

Fig. S1a. Breeding procedure of Huaduo 8



Fig. S1b Panicles of Huaduo 1 (H1), Huaduo 8 (H8) and their hybrid

A: Panicles of Huaduo 1 (H1), Huaduo 8 (H8) and their  $F_1$  hybrid ( $F_1$ ); B: Panicles of Luxiang 97-4x (T445, An autotetraploid rice), Huaduo 8 (H8) and their  $F_5$  generation with high fertility; C: Panicles of Huaduo 3 × Huaduo 8 in  $F_3$  generation

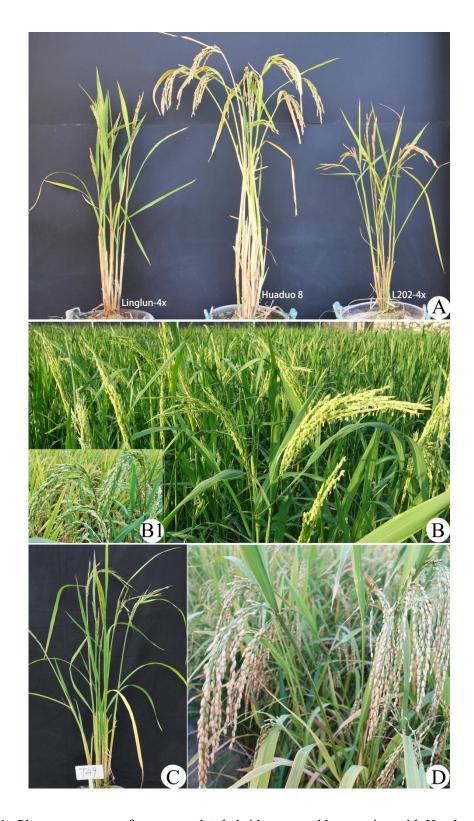


Fig. S1c Plant appearance of parents and F1 hybrid generated by crossing with Huaduo 8 (H8)

[A: Linglun-4x (T428), Huaduo 8 (H8) and L202-4x (T43); B: Huaduo 8 at flowering stage, B1: Huaduo 8 at 15 days after flowering; C: Bengal-4x (T49, an autotetraploid rice with low fertility); D:  $F_1$  of Bengal-4x × Huaduo 8]

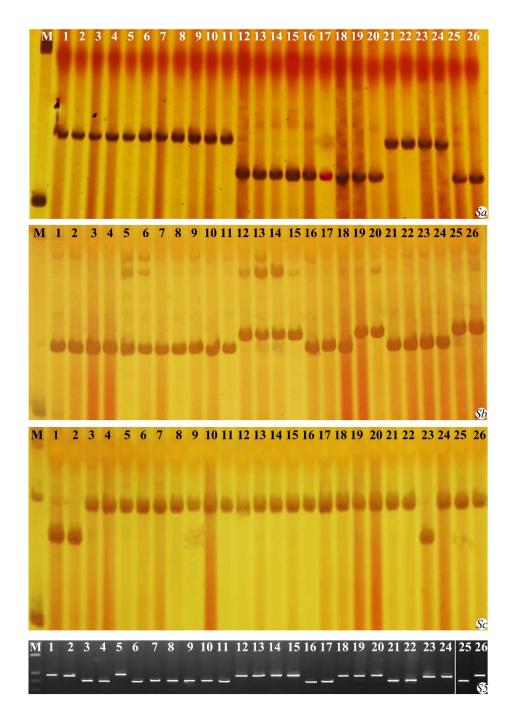


Fig. S2 Original images of genotypes of parental lines at pollen sterility loci (Sa, Sb and Sc) and S5 locus detected by molecular markers.

First image indicates the genotypes at *Sa* locus, followed by (upper to lower) *Sb*, *Sc* and *S5* locus. M indicates DNA ladder, lanes 1-4 indicate control cultivars (typical *indica* and *japonica* cultivars), while lanes 5-26 indicate neo-tetraploid/autotetraploid rice lines (see the name of materials in Additional file 2: Table S1). Lanes 25 and 26 in S5 were taken from another gel.

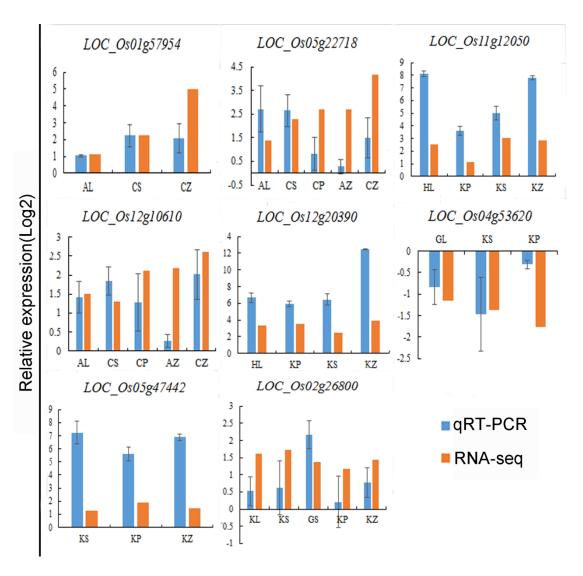
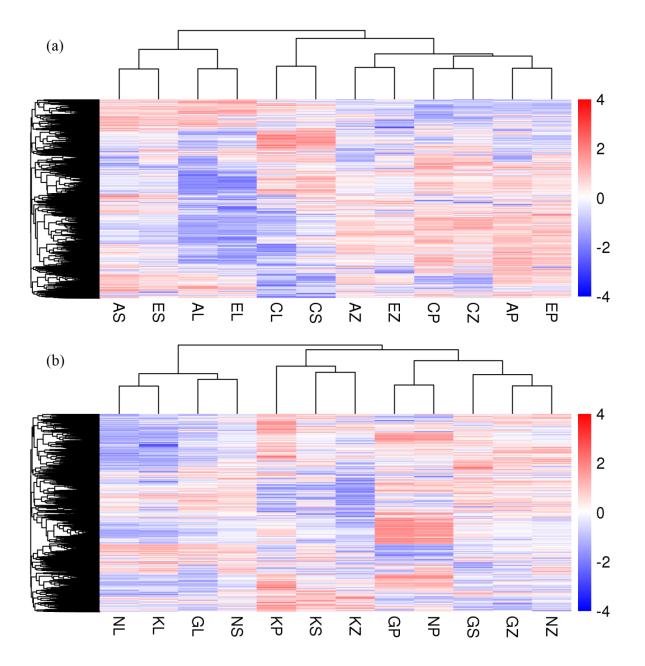


Fig. S3 qRT-PCR validation of the important genes detected in the present study (Codes of all X-axis are listed in Additional file 2: Table S4a)



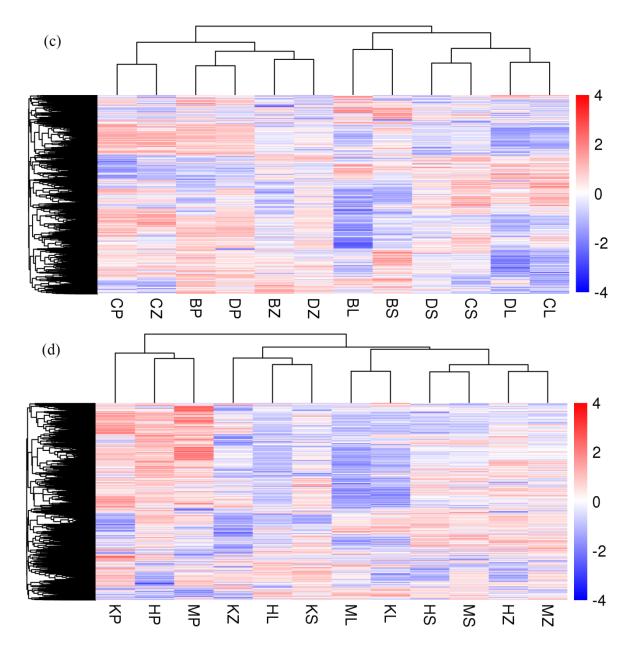


Fig. S4. Hierarchical clustering analysis of all expressed genes in  $F_1$  hybrid and their parents based on transcriptome data

(a):  $F_1$  hybrid (H1×H8) and its parents before flowering; (b):  $F_1$  hybrid (H1×H8) and its parents 5 days after flowering; (c):  $F_1$  hybrid (T485×H8) and its parents before flowering; (d)  $F_1$  hybrid (T485×H8) and its parents 5 days after flowering. Codes of all X-axis are listed in Additional file 2: Table S4a

Hybrids					(H1xl	H8)F <sub>1</sub>				1		C	Г485	xH8)I	₹1		_
A. Biological process			$S_0$	$P_0$	$Z_0$	L <sub>5</sub>	$S_5$	P <sub>5</sub>	$Z_5$	L <sub>0</sub>	$S_0$	P <sub>0</sub>	$Z_0$	L <sub>5</sub>	S <sub>5</sub>	P <sub>5</sub>	$Z_5$
GO:0055114 GO:0005975	oxidation reduction carbohy drate metabolic process	L <sub>0</sub>															
GO:0044267 GO:0016310	cellular protein metabolic process phosphory lation																
GO:0006796 GO:0006793	phosphate metabolic process phosphorus metabolic process																
GO:0065007 GO:0043687 GO:0006464	biological regulation  post-translational protein modification																
GO:0006464 GO:0006468	protein modification process macromolecule modification protein amino acid phosphory lation																
GO:0050794 GO:0010467	regulation of cellular process gene expression																
GO:0050789 GO:0006350	regulation of biological process transcription																
GO:0044262 GO:0006810	cellular carbohy drate metabolic process transport																
GO:0051234 GO:0051179	establishment of localization localization																
GO:0043436 GO:0019752 GO:0006082	oxoacid metabolic process carboxy lic acid metabolic process																
GO:0008082 GO:0042180 GO:0009308	organic acid metabolic process cellular ketone metabolic process anning metabolic process																
GO:0009308 GO:0044281 GO:0015979	amine metabolic process small molecule metabolic process photosy nthesis																
GO:0065004 GO:0006323	protein-DNA complex assembly DNA packaging																
GO:0034728 GO:0065003	nucleosome organization macromolecular complex assembly																
GO:0043933 GO:0019538	macromolecular complex subunit organization protein metabolic process																
GO:0022607 GO:0006334	cellular component assembly nucleosome assembly																
GO:0034621 GO:0034622 GO:0031497	cellular macromolecular complex subunit organization cellular macromolecular complex assembly chromatin assembly																
GO:0031497 GO:0071103 GO:0006412	chromatin assembly DNA conformation change translation			H													
GO:0000412 GO:0031326 GO:0080090	regulation of cellular biosy mhetic process regulation of primary metabolic process																
GO:0009889 GO:0010468	regulation of biosynthetic process regulation of gene expression							Ŀ		E							
GO:0045449 GO:0051171	regulation of transcription regulation of nitrogen compound metabolic process																
	regulation of macromolecule metabolic process regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process																
GO:0031323 GO:0010556 GO:0019222	regulation of cellular metabolic process regulation of macromolecule biosynthetic process																
GO:0019222 GO:0055085 GO:0006520	regulation of metabolic process transmembrane transport cellular amino acid metabolic process																
GO:0000520 GO:0034641 GO:0005976	cellular nitrogen compound metabolic process  cellular nitrogen compound metabolic process  poly saccharide metabolic process																
GO:0006519 GO:0006811	cellular amino acid and derivative metabolic process ion transport																
GO:0044106 GO:0006812	cellular amine metabolic process cation transport																
GO:0009607 GO:0016051	response to biotic stimulus carbohy drate biosynthetic process																
GO:0034637 GO:0046394 GO:0044283	cellular carbohy drate biosy nthetic process carboxy lic acid biosy nthetic process																
GO:0044283 GO:0044085 GO:0016053	small molecule biosynthetic process cellular component biogenesis organic acid biosynthetic process																
GO:0034660 GO:0006457	ncRNA metabolic process protein folding																
	ar function	$L_0$	$S_0$	$P_0$	$Z_0$	$L_5$	$S_5$	P <sub>5</sub>	$Z_5$	$L_0$	$S_0$	$P_0$	$Z_0$	$L_5$	$S_5$	P <sub>5</sub>	$Z_5$
GO:0016491 GO:0016773 GO:0004672	oxidoreductase activity phosphotransferase activity, alcohol group as acceptor protein kinase activity																
GO:0004674 GO:0016301	protein serine/threonine kinase activity kinase activity kinase activity																
GO:0030554 GO:0001883	adenyl nucleotide binding purine nucleoside binding																
GO:0001882 GO:0000166	nucleoside binding nucleotide binding																
GO:0016798 GO:0004553	hydrolase activity, acting on glycosyl bonds hydrolase activity, hydrolyzing O-glycosyl compounds																
GO:0032559 GO:0005524	adenyl ribonucleotide binding ATP binding																
GO:003700 GO:0032553 GO:0017076	transcription factor activity ribonucleotide binding purine nucleotide binding																
GO:0032555 GO:0009055	purine ribonuc leotholing purine ribonuc leotholing electron carrier activity																
GO:0003735 GO:0005198	structural molecule activity													E			
GO:0030528 GO:0005215	transcription regulator activity transporter activity																
GO:0020037 GO:0005506	heme binding iron ion binding																
GO:0046906 GO:0043565 GO:0048037	tetrapy rrole binding sequence-specific DNA binding cofactor binding															$\blacksquare$	
GO:0048037 GO:0004497 GO:0016757	cofactor binding monooxy genase activity transferase activity, transferring gly cosy l groups																
GO:0016737 GO:0045735 GO:0050662	nutrient reservoir activity  coenzy me binding																
C. Cell cor	mponent	$L_0$	$S_0$	P <sub>0</sub>	$Z_0$	$L_5$	$S_5$	P <sub>5</sub>	$Z_5$	L <sub>0</sub>	$S_0$	P <sub>0</sub>	$Z_0$	L <sub>5</sub>	$S_5$	P <sub>5</sub>	$Z_5$
GO:0016020 GO:0032991 GO:0005623	membrane macromolecular complex cell																
GO:0005623 GO:0044464 GO:0044425	cell cell part membrane part																
GO:0005840 GO:0030529	ribosome ribosome ribonucleoprotein complex								H	F							
GO:0032993 GO:0000786	protein-DNA complex nucleosome																
GO:0034357 GO:0009579	photosynthetic membrane thylakoid																
GO:0043234 GO:0005737	protein complex cy toplasm																
GO:0044444 GO:0009521 GO:0016021	cytoplasmic part photosystem																
GO:0016021 GO:0031224 GO:0044424	integral to membrane intrinsic to membrane intracellular part																
GO:0044424 GO:0015935 GO:0005622	mracenuar part small ribosomal submit intracellular																
GO:0003622 GO:0043229 GO:0043232	intracellular organelle intracellular organelle intracellular non-membrane-bounded organelle																
GO:0009522 GO:0015934	photosy stem I large ribosomal subunit																E
GO:0043228 GO:0033279	non-membrane-bounded organelle ribosomal subunit																
GO:0043226	organelle		L							1							

Fig. S5. Prominent functional categories of genes in the eight tissues detected by Gene ontology enrichment analysis before and 5 days after flowering in two hybrids

 $L_0$ , Flag leaf;  $S_0$ , Leaf sheath;  $P_0$ , Spikelet;  $Z_0$ , Main panicle axis before flowering;  $L_5$ , Flag leaf;  $S_5$ , Leaf sheath;  $P_5$ , Spikelet;  $Z_5$ , Main panicle axis 5 days after flowering

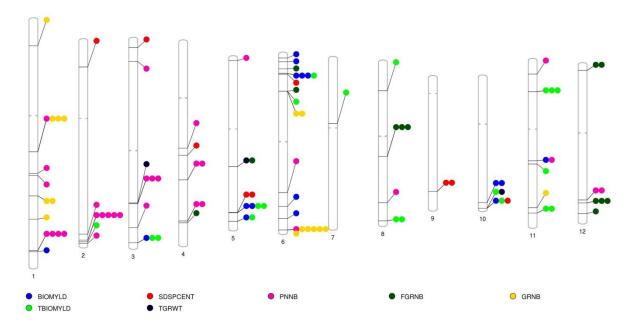


Fig. S6 Distribution of the common seven yield-related QTLs in our two  $F_1$  hybrids, T449  $\times$  H1 [17] and T452  $\times$  H3 [16], compared to the DEGFu-sp-related QTLs detected in the present study. Seven rice yield-related QTLs included biomass yield (TBIOMYLD), biomass yield (BIOMYLD), filled grain number (FGRNB), grain number (GRNB), panicle number (PNNB), seed set percent (SDSPCENT) and 1000-grain weight (TGRWT).

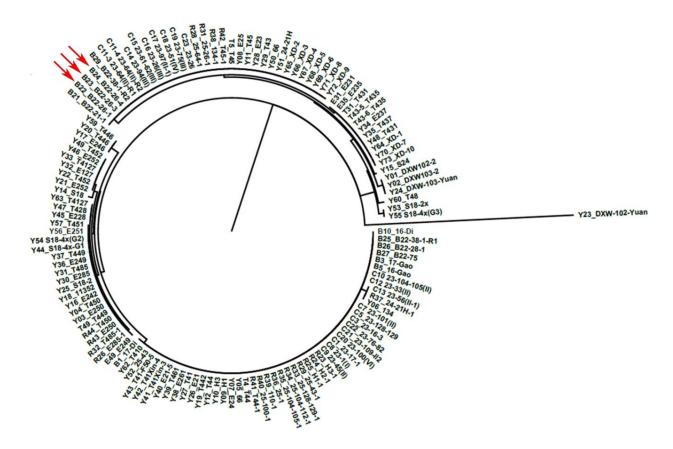


Fig. S7. Cluster of the DNA variations in LP gene of Huaduo 8 Arrows indicated Huaduo 8, in which DNA sequence was different from most of the other neo-tetraploid, autotetraploid and diploid rice lines. Three different plants of Huaduo 8 were re-sequenced



Fig. S8 Samples used for RNA-seq analysis
A. Flag leaf; B. Leaf sheath; C. Spikelets; D. Main panicle axis