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Comparative transcriptome analysis reveals key genes associated with meiotic stability and high seed setting rate in tetraploid rice

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Abstract

Background Polyploid rice has a high yield potential and excellent nutritional quality. The development of polyploid rice remained critically limited for several decades due to low seed setting rate until the successful breeding of polyploid meiosis stability (PMeS) lines. To determine the mechanism responsible for meiotic stability and high seed setting rate of PMeS line, agronomic traits, pollen fertility and viability, and meiotic behaviors of PMeS and non-PMeS lines were investigated. Further, comparative transcriptome analysis was performed to identify genes associated with meiotic stability and high seed setting rate in PMeS line.

Results The seed setting rate, fertile and viable pollen ratios of PMeS line were significantly higher than those of non-PMeS line. The PMeS line exhibited stable meiosis, and chromosomes mainly paired as bivalents, rarely as univalents and multivalents in prophase I. Few lagging chromosomes were observed in anaphase I. By contrast, the homologous chromosomes pairing was disorganized in the non-PMeS line, with low frequencies of bivalents and high frequencies of univalents and multivalents in prophase I, while more cells with increased lagging chromosomes were detected in anaphase I. Many differentially expressed genes (DEGs) between PMeS and non-PMeS lines were identified through comparative transcriptome analysis. Some meiosis-related genes were specifically investigated from all DEGs. Further, several meiotic genes were identified as candidate genes.

Conclusions The study not only demonstrates the morphological, cytological, and molecular differences between the PMeS and non-PMeS lines, but also provides several key genes associated with meiotic stability and high seed setting rate in tetraploid rice.

Keywords Tetraploid rice, High seed setting rate, Meiotic stability, Meiotic genes, Transcriptome analysis

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Background

Polyploidization (whole-genome duplication) is a predominant driver of plant evolution and speciation that enhance plant fitness through increased genetic diversity and adaptation to diverse environments [1–4]. Naturally occurring polyploids have been identified in a wide range of taxa and recent estimates suggest that almost all extant angiosperm species have experienced one or more polyploidization events in their evolutionary history [5–9]. Many crops, such as wheat (*Triticum aestivum* L.), cotton (*Gossypium hirsutum* L.), and rape (*Brassica napus* L.), are great examples of polyploid evolution in plants. After undergoing the evolution from diploid to polyploids, they have not only generated rich genetic diversity but their yields have also increased [10]. Artificially induced polyploidy is a valuable tool for facilitating plant breeding and development of new crops [11]. Polyploid rice (*Oryza sativa* L.) is characterized by thick stems, large grains, increased nutritive value, strong stress resistance, and high yield potential [12–14]. Following the discovery of autotetraploid rice [15], intense polyploid rice breeding programs were conducted before 1960s. But nearly all research ceased because of the low seed setting rate of polyploid rice [16]. The low seed setting rate was the bottleneck problem that seriously limited the development of polyploid rice breeding [12, 17]. According to the fact that natural allopolyploid plants often exhibit diploid-like meiotic behavior and normal sexual reproduction, Cai et al. proposed that the origin of the low seed setting rate in autotetraploid rice was the formation of unstable pairing forms, such as univalent and multivalent during meiosis [18]. Expanding the genetic distance between chromosomes and reducing the formation of unstable pairing forms is a key strategy to overcoming the low seed setting rate in polyploid rice [18]. After years of work, two polyploid meiosis stability (PMeS) lines, HN2026-4*x* and Sg99012-4*x* with stable meiosis and high seed setting rates (> 80%) were bred through extensive inter-subspecies and composite crosses between *indica* and *japonica* [19]. Moreover, hybrids between PMeS lines and common autotetraploid rice lines with low seed setting rates showed over 80% high seed setting rates [19, 20]. This indicated that the breeding of PMeS lines solved the bottleneck of low seed setting rate in polyploid rice [19, 21]. Based on the PMeS lines, over 5,000 polyploid rice lines have been developed and cultured, with numerous lines nearing production and application phases [22, 23]. Therefore, the determination of meiotic stability and high seed setting rate mechanism in PMeS line has important theoretical and practical significance for polyploid rice breeding. In the present study, the agronomic traits, pollen fertility and viability, and meiotic behaviors of PMeS line HN2026-4*x* and non-PMeS line 9311-4*x* were investigated. Moreover, deep illumina RNA sequencing was used to analyze their genetic differences at the transcriptome level. Some meiosis related genes were specifically investigated from all differentially expressed genes (DEGs). This study reveals key morphological, cytological and molecular diversity between PMeS and non-PMeS lines and provides candidate meiotic genes associated with stable meiosis and high seed setting rate in tetraploid rice.

Results

Agronomic traits

The main morphology and agronomic traits of HN2026-4*x* (PMeS line) and 9311-4*x* (non-PMeS line) are shown in Table 1; Fig. 1. The plant height, effective panicle number per plant, total grain number per panicle, and filled grain number per panicle of HN2026-4*x* were significantly higher than those of 9311-4*x*. However, the grain length and 1000-grain weight of HN2026-4*x* were significantly lower than those of 9311-4*x*. Especially, the seed setting rate of HN2026-4*x* was 83.73%, which was significantly higher than that of 9311-4*x* (32.15%). This is the most typical difference between PMeS and non-PMeS lines in polyploid rice.

Meiotic behavior of chromosomes in pollen mother cells

There were significant differences in meiotic behavior of pollen mother cells (PMCs) between HN2026-4*x* and 9311-4*x* (Fig. 2). Chromosomes mainly paired as bivalents (16.42/cell) in HN2026-4*x*, with some quadrivalents (3.62/cell), while only a few univalents (0.06/cell), trivalents (0.04/cell) and multivalents (0.08/cell) were detected in prophase I. Only 1.4 lagging chromosomes were detected in anaphase I and the percentage of cells with lagging chromosomes was only 10.71% (Tables 2 and 3). By contrast, irregular chromosome pairing was detected in 9311-4*x*, with low frequency of bivalents and

Table 1 Main agronomic traits of HN2026-4*x* and 9311-4*x*

Lines	PH (cm)	EPP	PL (cm)	GL (cm)	GW (cm)	TGP	FGP	SSR (%)	TGW (g)
HN2026-4*x*	118.72 ± 2.24	8.40 ± 0.89	24.01 ± 2.16	0.89 ± 0.02	0.39 ± 0.01	149.96 ± 13.68	125.44 ± 10.19	83.73 ± 2.53	35.90 ± 0.97
9311-4*x*	115.34 ± 2.30*	5.80 ± 0.84**	26.54 ± 1.60	1.08 ± 0.01**	0.39 ± 0.01	126.48 ± 10.30*	40.52 ± 2.39**	32.15 ± 2.42**	45.68 ± 1.30**

Values represent the means ± SD. Significant differences were determined using independent samples *t*-test; * and ** indicate significant differences at 0.05 and 0.01 probability levels, respectively. PH–plant height; EPP–the effective panicle number per plant; PL–panicle length; GL–grain length; GW–grain width; TGP–the total grain number per panicle; FGP–the filled grain number per panicle; SSR–seed setting rate; TGW–1000-grain weight

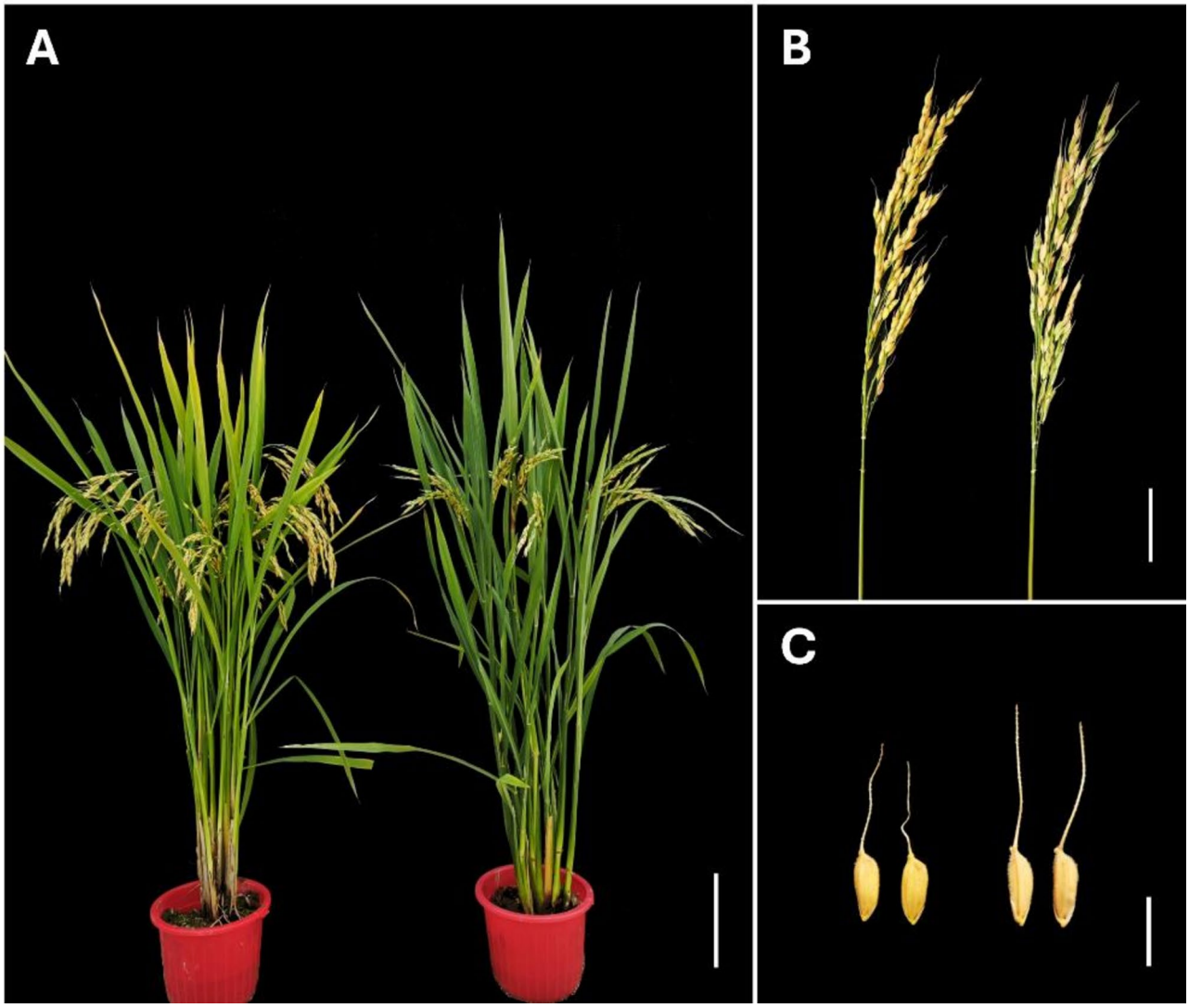

Fig. 1 Morphological characteristics of HN2026-4*x* and 9311-4*x*. **A** Plants; **B** Panicles; **C** Grains. Left: HN2026-4*x*; Right: 9311-4*x*; Bars: **A** = 20 cm; **B** = 5 cm; **C** = 1 cm

high frequencies of univalents, trivalents, quadrivalents, and multivalents in prophase I. In addition, the number of lagging chromosomes and the percentage of cells with lagging chromosomes in anaphase I were significantly greater than those of HN2026-4*x* (Tables 2 and 3).

Pollen fertility and viability

The results of pollen fertility and vitality analyses were shown in Fig. 3. The ratios of fertile pollen and viable pollen of HN2026-4*x* were 82.93% and 80.63%, respectively, while those of 9311-4*x* were 75.07% and 58.69%, respectively. The pollen fertility and viability of HN2026-4*x* were both significantly higher than those of 9311-4*x*.

Transcriptome sequencing and reads mapping

Comparative transcriptome analysis of HN2026-4*x* and 9311-4*x* was performed to identify the gene expression differences at meiosis stage. A total of 151,152,958 and 158,155,192 clean reads, with Q30 of 85.10% and 85.13%, and GC content of 57.83% and 57.35% were identified from HN2026-4*x* and 9311-4*x* transcriptome, respectively. The reads were successfully mapped to Nipponbare genome (IRGSP-1.0 pseudomolecule/MSU7) with 81.98–83.45% coverage, of which 80.28–81.80% were uniquely mapped (Table 4; Fig. S1).

Identification and functional annotation of DEGs

Using FPKM method, a total of 36,029 genes (including 1,742 new genes) were detected for expression (Table S1). Among them, a total of 1,441 genes were identified as DEGs between HN2026-4*x* and 9311-4*x* with a screening criteria of fold Change ≥ 2 ($|\log_2 FC| \geq 1$) and FDR < 0.01, with 864 and 577 up- and down-regulated genes, respectively in HN2026-4*x* (Fig. 4). A total of 1,153 unigenes

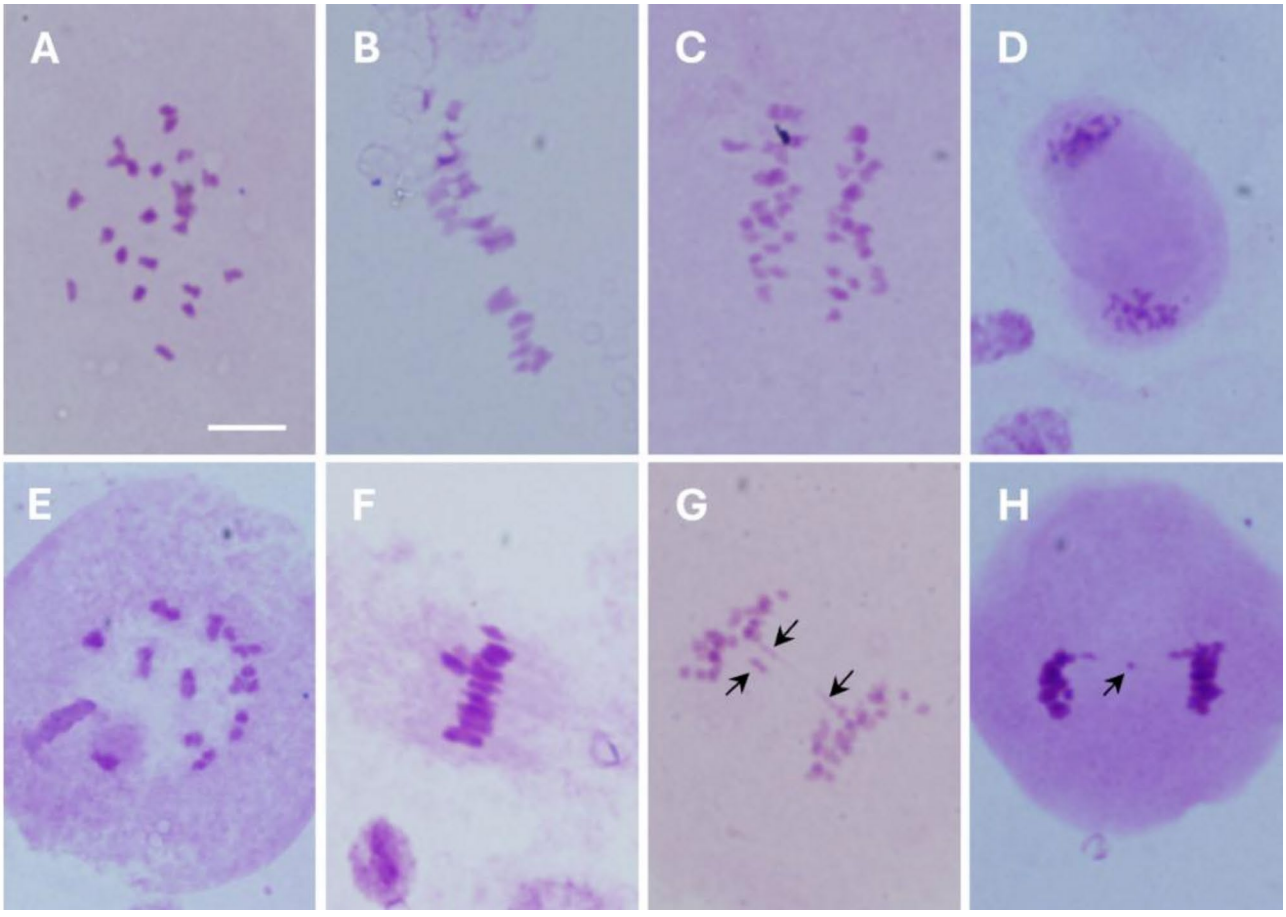

Fig. 2 The meiosis behavior of pollen mother cells in HN2026-4*x* and 9311-4*x*. **A**, **B**, **C**, **D** HN2026-4*x*; **E**, **F**, **G**, **H** 9311-4*x*. **A**, **E** Prophase I (Diakinesis); **B**, **F** Metaphase I; **C**, **G** Anaphase I; **D**, **H** Telophase I. The lagging chromosomes are indicated by arrows. Bar = 10 μm

Table 2 Chromosome configurations in PMCs meiosis of HN2026-4*x* and 9311-4*x*

Lines	No. of PMCs	Univalent		Bivalent		Trivalent		Quadrivalent		Multivalent	
		mean ± SD	range	mean ± SD	range	mean ± SD	range	mean ± SD	range	mean ± SD	range
HN2026-4*x*	104	0.06 ± 0.31	0 ~ 2	16.42 ± 5.64	10 ~ 22	0.04 ± 0.19	0 ~ 1	3.62 ± 2.48	1 ~ 7	0.08 ± 0.39	0 ~ 2
9311-4*x*	87	0.31 ± 0.88*	0 ~ 4	10.86 ± 5.21**	2 ~ 22	0.21 ± 0.93	0 ~ 3	5.93 ± 2.87**	1 ~ 11	0.24 ± 0.63*	0 ~ 3

Multivalent refers to the synapsis of more than four chromosomes. Significant differences were determined using independent samples *t*-test; * and ** indicate significant differences at 0.05 and 0.01 probability levels, respectively

Table 3 Lagging chromosomes in PMCs meiosis of HN2026-4*x* and 9311-4*x*

Lines	No. of PMCs	PMCs with lagging chr.		No. of lagging chr.	Normal PMCs	
		No.	Percentage (%)		No.	Percentage (%)
HN2026-4*x*	56	6	10.71	1.40 ± 0.55	50	89.29
9311-4*x*	54	21	38.89	2.36 ± 0.85*	33	61.11

Significant differences were determined using independent samples *t*-test; * indicates significant difference at 0.05 probability levels

were annotated, with 1,152, 801, 329, 905, and 96 unigenes being annotated in Nr, Swiss-Prot, COG, GO, and KEGG databases, respectively (Table S2).

GO annotation classified DEGs into three functional categories, including cellular component (CC), molecular function (MF), and biological process (BP), which could further be divided into 16, 16, and 24 subcategories, respectively (Fig. 5). Cell, cell part, and organelle were the most annotated unigenes in CC category, while binding, catalytic activity, and transporter activity were the most annotated in MF category. In addition, cellular process, metabolic process, and response to stimulus were the

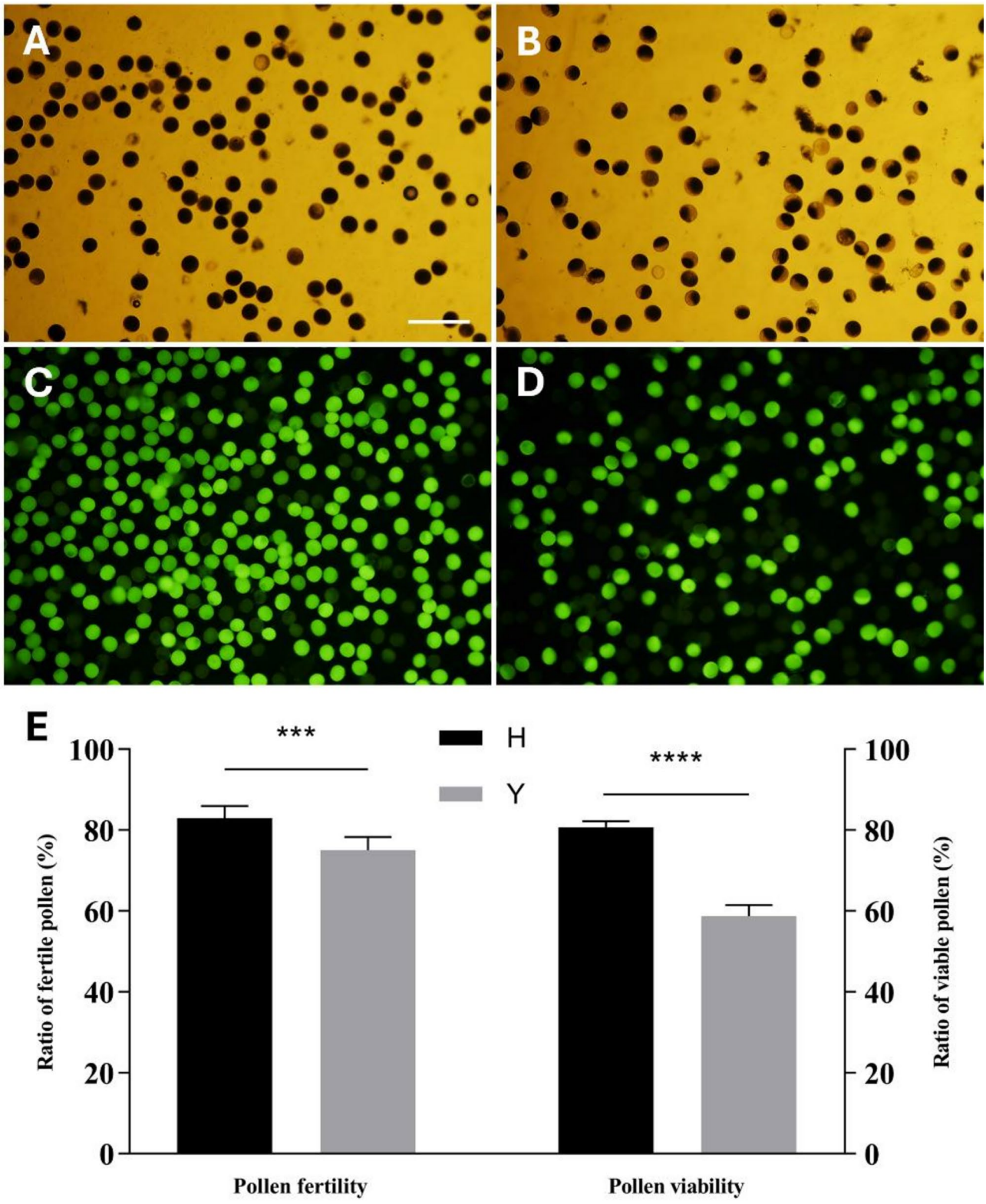

Fig. 3 Analysis of pollen fertility and viability of HN2026-4*x* and 9311-4*x*. **A** Pollens of HN2026-4*x* stained by I_2-KI; **B** Pollens of 9311-4*x* stained by I_2-KI; **C** Pollens of HN2026-4*x* stained by FDA; **D** Pollens of 9311-4*x* stained by FDA; **E** Comparison of pollen fertility and viability; **H**: HN2026-4*x*; **Y**: 9311-4*x*; Significant differences were determined using independent samples *t*-test; *** and **** indicate significant differences at 0.001 and 0.0001 probability levels, respectively. Bar = 200 μm

Table 4 Summary of transcriptome data by the illumina platform

Sample	HN2026-4*x*	9311-4*x*	Sum/average
Raw reads	174,957,372	181,908,738	356,866,110
Clean reads	151,152,958	158,155,192	309,308,150
Clean bases	15,113,076,099	15,813,322,409	30,926,398,508
Q20 (%)	93.02	93.00	93.01
Q30 (%)	85.10	85.13	85.11
GC (%)	57.83	57.35	57.59
Total Mapped (%)	126,135,195 (83.45%)	129,656,114 (81.98%)	255,791,309 (82.70%)
Unique mapped (%)	123,638,225 (81.80%)	126,974,299 (80.28%)	250,612,524 (81.02%)
Multiple mapped (%)	2,496,970 (1.65%)	2,681,815 (1.70%)	5,178,785 (1.67%)

most annotated unigenes in BP category. Significantly, high number of unigenes were annotated in the reproduction and reproductive process subcategory, which was consistent with the significant differences in reproductive characteristics between HN2026-4*x* and 9311-4*x*.

The DEGs in COG annotation were classified into 22 COG categories, with 'General function prediction only'(R) as the largest group, followed by 'Replication, recombination and repair' (L), and 'Function unknown' (S) (Fig. 6). The result indicated the differences in replication, recombination, and repair between HN2026-4*x* and 9311-4*x*.

KEGG analysis showed that 96 DEGs were assigned to 67 pathways (Table S3). The most enriched category

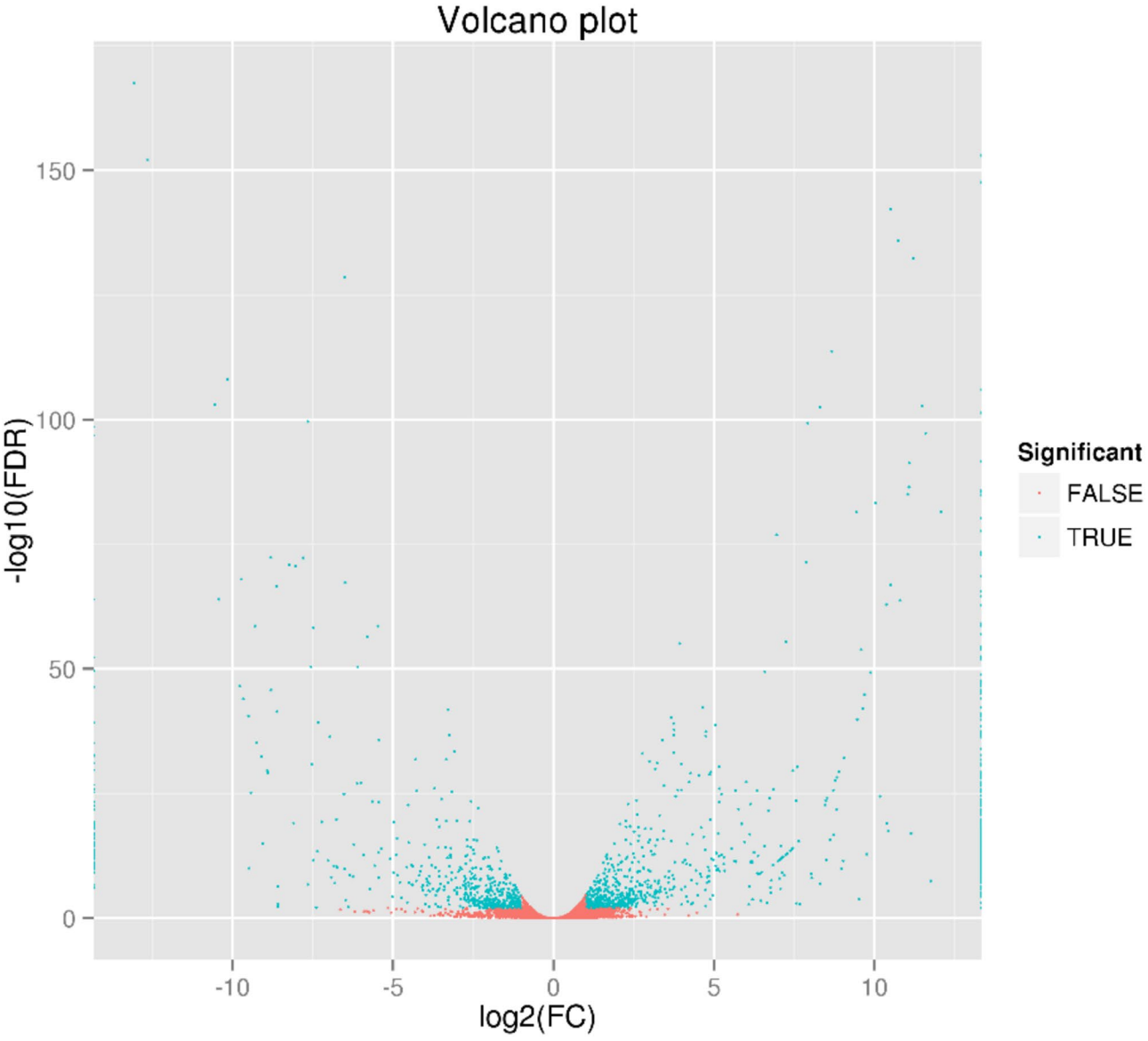

Fig. 4 Volcano map of differentially expressed genes between HN2026-4*x* and 9311-4*x*. Each dot represents a gene, with turquoise dots representing differentially expressed genes and red dots representing non differentially expressed genes

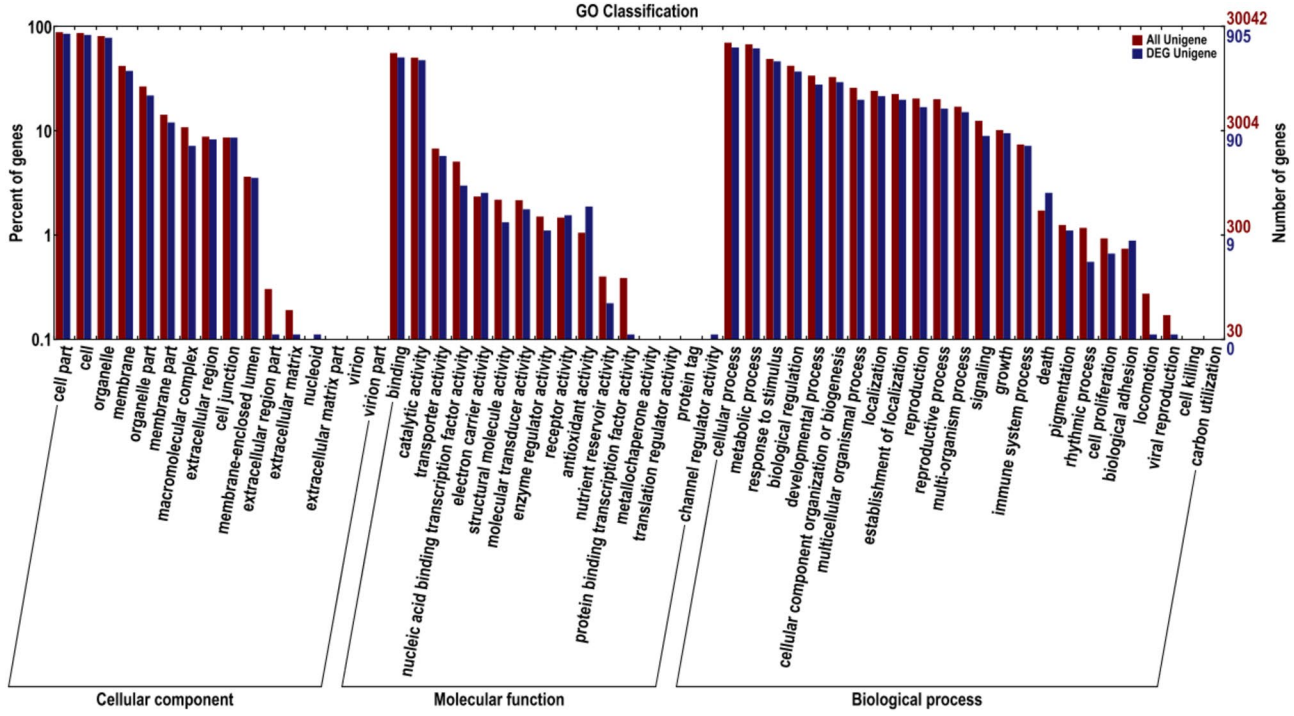

Fig. 5 Gene ontology (GO) analysis of differentially expressed genes between HN2026-4*x* and 9311-4*x*. The percentage and number of genes in each subcategory are shown on the left and right y-axes, respectively. The GO subcategories are shown on the x-axis

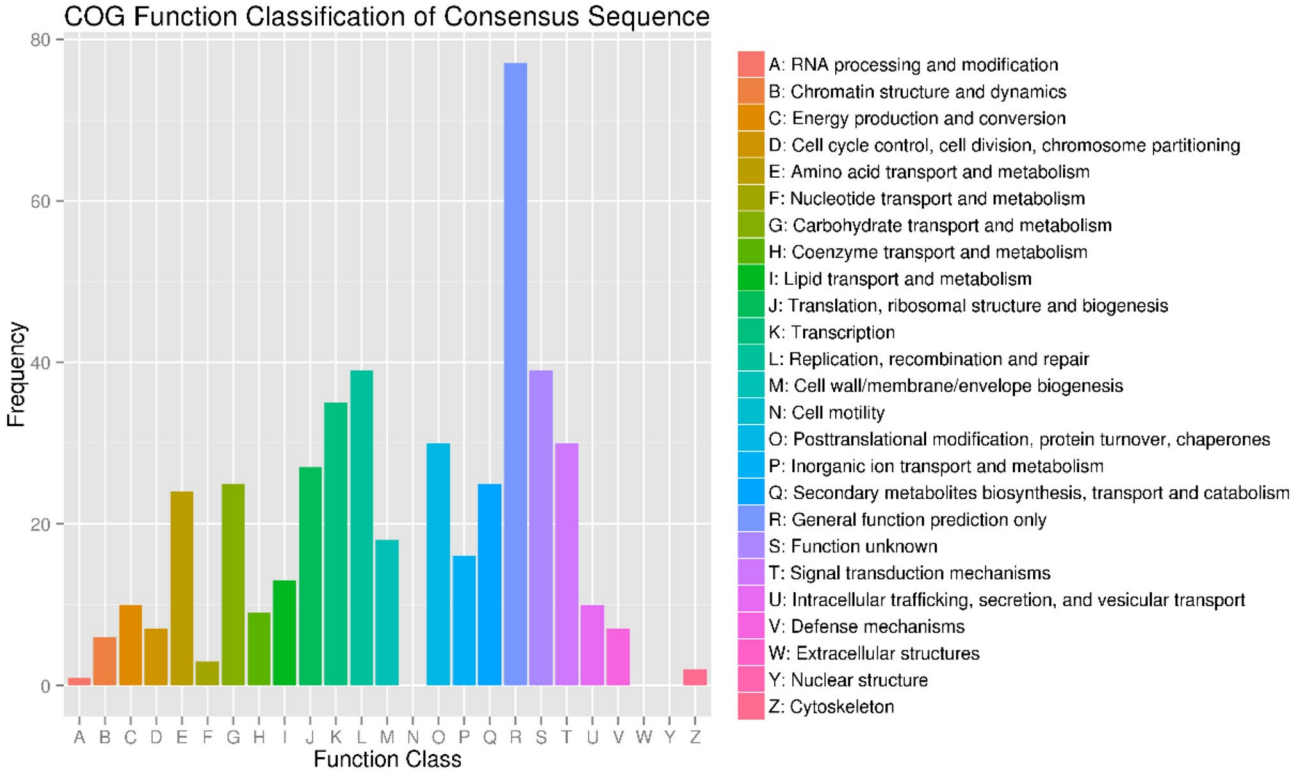

Fig. 6 Clusters of orthologous groups (COG) classification of differentially expressed genes between HN2026-4*x* and 9311-4*x*. A total of 329 unigenes are classified into 22 COG categories

Table 5 List of DEGs participated in tetraploid rice meiosis annotated by GO database

Category	GO term (No.)	Gene ID
Cellular Component	synaptonemal complex (GO: 0000795)	*Os07g0486000*
	mismatch repair complex (GO: 0032300)	*Os07g0486000*
Molecular Function	mismatched DNA binding (GO: 0030983)	*Os07g0486000*
Biological Process	meiotic mismatch repair (GO: 0000710)	*Os07g0486000*
	homologous chromosome segregation (GO: 0045143)	*Os07g0486000*
	meiotic chromosome segregation (GO: 0045132)	*Os08g0123300, Os03g0281500, Os07g0492100*
	synapsis (GO: 0007129)	*Os01g0347000*
	reciprocal meiotic recombination (GO: 0007131)	*Os08g0123300, Os03g0281500, Os07g0492100, Os07g0486000*
	male meiosis (GO: 0007140)	*Os02g0616300, Os03g0353900*
	female meiosis (GO: 0007143)	*Os02g0616300, Os03g0353900*
	meiosis I (GO: 0007127)	*Os11g0615800, Os11g0275400, Os11g0208400*
	meiosis (GO: 0007126)	*Os11g0615800, Os01g0818600*
	zygotene (GO: 0000238)	*Os09g0506800*
	leptotene (GO: 0000237)	*Os09g0506800*
	male meiosis cytokinesis (GO: 0007112)	*Os02g0644466*
	DNA recombination (GO: 0006310)	*Os10g0136950, Os10g0136150*
	double-strand break repair (GO: 0006302)	*Os07g0486000*
	double-strand break repair via homologous recombination (GO: 0000724)	*Os07g0273301, Os12g0279000, Os04g0629300, Os07g0416600*
	double-strand break repair via synthesis-dependent strand annealing (GO: 0045003)	*Os11g0615800*
	meiotic DNA double-strand break formation (GO: 0042138)	*Os08g0123300, Os03g0281500, Os07g0492100, Os07g0486000*

was metabolism with 48 unigenes, which included carbohydrate metabolism, amino acid metabolism, lipid metabolism, metabolism of other amino acids, nucleotide metabolism, energy metabolism, and other subcategories. The genetic information processing was the second most enriched category with 20 unigenes, which included translation, folding, sorting and degradation, replication and repair, and transcription. The result suggested significant differences in genetic processes between HN2026-4*x* and 9311-4*x*.

Screening of key genes associated with tetraploid rice meiosis

Since the seed setting rate is directly affected by meiosis, DEGs screening was performed to identify specific functional genes related to meiotic stage. Based on GO annotation, a total of 19 DEGs related to meiosis were screened, which involved in 20 GO terms in CC, MF, and BP categories (Table 5). Of these, *Os07g0486000* and *Os11g0615800* belonged to several terms, and their expression levels significantly differed between HN2026-4*x* and 9311-4*x*. Annotation of *Os07g0486000* and *Os11g0615800* in the RAP database (http://rapdb.dna.affrc.go.jp/) revealed that the two genes were *OsMSH4* and *OsRAD51A1*, respectively, which are key meiotic genes in rice. COG annotation revealed 39 unigenes were annotated in 'Replication, recombination and repair' category, including *Os07g0486000* and *Os11g0615800*, with the latter also being annotated in 'homologous recombination' (ko03440) pathway based on KEGG analysis (Fig. S2). Based on the comprehensive analysis of gene function and differential expression levels, three meiotic genes *Os07g0486000*, *Os11g0615800*, and *Os09g0506800* (*PAIR2*) were identified as candidates responsible for meiotic stability and high seed setting rate in tetraploid rice.

Table 6 The expression levels of DEGs in RNA-Seq and qRT-PCR

Gene ID	RNA-Seq (FPKM)		qRT-PCR ($2^{-\Delta\Delta CT}$)	
	HN2026-4*x*	9311-4*x*	HN2026-4*x*	9311-4*x*
Os07g0486000	10.05	0.31	1.00	0.03
Os11g0615800	47.74	7.93	1.00	0.16
Os09g0506800	34.22	3.01	1.00	0.09
Os01g0917500	3.58	1.35	1.00	0.53
Os06g0553200	609.82	418.67	1.00	0.67
Os04g0543700	3.11	219.10	1.00	76.18

Validation of candidate genes with quantitative real-time PCR

Six genes with obvious expression differences between HN2026-4*x* and 9311-4*x*, including *Os07g0486000*, *Os11g0615800*, *Os09g0506800*, *Os01g0917500*, *Os06g0553200*, and *Os04g0543700* were validated by quantitative real-time PCR (qRT-PCR). The result showed that the gene expression levels in qRT-PCR were highly consistent with those in RNA-Seq (Table 6; Fig. 7), suggesting reliability and accuracy of the RNA-Seq data in this study.

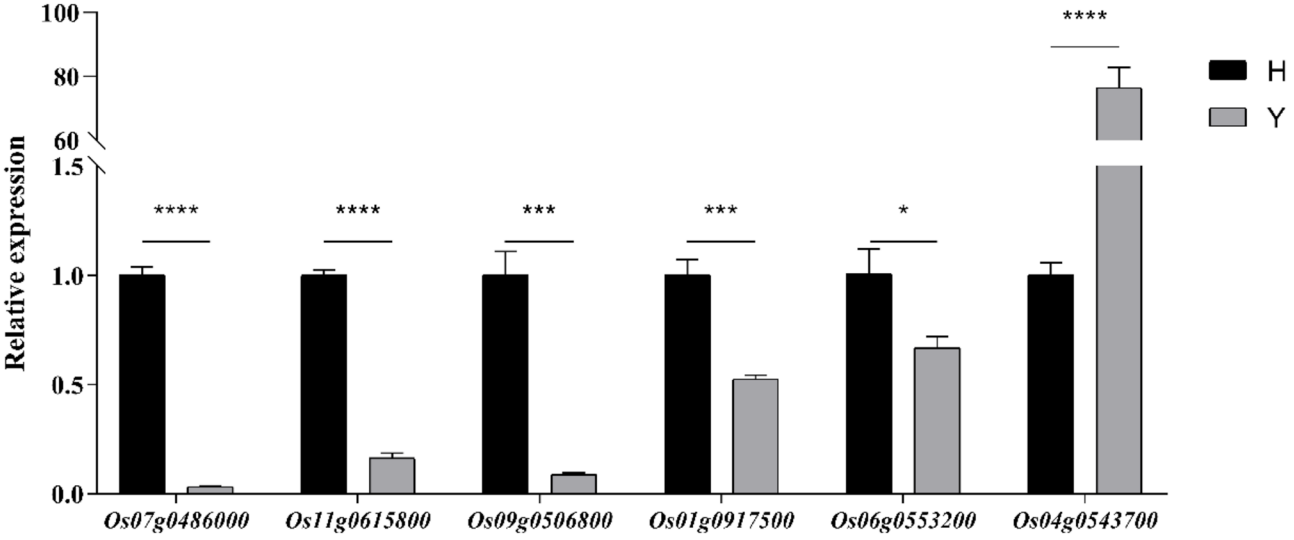

Fig. 7 Validation of six differentially expressed genes by qRT-PCR. **H**: HN2026-4*x*; **Y**: 9311-4*x*. Significant differences were determined using independent samples *t*-test; *, ***, and **** indicate significant differences at 0.05, 0.001, and 0.0001 probability levels, respectively

Discussion

Stable meiosis is crucial for high seed setting rate in tetraploid rice

Rice is one of the three major food crops globally and consumed by over half of the world's population [24]. The increasing human population is projected to be 9 billion by 2050, which puts pressure on the need to increase rice yield by at least 60% to guarantee future food security [25, 26]. However, limited research breakthroughs have been witnessed since the introduction of the dwarf breeding and rice hybridization technology. Thus, new technological approaches are warranted to ensure significant increase in rice yield. Given their high yield and nutritional potential, polyploid rice breeding is considered as one of the new breeding strategies in the 21st century [12–14, 18, 27, 28]. However, the low seed setting rate seriously prevented the development of polyploid rice breeding for a long time [12, 17, 18]. Therefore, to achieve the application of polyploid rice, it is necessary to solve the bottleneck problem of low seed setting rate.

The low seed setting rate in polyploid rice has been associated various factors, such gamete and zygote abortion, environmental effects, and abnormal cell separations in the meiotic anaphase I [16]. Moreover, autotetraploid rice exhibited more aborted pollen as well as more degenerated and aberrant embryo sacs, which suggested that the low seed setting rate resulted from reduced sexual reproduction ability due to abnormal development of male and female gametophytes [29, 30]. Numerous abnormalities that potentially influence seed setting rate have also been observed in the autotetraploid rice during embryo sac, fertilization, embryogenesis, and endosperm formation [31–37]. Recent molecular studies detected abnormal expression profiles of fertility related genes, altered methylation levels, and differentially expressed non-coding RNAs during pollen and embryo sac development in autotetraploid rice [38–42], which provide crucial basis for uncovering the molecular mechanisms of low seed setting rate.

After analyzing the evolution of allopolyploids in plants, Cai et al. proposed that the low seed setting rate of autotetraploid rice was ultimately caused by its chromosome composition [18]. Unlike allopolyploid, the chromosome set of autopolyploid is derived from the same plant species. In the first meiotic division (MI), a pair of homologous chromosomes forms a bivalent in diploid or allopolyploid that regularly segregate away from one another. However, in autopolyploid, typical formation of bivalents is often inhibited due to homology of three or more chromosomes, which prevent intrinsic cues necessary for normal, diploid-like segregation [43]. The formation of univalent and multivalent at prophase I as well as unequal separation of chromosomes at anaphase I results in the formation of unbalanced gametes, gamete sterility, and low seed setting rate in autotetraploid plants [44–46]. Consequently, a novel pathway for "breeding super rice using double advantages of wide cross and polyploidization" has been proposed [18], to overcome low seed setting rate in tetraploid rice by expanding the genetic distance between chromosomes to enhance bivalent formations and reduce unstable pairing forms, leading to improved chromosome segregation. Under the guidance of this strategy, two PMeS lines were bred through extensive inter-subspecies and composite crosses of *indica* and *japonica* [19]. The PMeS lines have stable meiosis and high seed setting rates. Crucially, this trait exhibits stable dominant inheritance [19, 20].

Our results showed obvious differences between the meiotic behaviors of PMeS line, HN2026-4*x* and non-PMeS line, 9311-4*x*. For example, chromosome pairings mostly formed bivalents in HN2026-4*x*, but with rare univalents and multivalents in prophase I, as well as few lagging chromosomes in anaphase I. By contrast, the homologous chromosome pairing in 9311-4*x* was disorganized and formed more univalents and multivalents in prophase I, as well as more cells with more lagging chromosomes in anaphase I. This suggested that the PMeS line rather than the non-PMeS line had more regular and stable meiosis process. Further, the pollen fertility and viability of PMeS line were significantly higher than those of non-PMeS line, which is likely due to the stable meiosis in PMeS line. Therefore, it can be inferred that unstable meiosis is the origin of low seed setting rate in autotetraploid rice. The occurrence of univalents, multivalents, and lagging chromosomes caused unbalanced distribution of chromosomes to daughter cells, which further decreased gamete fertility, inevitably affecting fertilization and embryonic development, leading to low seed setting rate. These results suggest that stable meiosis is crucial for high seed setting rate in the tetraploid rice.

Stable meiosis is controlled by key meiotic genes

Meiosis is a key biological process in sexual reproduction and is important for genetic diversity of populations. During meiosis, a single round of DNA replication is followed by two successive rounds of nuclear division to generate haploid gametes. The first division (meiosis I) has been recognized as the crucial stage of meiosis, in which prophase I is particularly important [47–50]. In prophase I, homologous chromosomes recognition, pairing, synapsis, and recombination are delicately linked to ensure formation of stable bivalents and precise separation of homologous chromosomes. Any errors in these processes will lead to irregular segregation of homologous chromosomes and affect fertility [49–53]. To date, numerous genes involved in the regulation of meiosis have been characterized in diploid species, including rice [54, 55], while only limited studies on meiosis mechanism are available in polyploids. Due to the presence of more than two homologous chromosomes (homologs) in autopolyploids, or homoeologous chromosomes (homoeologs) in allopolyploids, faithful chromosome segregation in meiosis is especially demanding to ensure genome stability and fertility in polyploid species [56, 57]. Chromosomes need to be sorted out during meiosis to produce balanced gametes, otherwise multiple or illegitimate chiasmatic associations would result in homologous chromosome missegregation, which further leads to aneuploidy and partial fertility [58, 59]. Crossovers (COs) play a key role in faithful segregation of homologous chromosomes at the first meiotic division. In autopolyploids, homologous chromosome segregation can be improved by reducing CO frequency to inhibit multivalent and thereby enhance bivalent formation [59]. The tetraploid *Arabidopsis arenosa* is a natural autotetraploid with fewer chiasmata per bivalent than those observed in diploids, and exhibits stable meiosis and fully fertile. Eight meiotic genes which sharply differentiated between diploid and tetraploid *A. arenosa* were identified through genome scan. They encoded proteins that are critical to CO formation, including meiotic chromosome axis components (ASY1, ASY3, SMC3, and SYN1) and the synaptonemal complex (ZYP1a and ZYP1b) [60, 61]. They may play a critical role in meiotic stability by reducing CO frequencies in autotetraploid *A. arenosa*.

In allopolyploids, stable meiosis requires both inter-homoeologue CO inhibition and homologous CO activation [59, 62]. A typical example is the *Pairing homoeologous 1* (*Ph1*) locus in allohexaploid wheat (*T. aestivum*), which can promote CO formation between homologous chromosomes and prevent maturation of CO between homoeologous chromosomes leading to faithful segregation [63, 64]. The *Pairing Regulator in B. napus* (*PrBn*) locus was identified as a controlling factor of homoeologous chromosome pairing in allohaploids *B. napus* [65, 66]. Meiotic stability in polyploid plants is also regulated by *MSH4* and *MSH5* genes. For example, *MSH4* in allotetraploid *B. napus* could prevent homoeologous CO by promoting homologous CO formation [62]. In allotetraploid wheat (*Triticum turgidum* L.) and allohexaploid wheat (*T. aestivum*), both *MSH4* and *MSH5* genes are required for the obligate CO between homologous chromosomes [67]. Most eukaryotes contain two kinds of CO, class I and II Cos [68]. The majority (85–90%) COs belong to class I, exhibit CO interference, and their formation is dependent on ZMM proteins (ZIP1, ZIP2, ZIP3, ZIP4, MSH4, MSH5, and MER3) [55, 69]. MSH4 and MSH5 are meiosis-specific MutS homologues of bacterial mismatch repair proteins that form heterodimers (MutSγ) to promote class I COs formation. Data from *Arabidopsis*, rice, tomato (*Solanum lycopersicum* L.), *T. turgidum*, and *B. napus* show that MutSγ is required for 85% of COs and the obligate chiasma, indicating that it is mostly likely the major meiotic recombination pathway in plants [67]. Moreover, MSH4 and MSH5 act upstream of other ZMMs, and the loss of MSH4 function resulted in a more severe diminution in CO formation than other zmm mutants [50, 68]. Overall, these observations demonstrated the key roles of MSH4 and MSH5 in CO formation in both diploid and polyploid plants.

In the present study, cytological observation revealed obvious different meiotic behaviors between PMeS line HN2026-4*x* and non-PMeS line 9311-4*x*. Through further transcriptome analysis, some meiosis-related genes were screened from DEGs between HN2026-4*x* and

9311-4*x*. Based on the comprehensive analysis of gene function and differential expression level, three meiotic genes *Os07g0486000* (*OsMSH4*), *Os11g0615800* (*OsRAD51A1*), and *Os09g0506800* (*PAIR2*) were identified as candidate genes for meiotic stability and high seed setting rate in tetraploid rice. Notably, *MSH4* gene exhibited most significant expression differences between PMeS and non-PMeS lines. Due to the pivotal role of MSH4 in CO formation, it is reasonable to speculate that *MSH4* gene is a key gene for regulating meiotic stability in PMeS lines. For RAD51, it is a eukaryotic homolog of bacterial RecA recombinase and functions as an essential protein in homologous recombination and recombinational repair of DNA double-stranded breaks (DSBs) [70–72]. In rice, *OsRAD51* gene includes *OsRAD51A1* and *OsRAD51A2* two copies. Both single mutants *Osrad51a1* and *Osrad51a2* exhibited normal vegetative growth and fertility, while double mutants *Osrad51* (*Osrad51a1 Osrad51a2*) showed normal vegetative growth but with complete sterility, indicating functional redundancy of *OsRAD51A1* and *OsRAD51A2* in rice fertility [73]. While in vitro experiment have shown that homologous-pairing activity of RAD51A2 is about 10-fold higher than that of RAD51A1 [72]. Compared to the wild type, *Osrad51* chromosomes showed imperfect pairing at the pachytene and deficient synaptonemal complex formation. Univalents and multivalents were observed at metaphase I, chromosome fragments and unbalanced chromosome segregation were detected at anaphase I, and CO formation was suppressed in *Osrad51* PMCs [73]. Moreover, OsRAD51 paralogues, especially OsRAD51D, have also been shown to inhibit nonhomologous connections, thus ensuring faithful pairing and recombination during meiosis [74]. Although the activity of OsRAD51A1 is much lower than OsRAD51A2 in diploid rice, has its activity and function altered in tetraploid rice to adapt to the evolution of polyploids? *PAIR2* is the orthologue of *Arabidopsis ASY1*. PAIR2 protein associates with axial elements at leptotene and zygotene and is required for the formation of synaptonemal complex. The *pair2* mutant in diploid rice exhibited 24 completely unpaired univalents at pachytene and diakinesis [75, 76]. Given the adaptive evolution of meiosis in autotetraploid *A. arenosa*, it remains unclear whether the *PAIR2* also play similar roles in reducing CO frequencies in the tetraploid rice. Next, functional analysis of candidate genes will be performed to uncover the molecular mechanism of meiotic stability and high seed setting rate in PMeS lines, which can provide theoretical and practical basis for promoting polyploid rice breeding.

Conclusion

The seed setting rate, fertile and viable pollen ratios of PMeS line were significantly higher than those of non-PMeS line. PMeS line exhibited stable meiosis, with chromosomes mainly pairing as bivalents, but with rare univalents and multivalents in prophase I. In addition, few lagging chromosomes were detected in anaphase I. DEGs between PMeS and non-PMeS lines were identified, and some meiosis-related genes were specifically investigated. Three meiotic genes *Os07g0486000*, *Os11g0615800*, and *Os09g0506800* were identified as candidate genes. The study reveals the differences between PMeS and non-PMeS lines from morphology, cytology and molecular biology, and also provides several key genes essential for meiotic stability and high seed setting rate in tetraploid rice.

Materials and methods

Plant materials

Two tetraploid rice lines HN2026-4*x* (H) and 9311-4*x* (Y) were used in this research. HN2026-4*x* is PMeS line with high seed setting rate and was bred through *indica-japonica* cross [19]. Autotetraploid rice line 9311-4*x* (non-PMeS line), which has low seed setting rate, was induced from 9311-2*x* by chromosome-doubling. All plant materials were provided by the Laboratory of Polyploid Genetics, School of Life Sciences, Hubei University, China. The plant materials were grown in the experimental field of Hubei University, Wuhan, China (30°34′N, 114°20′E). Seedlings were planted with 16.7 cm × 30.0 cm spacing and managed with general field production practices.

Evaluation of agronomic traits

Main agronomic traits of HN2026-4*x* and 9311-4*x* were measured according to the methods of He et al. [77], including plant height, number of effective panicles per plant, panicle length, grain length and width, total grains per panicle, filled grains per panicle, seed setting rate, and 1,000-grain weight. Five plants of each line were measured.

Observation of meiotic behaviors in PMCs

Young panicles at meiotic stage were collected and fixed in Carnoy's fixative (95% ethanol: glacial acetic acid, 3:1 [*v/v*]) for 24 h, then stored in 70% ethanol at 4 °C. Meiotic chromosome preparations were made according to the protocol of Li and Zhang [78] with modification. The fixed panicles were rinsed in distilled water for 20 min. Then, anthers were picked and digested in an enzyme mixture containing 2% cellulase and 2% pectinase for 3 h at 28 °C, washed three times in distilled water, and incubated in 75 mM KCl for 10 min. The anthers were then placed on precooled slides and squashed in the presence

of the fixative. The slides were heated over an alcohol flame to dry the fixative, stained with carbol fuchsin for 20 min, washed under a stream of tap water, and then dried. The meiotic processes were observed under Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan) to record the chromosome configurations and lagging chromosomes.

Analysis of pollen fertility and viability

Pollen fertility and viability were analyzed by staining mature pollens with 1.0% (*w/v*) I_2-KI and 50 μg mL^{-1} fluorescein diacetate (FDA) solutions, respectively followed by observation under microscope. Three slides were made for each observation, and pollens were counted in 10 random micro-optical fields on each slide. Ratios of fertile pollen and viable pollen were calculated using the following formulas: Ratio of fertile pollen (%) = (number of stained pollen grains / number of total pollen grains) × 100; Ratio of viable pollen (%) = (number of pollen grains that emitted bright green fluorescence / number of total pollen grains) × 100.

RNA extraction and library construction

Anthers at the meiotic stage were collected from HN2026-4*x* and 9311-4*x* in three biological replicates, frozen in liquid nitrogen, then stored at − 80 °C for RNA extraction. Total RNA from each sample was extracted using TRIzol Reagent (Life technologies, California, USA) according to the manufacturer's instructions and then treated with RNase-free DNase I (TAKARA, Tokyo, Japan) to remove DNA. RNA integrity and concentration were checked using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The mRNA was isolated using NEBNext Poly (A) mRNA Magnetic Isolation Module (NEB, E7490). The enriched mRNA was fragmented into approximately 200 nt RNA inserts, which were then reverse transcribed into cDNA. cDNA libraries were constructed with the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, E7530) and NEBNext Multiplex Oligos for Illumina (NEB, E7500) following the manufacturer's instructions.

Transcriptome sequencing analysis and DEGs identification

Sequencing was performed on an Illumina HiSeq™ 2500 platform by Biomarker Technologies, Beijing, China. Low quality reads, such as adaptor sequences, unknown nucleotides > 5%, or Q20 < 20%, were removed using perl script. The filtered clean reads were mapped to Nipponbare genome (IRGSP-1.0 pseudomolecule/MSU7) using Tophat2 software [79]. The aligned records from the aligners in BAM/SAM format were further examined to remove potential duplicate molecules, then gene expression levels estimated using FPKM values in Cufflinks software [80]. Differential expression analysis was performed using the DESeq2 [81]. The false discovery rate (FDR) method was used to identify threshold *P*-value in multiple tests to compute significance differences, and genes with a Fold Change ≥ 2 ($|\log_2 FC| \geq 1$) and FDR ≤ 0.01 were selected as significant DEGs.

Gene functional annotation

The unigenes were annotated using BLAST program against the NCBI database followed by homology searches in various databases, such as NCBI Non-redundant protein database (Nr), Swiss-Prot database, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Clusters of Orthologous Groups of proteins database (COG) with a BLAST threshold of (E-value < 1E-5), and the best alignment results were selected to annotate the unigenes. Functional annotation by gene ontology terms (GO) was analyzed by Blast2Go software [82].

qRT-PCR validation of DEGs

The profiles of six genes with obvious expression differences between the PMeS and non-PMeS lines were validated using qRT-PCR. Gene-specific primers were designed using Primer3Plus software and the used primer sequences are listed in Table S4. The rice *β-actin* gene (*Os03g0718100*) was used as the internal reference gene. The qRT-PCR was performed using SYBR Green (Roche) in ABI 7300 real-time PCR system (Applied Biosystems). The relative gene expression levels were calculated with three technical replicates using the $2^{-\Delta\Delta Ct}$ method [83].

Statistical analysis

Data were analyzed using SPSS Statistics 26.0 (IBM, Armonk, NY, USA) and MS Excel 2019 (Microsoft Corp., Redmond, WA, USA). Significant differences between the means of HN2026-4*x* and 9311-4*x* were determined using independent samples *t*-test.

Abbreviations

COG	Cluster of orthologous groups of proteins
DEGs	Differentially expressed genes
FC	Fold change
FDA	Fluorescein diacetate
FDR	False discovery rate
FPKM	Fragments per kilobase of transcript per million fragments mapped
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
NCBI	National Center for Biotechnology Information
Nr	Non-redundant protein sequence database
PMCs	Pollen mother cells
PMeS	Polyploid meiosis stability
qRT-PCR	Quantitative real-time PCR

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
Z.S. and D.C. conceived and designed the study. D.C. bred PMeS line HN2026-4***x*** and autotetraploid 9311-4***x***. P.L., M.W., R.Q., C.Y., M.F., Y.X., X.Z. and Y.H. performed the experiments. P.L. and Z.S. analyzed the data and wrote the manuscript. Z.S. revised the manuscript. All authors read and approved the final manuscript.

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Data availability
RNA-seq data of this study can be found at the National Center for Biotechnology Information with the BioProject accession No. PRJNA1198123 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1198123).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Comai L. The advantages and disadvantages of being polyploid. Nat Rev Genet. 2005;6:836–46.
2. Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, et al. Polyploidy and angiosperm diversification. Am J Bot. 2009;96:336–48.
3. Fang Z, Morrell PL. Domestication: polyploidy boosts domestication. Nat Plants. 2016;2:16116.
4. Yu H, Lin T, Meng X, Du H, Zhang J, Liu G, et al. A route to *de Novo* domestication of wild allotetraploid rice. Cell. 2021;184:1156–70.
5. Otto SP. The evolutionary consequences of polyploidy. Cell. 2007;131:452–62.
6. Soltis DE, Burleigh JD. Surviving the K-T mass extinction: new perspectives of polyploidization in angiosperms. Proc Natl Acad Sci USA. 2009;106:5455–6.
7. Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, et al. Ancestral polyploidy in seed plants and angiosperms. Nature. 2011;473:97–100.
8. Jiang W, Liu Y, Xia E, Gao L. Prevalent role of gene features in determining evolutionary fates of whole-genome duplication duplicated genes in flowering plants. Plant Physiol. 2013;161:1844–61.
9. Cao Y, Zhao K, Xu J, Wu L, Hao F, Sun M, et al. Genome balance and dosage effect drive allopolyploid formation in *Brassica*. Proc Natl Acad Sci USA. 2023;120:e2217672120.
10. Lagudah ES, Appels R. Wheat as a model system. In: Chapman GP, editor. Grass evolution and domestication. Cambridge: Cambridge University Press; 1992. pp. 225–65.
11. Touchell DH, Palmer IE, Ranney TG. In vitro ploidy manipulation for crop improvement. Front Plant Sci. 2020;11:722.
12. Song W, Zhang Y. Rice tetraploid and its effect on agronomic traits and nutritional constituents. Acta Agron Sin. 1992;18:137–44.
13. Li H, Liu X, He W, Liu K, Qiao Z, Lyu P, et al. Induction, identification and salt-alkali tolerance evaluation of tetraploid Haidao86. Acta Agron Sin. 2024;50:914–31.
14. Zhan M, Lv P, Zhou X, Liu K, Qiu R, Wang M, et al. Variations in endosperm structure facilitate the formation of high protein quality in tetraploid rice. Crop Sci. 2025;65:e21410.
15. Nakamori E. On the occurrence of the tetraploid plant of rice, *Oryza sativa* L. Proc Imperial Acad. 1933;9:340–1.
16. Nayar NM. Origin and cytogenetics of rice. Adv Genet. 1973;17:153–292.
17. Koide Y, Kuniyoshi D, Kishima Y. Fertile tetraploids: new resources for future rice breeding. Front Plant Sci. 2020;11:1231.
18. Cai D, Yuan L, Lu X. A new strategy of rice breeding in the 21st century II. Searching a new pathway of rice breeding by utilization of double heterosis of wide cross and polyploidization. Acta Agron Sin. 2001;27:110–6.
19. Cai D, Chen J, Chen D, Dai B, Zhang W, Song Z, et al. The breeding of two polyploid rice lines with the characteristic of polyploid meiosis stability. Sci China (Ser C Life Sci). 2007;50:356–66.
20. Song Z, Du C, Dai B, Chen D, Chen J, Cai D. Studies on the growth habits and characteristics of two polyploid *Indica-Japonica* hybrid rice with powerful heterosis. Agr Sci China. 2007;6:265–74.
21. Song Z, Du C, Zhang X, Chen D, He Y, Cai D. Studies on awns in polyploid rice (*Oryza sativa* L.) and preliminary cross experiments of a special awnless tetraploid rice. Genet Resour Crop Evol. 2014;61:797–807.
22. Chen R, Feng Z, Zhang X, Song Z, Cai D. A new way of rice breeding: polyploid rice breeding. Plants. 2021;10:422.
23. Song Z, Feng Z, Qu T, Lyu P, Yang X, Zhan M, et al. *Indica-japonica* attribute identification and heterosis utilization of diploid rice lines reverted from tetraploid rice. Acta Agron Sin. 2023;49:2039–50.
24. Chen R, Deng Y, Ding Y, Guo J, Qiu J, Wang B, et al. Rice functional genomics: decades' efforts and roads ahead. Sci China Life Sci. 2022;65:33–92.
25. Karki S, Rizal G, Quick WP. Improvement of photosynthesis in rice (*Oryza sativa* L) by inserting the C_4 pathway. Rice. 2013;6:28–36.
26. Khush GS. Strategies for increasing the yield potential of cereals: case of rice as an example. Plant Breed. 2013;132:433–6.
27. Muthayya S, Sugimoto JD, Montgomery S, Maberly GF. An overview of global rice production, supply, trade, and consumption. Ann NY Acad Sci. 2014;1324:7–14.
28. Liu X, Wu J, Shahid MQ. Development of neo-tetraploid rice and research progress on its heterosis mechanism. Biotechnol Bul. 2022;38:44–50.
29. Huang Q, Sun J, Bai S. Study on reproductive characters of autotetraploid rice. Sci Agric Sin. 1999;32:14–8.
30. Huang C, Huang Q, Li Z. Polymorphism of male and female gametophytes in autotetraploidy rices. J Fujian Agric Univ. 1999;28:18–21.
31. Zhang H, Feng J, Lu Y, Yang B, Liu X. Observation on formation and development of autotetraploid rice embryo sac using laser scanning confocal microscope. J Chin Electr Microscopy Soc. 2003;22:380–4.
32. Zhang H, Liu X, Lu Y, Feng J. Observation on the double fertilization and embryogenesis in autotetraploid rice. Acta Laser Biol Sini. 2006;15:9–14.
33. Wang L, Liu X, Lu Y, Feng J, Xu X, Xu S. Endosperm development in autotetraploid rice: the fusion of polar nuclei and the formation of endosperm cell wall. Chin J Rice Sci. 2004;18:281–9.
34. Wang L, Liu X, Lu Y, Feng J, Xu X, Xu S. Endosperm development in autotetraploid rice: the development of the cellulose wall of aleuronic layer cell, starch accumulation of endosperm and formation of a callose sheath-like structure. Chin J Rice Sci. 2004;18:507–14.
35. Guo H, Liu X, Lu Y, Feng J. Structure of mature embryo sac and its abnormal phenomena in autotetraploid rice. Chin J Rice Sci. 2006;20:283–9.
36. Guo H, Lu Y, Feng J, Yang B, Liu X. Further observation on the formation and development of autotetraploid rice embryo sac using laser scanning confocal microscopy. Acta Laser Biol Sini. 2006;15:111–7.
37. Shahid MQ, Sun J, Wei C, Zhang P, Liu X. Studies on the abnormality of embryo sac and pollen fertility in autotetraploid rice during different growing seasons. Pak J Bot. 2010;42:7–19.
38. Wu J, Shahid MQ, Guo H, Yin W, Chen Z, Wang L, et al. Comparative cytological and transcriptomic analysis of pollen development in autotetraploid and diploid rice. Plant Reprod. 2014;27:181–96.
39. Li X, Shahid MQ, Xia J, Lu Z, Fang N, Wang L, et al. Analysis of small RNAs revealed differential expressions during pollen and embryo sac development in autotetraploid rice. BMC Genomics. 2017;18:129.

40. Li X, Yu H, Jiao Y, Shahid MQ, Wu J, Liu X. Genome-wide analysis of DNA polymorphisms, the methylome and transcriptome revealed that multiple factors are associated with low pollen fertility in autotetraploid rice. PLoS ONE. 2018;13:e201854.
41. Li X, Shahid MQ, Wen M, Chen S, Yu H, Jiao Y, et al. Global identification and analysis revealed differentially expressed LncRNAs associated with meiosis and low fertility in autotetraploid rice. BMC Plant Biol. 2020;20:82.
42. Chen L, Shahid MQ, Wu J, Chen Z, Wang L, Liu X. Cytological and transcriptome analyses reveal abrupt gene expression for meiosis and saccharide metabolisms that associated with pollen abortion in autotetraploid rice. Mol Genet Genomics. 2018;293:1407–20.
43. Bomblies K, Jones G, Franklin C, Zickler D, Kleckner N. The challenge of evolving stable polyploidy: could an increase in crossover interference distance play a central role. Chromosoma. 2016;125:287–300.
44. Hazarika MH, Rees H. Genotypic control of chromosome behaviour in rye X. Chromosome pairing and fertility in autotetraploids. Heredity. 1967;22:317–32.
45. Biswas SC, Biswas AK. Colchicine induced autotetraploids in *Lathyrus sativus* L. Bangladesh J Plant Breed Genet. 2001;14:9–14.
46. Talukdar D. Cytogenetic characterization of induced autotetraploids in grass pea (*Lathyrus sativus* L). Caryologia. 2010;63:62–72.
47. Zickler D, Kleckner N. Meiotic chromosomes: integrating structure and function. Annu Rev Genet. 1999;33:603–754.
48. Li W, Ma H. Double-stranded DNA breaks and gene functions in recombination and meiosis. Cell Res. 2006;16:402–12.
49. Yuan W, Li X, Chang Y, Wen R, Chen G, Zhang Q, et al. Mutation of the rice gene *PAIR3* results in lack of bivalent formation in meiosis. Plant J. 2009;59:303–15.
50. Zhang L, Tang D, Luo Q, Chen X, Wang H, Li Y, et al. Crossover formation during rice meiosis relies on interaction of OsMSH4 and OsMSH5. Genetics. 2014;198:1447–56.
51. Pawlowski WP, Cande WZ. Coordinating the events of the meiotic prophase. Trends Cell Biol. 2005;15:674–81.
52. Wang K, Tang D, Wang M, Lu J, Yu H, Liu J, et al. MER3 is required for normal meiotic crossover formation, but not for presynaptic alignment in rice. J Cell Sci. 2009;122:2055–63.
53. Shen Y, Tang D, Wang K, Wang M, Huang J, Luo W, et al. ZIP4 in homologous chromosome synapsis and crossover formation in rice meiosis. J Cell Sci. 2012;125:2581–91.
54. Wang Y, van Rengs WMJ, Zaidan MWAM, Underwood CJ. Meiosis in crops: from genes to genomes. J Exp Bot. 2021;72:6091–109.
55. Chen L, Wang K, Wang C. Meiosis in plants: from understanding to manipulation. New Crops. 2025;2:100055.
56. Szadkowski E, Eber F, Huteau V, Lodé M, Huneau C, Belcram H, et al. The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol. 2010;186:102–12.
57. Zhang H, Bian Y, Gou X, Zhu B, Xu C, Qi B, et al. Persistent whole-chromosome aneuploidy is generally associated with nascent allohexaploid wheat. Proc Natl Acad Sci USA. 2013;110:3447–52.
58. Cifuentes M, Grandont L, Moore G, Chèvre AM, Jenczewski E. Genetic regulation of meiosis in polyploid species: new insights into an old question. New Phytol. 2010;186:29–36.
59. Mercier R, Mézard C, Jenczewski E, Macaisne N, Grelon M. The molecular biology of meiosis in plants. Annu Rev Plant Biol. 2015;66:297–327.
60. Hollister JD, Arnold BJ, Svedin E, Xue KS, Dilkes BP, Bomblies K. Genetic adaptation associated with genome-doubling in autotetraploid *Arabidopsis arenosa*. PLOS Genet. 2012;8:e1003093.
61. Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, Franklin FCH, et al. Meiotic adaptation to genome duplication in *Arabidopsis arenosa*. Curr Biol. 2013;23:2151–6.
62. Gonzalo A, Lucas M-O, Charpentier C, Sandmann G, Lloyd A, Jenczewski E. Reducing *MSH4* copy number prevents meiotic crossovers between non-homologous chromosomes in *Brassica napus*. Nat Commun. 2019;10:2354.
63. Martín AC, Rey M-D, Shaw P, Moore G. Dual effect of the wheat *Ph1* locus on chromosome synapsis and crossover. Chromosoma. 2017;126:669–80.
64. Rey M-D, Martín AC, Higgins J, Swarbreck D, Uauy C, Shaw P, et al. Exploiting the *ZIP4* homologue within the wheat *Ph1* locus has identified two lines exhibiting homoeologous crossover in wheat-wild relative hybrids. Mol Breed. 2017;37:95.
65. Jenczewski E, Eber F, Grimaud A, Huet S, Lucas MO, Monod H, et al. *PrBn*, a major gene controlling homeologous pairing in oilseed rape (*Brassica napus*) haploids. Genetics. 2003;164:645–53.
66. Liu Z, Adamczyk K, Manzanares-Dauleux M, Eber F, Lucas MO, Delourme R, et al. Mapping *PrBn* and other quantitative trait loci responsible for the control of homeologous chromosome pairing in oilseed rape (*Brassica Napus* L.) haploids. Genetics. 2006;174:1583–96.
67. Desjardins SD, Ogle DE, Ayoub MA, Heckmann S, Henderson IR, Edwards KJ, et al. *MutS homologue 4* and *MutS homologue 5* maintain the obligate crossover in wheat despite stepwise gene loss following polyploidization. Plant Physiol. 2020;183:1545–58.
68. Wang C, Wang Y, Cheng Z, Zhao Z, Chen J, Sheng P, et al. The role of OsMSH4 in male and female gamete development in rice meiosis. J Exp Bot. 2016;67:1447–59.
69. Luo Q, Li Y, Shen Y, Cheng Z. Ten years of gene discovery for meiotic event control in rice. J Genet Genomics. 2014;41:125–37.
70. Shinohara A, Ogawa H, Ogawa T. Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell. 1992;69:457–70.
71. Baumann P, West SC. Role of the human RAD51 protein in homologous recombination and double-stranded-break repair. Trends Biochem Sci. 1998;23:247–51.
72. Morozumi Y, Ino R, Ikawa S, Mimida N, Shimizu T, Toki S, et al. Homologous pairing activities of two rice RAD51 proteins, RAD51A1 and RAD51A2. PLoS ONE. 2013;8:e75451.
73. Liu X, Cao Y, Du G, Zhang C, Xu M, Cheng Z, et al. OsRAD51 plays a vital role in promoting homologous recombination in rice meiosis. Int J Mol Sci. 2022;23:9906.
74. Zhang F, Shen Y, Miao C, Cao Y, Shi W, Du G, et al. OsRAD51D promotes homologous pairing and recombination by preventing nonhomologous interactions in rice meiosis. New Phytol. 2020;227:824–39.
75. Nonomura KI, Nakano M, Murata K, Miyoshi K, Eiguchi M, Miyao A, et al. An insertional mutation in the rice *PAIR2* gene, the ortholog of *Arabidopsis ASY1*, results in a defect in homologous chromosome pairing during meiosis. Mol Genet Genomics. 2004;271:121–9.
76. Nonomura KI, Nakano M, Eiguchi M, Suzuki T, Kurata N. PAIR2 is essential for homologous chromosome synapsis in rice meiosis I. J Cell Sci. 2006;119:217–25.
77. He W, Zhang X, Lv P, Wang W, Wang J, He Y, et al. Full–length transcriptome reconstruction reveals genetic differences in hybrids of *Oryza sativa* and *Oryza punctata* with different ploidy and genome compositions. BMC Plant Biol. 2022;22:131.
78. Li M, Zhang Z. Research technology of crops chromosomes. Beijing: China Agriculture; 1996. pp. 91–7.
79. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14:R36.
80. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28:511–5.
81. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
82. Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008;36:3420–35.
83. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–8.

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