivalent; *Uv* univalent. *B-CK* Balilla-4x; *B-O* Balilla-4x *OsMND1* overexpression; *HN-CK* HN2026-4x; *HN-RNAi* HN2026-4x *OsMND1* RNAi

division time was inconsistent. Prior to the four sister cells forming, abnormal cells were produce because of lagging chromosomes and inconsistent divisions (Fig. 6j1). These observations suggested that the effects of *OsMND1* might be to maintain the balance of synapsis and recombination.

OsMND1 affected other meiosis-related gene expression levels

To understand why *OsMND1* affects the dynamic behavior of meiosis and its signal transduction pathway, the key related genes, such as *HOP2, DMC1, RAD51* and *ZEP1* were investigated (Fig. 8). The qRT-PCR results indicated that the relative expression levels of *HOP2, DMC1*, and *ZEP1* in *OsMND1*-overexpression lines were 4.36, 4.51, and 8.71 times higher than in the wild type, respectively; however, the expression level of *RAD51* did not change significantly. In the *OsMND1* RNAi lines, the relative expression levels of *HOP2, DMC1*, and *ZEP1* were 0.46, 0.85, and 0.89 times than in the wild type, respectively, but unexpectedly the expression level of *RAD51* increased markedly.

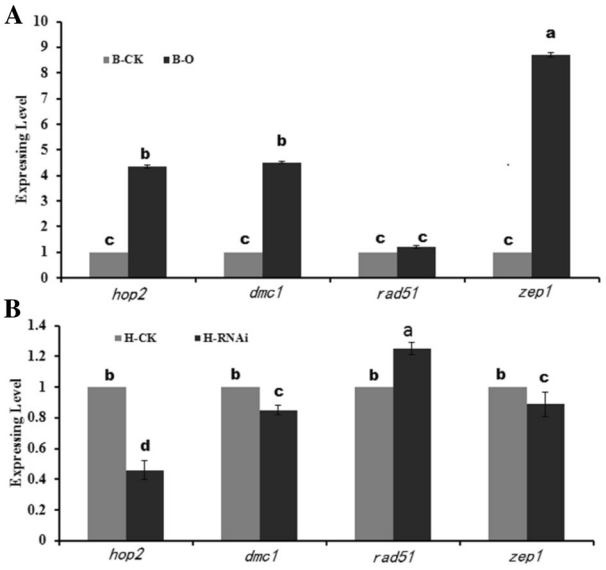

Fig. 8 *OsMND1* overexpression and RNAi affect other meiosis-related gene expression levels. **a** The relative expression levels of *HOP2, DMC1, RAD51*, and *ZEP1* in B-O (Balilla-4x overexpression line) compared with B-CK (Balilla-4x). **b** The relative expression levels of *HOP2, DMC1, RAD51*, and *ZEP1* in H-RNAi (HN2026-4x RNAi) compared with H-CK (HN2026-4x)

Discussion

The PMeS background plays a critical role in the stability of meiosis in polyploid rice

Because the gene duplications result in complicated chromosomal behaviors, most polyploid rice have disorganized meiosis, leading to a high frequency of abnormal pollen (Cai et al. 2007). The breeding of polyploid rice had no breakthroughs for a long time because of the low seed set rate. The discovery and application of PMeS materials played a pivotal role in addressing this problem (Cai et al. 2007;

He et al. 2010). PMeS line HN2026-4x undergoes a normal meiosis, similar to that of its diploid, but Balilla-4x does not have the PMeS background. Thus, it has a low pollen fertility level and seed set rate because of the aberrant meiosis (He et al. 2010). Nevertheless, little is known about the meiotic stability-controlling mechanism of polyploid rice. Determining the meiotic mechanisms will be difficult until breakthroughs in PMeS line breeding have been realized (Cai et al. 2007; He et al. 2010).

Polyploidization is the multiplication of the whole chromosomal complement (Doyle et al. 2008; Leitch and Leitch 2008; Li et al. 2016b). The new polyploid has to be equipped with mechanisms enabling it to cope with the genomic stress of chromosomal doubling in the early stages of its formation to overcome reduced fitness and realize stability. The gradual stabilization of polyploids is thought to be associated with chromosomal DNA changes, and meiotic regulation for a balanced anaphase segregation is very important (Lukaszewski and Kopecky 2010; Grandont et al. 2013). Cytological diploidization has long been viewed as a critical step for polyploid speciation. This process is fundamentally different in autopolyploid and allopolyploid species (Cifuentes et al. 2010). Polyploid genomes face special challenges during meiosis that are solved by specific restrictions on the positions of crossover recombination events and, thus the positions of chiasmata. A critical feature is an increase in the effective distance of meiotic crossover interference (Bomblies et al. 2016). Recombination is a main mechanism generating diversity in all of the sexual organisms through crossovers (COs). The frequency of COs is thus a key factor in increasing the variability in natural populations and in breeding (Suay et al. 2014). However, the meiotic regulation of polyploid wheat and rape (*Brassica* spp.) has been studied for a long time and a degree of understanding has been achieved (Griffiths et al. 2006; Nicolas et al. 2009; Xin et al. 2016). Previous reports have indicated that changes in the ploidy level can be associated with an increase in the recombination rate, suggesting that this could be a general trend (Pecinka et al. 2011). The frequency of recombination is higher in auto- or allotetraploids than in diploids, and the same result was also reported in rape and in cotton (*Gossypium* spp.) (Pecinka et al. 2011). *Ph1*, the main locus responsible for the cytological diploidization of wheat (Griffiths et al. 2006; Greer et al. 2012) was shown to affect CO formation both between homeologs (Greer et al. 2012) and homologs (Lukaszewski and Kopecky 2010). This result was consistent with the general trend to improve the CO frequency in polyploid species, and the genome repeats contribute in different levels of CO suppression between homeologs in *B. napus* allohaploids (Suay et al. 2014). In the non-PMeS background Balilla-4x, the disturbance in meiosis decreased the seed set rate, with 46.81% of the whole cells containing lagging chromosomes. By contrast, the meiosis of PMeS is normal and relatively similar to that in its diploid, and the seed set rate is maintained. Until now, the control mechanisms of polyploid rice meiosis have been poorly understood, and we believe that PMeS rice has different control skills regarding the formation and repair of DSBs and interference from the non-PMeS background line. There will be much research activity in this field in the future.

OsMND1 regulates the early stage of meiosis

OsMND1, which is involved in rice meiosis, is the homologous gene of yeast *MND1*, and has high homology with the gene in *Arabidopsis. MND1* plays an important role in homologous chromosome pairing, synapsis and recombination, but its function in rice is not understood. In the PMeS line HN2026-4x, *OsMND1* was expressed mainly in meiotic spikes, and at the same time, *OsMND1* was found to be expressed in roots and early stage embryos undergoing vigorous division. There were significant differences in the expression levels of *OsMND1* in the diploid and polyploid meiotic spikes. *OsMND1* expression level in the meiotic panicles of the PMeS line HN2026-4x was significantly higher than in the other non-PMeS line (Balilla-4x) or the diploid lines (HN2026-2x and Balilla-2x) (Supple. Fig. 3A). We will confirm whether *OsMND1* plays roles both in meiosis and mitosis in the future. In this study, *OsMND1*-overexpression improved meiotic chromosomal behavior and pollen development of non-PMeS Balilla-4x with low seed set rate. However, *OsMND1*-RNAi led to unequal chromosome segregation and chromosomal lagging. Each cell of *OsMND1*-RNAi rice had an average of 4.58 univalents and 0.62 trivalents, leading to 51.22% of the cells having lagging chromosomes. The yeast *mnd1* mutant is defective in meiotic inter-homolog interactions, and arrests in meiotic prophase I due to the activation of a DNA damage checkpoint. With most of the DSBs unrepaired, a low level of mature recombinants is produced and the meiotic nuclear division fails, resulting in defective SC formation (Tsubouchi and Roeder 2002). These results confirmed that *OsMND1* participated in the meiosis of polyploid rice and that the gene may play a role in the chromosomal recombination process at the early stage. These meiotic behavioral observations in *OsMND1* overexpression and *OsMND1*-RNAi lines suggested that the effects of *OsMND1* might be to maintain the balance of synapsis and recombination. The formation of COs exclusively between homologous chromosomes is very important, and reducing the number of CO guarantees correct chromosomal segregation (Pecinka et al. 2011). The *AHP2* of *Arabidopsis* is homologous to *S. cerevisiae*'s *HOP2* and *S. pombe*'s *MEU13* (Nabeshima et al. 2001), which act in regulating homology between pairing partners, and their mutation leads to chromosomal fragmentation and unbalanced chromosome segregation during early meiosis (Schommer et al. 2003).

We speculated that PMeS line HN2026-4x was bred in a different genetic background, which was considered as a distant hybridization offspring. Maybe this allopolyploid species inherited or evolved recombination-modifying loci that suppressed CO formation between the "homeologous chromosomes" inherited from the different parental species, and the loci regulated some basic mechanisms of chromosome recognition. Thus, we hypothesize that the PMeS background contributed to maintaining meiotic stability for polyploid rice and that *OsMND1* plays a key role in regulating the recombination rate, the number of the COs and DSB repairs.

To compare the *OsMND1* functions between diploid and polyloid, the seeding rates of the overexpression and RNAi lines in the diploid were identified and investigated (Supple. Figs. 6, 7; Supple. Table 4). *OsMND1* overexpression in the diploid line (Balilla-2x) did not significantly affect the pollen fertility level and the seed set rate compared with the wild type. *OsMND1* RNAi in the diploid line (HN2026-2x) significantly reduced the seed set rate to only between 11.50–31.43%, while it was 88.41% in the wild type (Supple. Figs. 6, 7). Intriguingly, the tiller number with *OsMND1* overexpression in the diploid line (Balilla-2x) increased by 32% compared with the wild type. We will conduct further research in this area.

Coaction factor of *OsMND1*

MND1 and HOP2 are conserved proteins that form a complex by interacting with each other, and the HOP2/MND1 complex acts on the unstable interactions between intact DNA duplexes that occur prior to DSB induction (Nabeshima et al. 2001; Tsubouchi and Roeder 2002). The comparison suggests that HOP2 and MND1 affect recombination through a mechanism distinct from those of the RAD51 and DMC1 proteins (Tsubouchi and Roeder 2002; Lee et al. 2015). The HOP2/MND1 complex joins the meiotic homologous recombination, when the function of either *MND1* or *HOP2* is lost, which leads to unsuccessful DNA repair in *Arabidopsis* (Uanschou et al. 2013). *RAD51*-mediated IS repair is independent of HOP2/MND1 (Vignard et al. 2007; Uanschou et al. 2013). However, emerging evidence has revealed that HOP2/MND1 is expressed in somatic tissues in humans, and that it functions in conjunction with the Rad51 recombinase to repair damaged telomeres through the alternate mechanism of telomere lengthening (Zhao and Sung 2015). In our research, the qRT-PCR results indicated that the relative expression levels of *HOP2, DMC1*, and *ZEP1* in *OsMND1* overexpression lines were 4.36, 4.51, and 8.71 times those in wild type, respectively; however, the expression level of *RAD51* did not change distinctly. The relative expression levels of *HOP2, DMC1*, and *ZEP1* in the *OsMND1* RNA lines were reduced significantly to 0.46, 0.85, and 0.89 times those in wild type, respectively. Unexpectedly, the expression level of *RAD51* was markedly upregulated. This indicated that *OsMND1* as a crucial player in establishing homologous chromosome pairs and in repairing meiotic DSBs, using the pathways of *HOP2, DMC1*, and *ZEP1*. Our outcome corroborated to some degree, those found in other organisms. We speculate that OsMND1 may play an essential role in the establishment of homologous chromosome pairing and in repairing meiotic DSBs through homologous chromosomes. MEICA1 interacts with TOP3α, which is essential for processing recombination intermediates and limiting meiotic crossover formation. Meiotic DSB formation is a key factor for chromosome associations in *meica1*. Furthermore, MEICA1 acts downstream of DMC1 (Hu et al. 2017). The detailed relationship between *OsMND1* and MEICA1 is an interesting future topic. *OsMND1* is expressed in pollen and root, and we hypothesize that *OsMND1* may have roles in the regulation of both meiosis and mitosis. This hypothesis needs to be studied in the future. However, DMC1 only functions in meiosis. According to our results, *OsMND1* is expressed in the anther, root, and embryo, but in *Arabidopsis*, it is only expressed in meiosis. Thus, we speculate that *MND1* in rice may have different functions.

Polyploidy may confer a new degree of plasticity to an organism, although polyploidization may also confer unstable meiotic challenges (Chen 2010; Hollister et al. 2012). Rice is considered as an ancient aneuploidy (Vandepoele et al. 2003), and polyploidy has tremendous advantages in yield and stress resistance (Zhang et al. 2015). The extensive application of PMeS lines in polyploid rice breeding has great potential. How duplicated gene(s) can affect rice meiosis and the pollen development pattern could be interesting for biological and agricultural applications. Solving the mechanism of meiosis in PMeS rice requires more integrative research.

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Author Contributions Yuguo Xiong and Lu Gan contributed equally to this paper, and cooperated to perform all of the experiments. Yaping Hu completed the gene cloning and vector construction. Wenchao Sun, Xue Zhou, Jinming Zhang, and Bo Peng contributed to making figures and tables. Zhaojian Song and Xianhua Zhang were responsible for plant materials and nursery maintenance. Yang Li, Zhifan Yang, and Weifeng Xu provided important guidance for molecular experiments and data analyses. Jianhua Zhang provided many suggestions and revised the manuscript. Yuchi He and Detian Cai cooperated to design the research and write the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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