Comparative cytological and transcriptome analysis reveals high pollen fertility and upregulation of related genes in neo-tetraploid rice

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SUBJECT AREAS

Plant Physiology and Morphology Plant Molecular Biology and Genetics

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neo-tetraploid rice, pollen fertility, polyploidy, transcriptome analysis

Abstract

Background: Autotetraploid rice is a useful germplasm for polyploid rice breeding; however, low seed setting is a major hindrance for the utilization of autotetraploid rice. Our previous study demonstrated that neo-tetraploid rice have great yield potential, which is thought to be one effective way to overcome the low fertility of autotetraploid rice. However, there is little known about the cause of high pollen fertility in neo-tetraploid rice. Here, we employed cytology and RNA-seq to study the molecular genetic mechanism of high pollen fertility in neo-tetraploid rice.

Results: Cytological observations indicate that H1 displayed high pollen fertility (95.62%), lower percentage of PMC cell abnormalities, and stable chromosome configurations during the pollen development process compared with its two parents. RNA-seq analysis detected 1483 differentially expressed genes (DEGs) in neo-tetraploid rice compared with its two parents. Of these DEGs, 433 were annotated as pollen fertility-related genes, and 240 (~55.4%) exhibited significant upregulation in neo-tetraploid rice compared with its two parents, including nine cloned genes (CSA, TMS5 etc.) that were validated by qRT-PCR and had been demonstrated to be pollen fertility-related genes. We further selected TMS5 as a candidate gene and analysed its phenotype in neo-tetraploid rice using the CRISPR/Cas9 technique. Significant variations have been detected in phenotypic charts, pollen development process and expression level in H1 and its TMS5 knockout lines.

Conclusions: Our finding provides strong evidence for the regulatory mechanisms of neo-tetraploid rice, and upregulation of pollen fertility-related genes should be associated with high fertility.

Moreover, the present study provides a new useful germplasm for polyploidy rice breeding.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Tables

Table 1 Pollen fertility of neo-tetraploid rice and its parents

Material name	Pollen fertility (%±SE)	Typical aborted pollens (%±SE)	Stained aborted pollens (% ±SE)	Small pollens (%±SE)
T45-4x	54.10±3.81	8.18±0.72	37.26±3.92	0.46±0.12
T44-4x	57.66±2.78	15.08±1.56	23.07±1.98	4.19±0.51**
H1	95.62±0.34**	3.16±0.34**	0.60±0.13**	0.62±0.12

Note: ** indicated significant difference existed in pollen fertility between neo-tetraploid rice and its parents from zero at $P < 0.01$.

Table 2 Genetic variation in agronomic traits of neo-tetraploid rice and their parents

Seasons	Material name	PH (%±SE)	EPN (%±SE)	PL (%±SE)	FG (%±SE)	TGP (%±SE)	SS (%±SE)	GWT (%±SE)
Late season	T45-4x	94.40±1.	3.40±0.27	25.97±0.	63.27±4.	120.90±	53.27±2.	30.74±1.
		19**	**	60	78	9.17*	34**	39*
	T44-4x	78.10±1.	5.65±0.4	23.53±0.	15.58±1.	55.89±1.	26.45±1.	31.39±0.
		58**	4	33	41**	94*	88**	46
Early season	H1	104.40±	6.60±0.4	25.46±0.	66.34±4.	87.02±6.	76.46±1.	35.44±0.
		0.93	0	52	63	34	43	34
	T45-4x	117.70±	4.45±0.2	31.74±0.	40.26±1.	132.38±	30.80±0.	30.82±1.
		0.64**	8	46**	33**	4.05	97**	04**
	T44-4x	96.05±0.	5.85±0.27	28.81±0.	30.17±0.	77.90±0.	39.16±7.	33.74±0.
		46**	*	34	65**	55**	16**	49
	H1	124.45±	4.95±0.2	28.09±0.	92.75±3.	120.41±	76.55±0.	33.86±0.
		0.84	2	24	57	4.23	84	29

*, ** Significantly different from zero at $P < 0.05$ and $P < 0.01$, respectively.

PH = plant height, EPN = effective panicles number, PL = panicle length, FG = Filled grains, TGP = total number of grains per plant, GWT = 1000-grain weight and SS = seed set.

Figures

Figure 1

Breeding procedure and phenotype of neo-tetraploid rice and its parent. a Breeding procedure of neo-tetraploid rice, Huaduo1 (H1). b Morphologies of whole plant between neo-tetraploid rice, and its two parents. c-e Grain size of H1, and its two parents. f-h pollen grains stained with I2-KI in H1 and its two parents. i-k Comparison of the pollen fertility (i), seed set ratio (j) and plant height (k) between H1 and its two parents.

Figure 2

Chromosome behaviors and chromosome configurations in H1 and its parents during PMC meiosis ($\times 3000$). a Zygotene. b-c Diakinesis. d Metaphase I. e Anaphase I. f Telophase I. g Prophase II. h Metaphase II. i Anaphase II. j Telophase II. k Tetrad stage. l Diakinesis, 24 II. m Diakinesis, 12 IV. n Diakinesis. o Diakinesis. p Abnormal Anaphase I, straggling chromosome (arrow). Bars = 10 μm .

Figure 3

Frequency of PMCs in H1 compared with its parents at the meiosis stage. a Frequency of normal cells and main type of abnormal cells at Metaphase I. b Frequency of normal cells and main type of abnormal cells at Anaphase I. c Frequency of normal cells and main type of abnormal cells at Telophase I. d Frequency of normal cells and main type of abnormal cells at Metaphase II. e Frequency of normal cells and main type of abnormal cells at Anaphase II. f Frequency of normal cells and main type of abnormal cells at Telophase II.

Figure 4

Number and distribution of SNPs and Indels detected on the rice chromosomes. a Total number of SNPs detected on each rice chromosome. b Total number of SNPs detected on each rice chromosome.

Figure 5

Chromosome-wide distribution of SNP and Indels between the H1 and its two parents. Total number of SNPs (blue color) and Indels (red color) detected on each rice chromosome are shown in the bar graphs.

Figure 6

Differentially expressed genes in neo-tetraploid rice detected by Re-sequencing and RNA-sequencing analysis compared to its two parents. a Venn diagram of differentially expressed genes in neo-tetraploid rice detected by Re-sequencing and RNA-sequencing analysis. b Number of differentially expressed genes in neo-tetraploid rice. c Expression patterns of different groups in neo-tetraploid rice and its two parents. Red and green colors indicate up- and down-regulated genes, respectively.

GO Description		
cell division		
response to auxin		
reproductive process		
reproduction		
pollination		
pollen exine formation		
pollen-pistil interaction		
recognition of pollen		
cell cycle		
purine nucleobase biosynthetic process		
regulation of protein dephosphorylation		

GO Description		
vacuolar membrane		
apoplast		
chloroplast thylakoid membrane		
microtubule associated complex		

GO Description		
transcription regulator activity		
DNA binding transcription factor activity		
obsolete transcription regulator activity		
cation binding		
protein kinase binding		
peptidase inhibitor activity		
xyloglucan:xyloglucosyl transferase activity		
hydrolase activity		
peptide receptor activity		
transmembrane signaling receptor activity		
motor activity		

Figure 7

GO analysis of differentially expressed genes in neo-tetraploid rice comparative its two parents. Genes were divided into three categories: biological process, cellular component, and molecular function.

Figure 8

Expression patterns of genes may be involved in meiosis and microspore development. a

Expression patterns of meiosis genes. b Expression patterns of the 12 known tapetum-related genes. c Expression patterns of the 12 known pollen fertility genes.

Figure 9

The distribution of up-regulated genes involved in meiosis process and anther specific in neo-tetraploid rice comparative with its two parents.

Figure 10

Phenotypic comparison and developing rice anthers between *nt-tms5-1* and its wild type (WT) in neo-tetraploid rice. a and b Floral organs between the wild type and *nt-tms5-1* after removed the lemma. Bars = 1 mm. c and d Anthers between the wild type and *nt-tms5-1* using the WE-CLSM analysis. Bars = 100 μ m. e and f Comparison of the panicle between the wild type and *nt-tms5-1*. g and h Pollen grains stained with 1% I₂-KI solution showing mature pollen grains in WT and typical abortion of mature pollen grains in *nt-tms5-1*. Bars = 100 μ m. i and j Pollen grains stained with 10 mg/L eosin B solution showing mature pollen grains in WT and typical abortion of mature pollen grains in *nt-tms5-1*. Bars = 50 μ m. k to p Semi-thin sections of wild type anthers. k, meiosis stage; l, meiosis stage; m, meiosis stage; n, meiosis stage; o, late bicellular stage; p, mature pollen stage. q to v, Semi-thin sections of *nt-tms5-1* anthers. q, pre-meiotic interphase; r, meiosis stage; s, meiosis stage; t, single

microspore stage; u, late bicellular; p, v, mature pollen stage. E, En, M and T indicate the epidermis, endothecium, middle layer and tapetum, respectively. Bars = 50 μ m.

Figure 11

Verification of the predicated TMS5 regulation network during the pollen development process in nt-tms5-1 and its wild type. a Predicted protein-protein interaction network of TMS5 gene. b to j, Quantitative real-time PCR (qRT-PCR) confirmation of the regulation network difference between the nt-tms5-1 and its wild type using the qRT-PCR analysis. b, c, d, f, indicated lower gene expression level in nt-tms5-1 compared with its wild type. e, g, h, i and j, indicated higher gene expression level in nt-tms5-1 compared with its wild type. PMA, indicated the pre-meiosis stage. MA, indicated the meiosis stage. SCP, indicated single microspore stage.

Supplementary Files

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