

## **Supporting Information**

### **Experimental Procedures**

#### **SNP Markers screening and Individual Genotyping**

Based on the alignment result, we used SOAPSnp (15) to calculate the posterior probability of each genotype using a Bayesian formula and construct a consensus sequence of each parent by locating the allele type with the highest probability at each position. Homozygous genotypes that were different between two parents were treated as potential SNP markers if the following criteria were satisfied: sequencing depth  $\geq 4$  and  $\geq 50$ ; base quality  $\geq 25$ ; copy number  $< 2$ . Each RIL was genotyped at the potential SNP markers following two consecutive steps. We calculated the likelihood of genotypes of each RIL and then we allocated the allele type with the greatest likelihood back to each individual as the consensus genotype of each RIL.

To obtain a set of high-quality SNP markers and facilitate the following map construction, we filtered the candidate SNP markers based on several criteria including the ratio of heterozygous genotypes ( $\leq 0.5$ ), minor allele frequency ( $\geq 0.05$ ) and linkage relationships with adjacent markers. Markers outside the prediction interval were excluded for their abnormal linkage relationships.

#### **Graphic Genotyping Procedure**

In the graphic genotyping procedure, four primary steps were adopted as the window slides along each chromosome. 1) A window with a *93-11:PA64s* SNP ratio of 4:1 or higher was called *93-11* genotype, 1:4 or lower was called *PA64s* genotype. After that, we combined the adjacent bins with the same genotype into a block. 2) When the length of a block was shorter than 100 kb or the number of markers in a block was less than 20% of the markers in a window, the block was treated as a fragment. The fragments would be merged into adjacent blocks if both of the blocks were four times longer than the fragments. 3) The frequently transient genotypes of adjacent windows and successive small fragmented bins were merged into a heterozygous block in graphic genotypes when they satisfied the following conditions: the *93-11:PA64s* SNP ratio between 4:1 and 1:4, the total length of these fragments longer than 400 kb, and the number of markers in these fragments more than 80% of the markers in a window. Otherwise, the fragments were integrated into a block and genotyped based on the *93-11:PA64s* SNP ratio and adjacent block information. 4) The small blocks shorter than 400 kb were merged when both of the adjacent blocks have the same genotype. We then obtained a super-high resolution map of genome-wide graphic genotypes.

## Genome Annotation

For the protein-coding gene prediction, we first used Augustus-2.5.5 (37), GENSCAN (38), and Fgenesh (39) to *de novo* predict genes with parameters trained for monocots. Protein sequences from *Oryza sativa*, *Zea mays*, and *Sorghum bicolor* were used to perform homology-based prediction. Homologous genome sequences were aligned against the matched proteins using GeneWise (version 2.0) (40) for accurate spliced alignments. Finally, we merged these gene models into a unigene set using GLEAN (41) for each parental genome.

## Phenotyping and Correlation Analysis

Phenotypic analysis was conducted with two replications in the Hangzhou field from August to October, 2010, and in the Hainan field from March to June, 2011. Of 24 individuals of each RIL, *93-11*, *PA64s* and *LYP9*, 6 plants were randomly chosen for phenotyping. A total of 12 traits were evaluated. Among them, heading date (HD), plant height (PH), and effective tiller number (ETN) were measured directly in the field. The other 9 traits included spikelet number per panicle (SN), panicle length (PL), primary panicle branch number (PPB), secondary panicle branch number (SPB) and seed set (SS), 1000-grain weight (TGW), grain length (GL), grain width (GW), and yield per plant (PY) were measured in the laboratory following harvest.

HD was recorded as days from sowing to time when inflorescences had emerged above the flag leaf sheath for more than half of the individuals of line. PH was measured from the soil surface to the apex of the tallest panicle when panicles fully emerged. Effective tiller number was evaluated when grains were fully matured. The longest panicle was measured in length as PL. The total number of spikelet produced on the main tiller was counted. The panicle and grain related traits were measured in the laboratory. For the main panicles of an individual, PL was measured with ruler; PPB and SPB on the main tiller were counted manually. Grains were mixed and 10 grains were randomly sampled for phenotypic analysis. GL and GW were recorded at the maximal values for each grain using an electronic digital caliper. The TGW value was obtained by converting the weight of 200 grains. SS was counted as number of grains formed divided by the number of spikelet per panicle. Basic statistical analysis and correlation analysis were conducted with SAS 6.12 (SAS Institute).

## InDel/SNP Marker Development for Fine Mapping

Several InDel and SNP markers were developed for fine mapping of *qSN8* and *qSPB1*. Primers are shown in **SI Appendix. Table S18**. A high-resolution melting (HRM) analysis system for SNP genotyping was applied. PCR amplifications using LCGreen (Idaho Technology Inc.) were carried out in 10  $\mu$ L volumes containing 1  $\mu$ L rTaq (Takara-Bio, Dalian, China), 1  $\mu$ L 10 $\times$ PCR buffer, 1  $\mu$ L 10 $\times$  LCGreen Plus, 1  $\mu$ L

each 5  $\mu$ M primer, 2  $\mu$ L (100 Mm) of each dNTPs, 1  $\mu$ L (1.5 mM)  $MgCl_2$ , and 2  $\mu$ L (approximately 10 ng) template DNAs. PCR reactions were performed using 96 well PCR plates (ABgene), and the reactions were covered with 10  $\mu$ L mineral oil. PCR profile for rice DNA samples was one cycle of 95°C for 5 min, 30 cycles of 95°C for 30 s, 50-60°C for 30 s, and 72°C for 30 s, one cycle of 72°C for 10 min, one cycle 95°C for 30 s, and then down to 40°C holding at the 0.1°C/s. Subsequently, the PCR plates were subjected for HRM analysis in LightScanner (Idaho Technology Inc.).

### **RNA Extraction and Real-Time RCR Analysis**

Total RNA was isolated from panicles (for examining *GS3*, *DTH8*, *LAX1*), first internodes (for *EUII*, *NRL1*) in the booting stage, and tiller buds (for *HTDI*) at the tillering stage with the MicroRNA Extraction kit (Axygen). After RNase-free DNase (Promega) treatment, first-strand cDNAs were synthesized from equal amounts of total RNA with a ReverTra Ace® qPCR-RT kit (TOYOBA, Japan) according to procedures described by the manufacturer. Real-time PCR was performed using 2  $\times$  SYBR Green PCR Master Mix on a 7900HT Real-Time PCR System (Applied Biosystems) with three replicates for each sample. The relative expression level of each transcript was obtained by comparing to the expression of the *OsACTIN1* gene. Primers for candidate genes and *OsACTIN1* are listed in **SI Appendix. Table S19**.

### **Vector Construction and Transformation**

A 3.75-kb genomic DNA fragment of *DTH8* from *93-11* was amplified by PCR with primers DTH8F (5'-TAGAATTCGCAAGTGGTAAGGAAAGGTAGGAAC-3') and DTH8R (5'-ATGGATCCAATGTGCCAAGCCCAGATGAGCGTGAGCCG-3'), and cloned into pCAMBIA1300 after digestion with EcoR I and BamH I. The resultant plasmid, pCAMBIA1300-*DTH8* was introduced into *PA64s* by *Agrobacterium*-mediated transformation (42). The genotype of transgenic plants was determined by PCR amplification of the hygromycin phosphotransferase gene (*hpt*) with primers HPTF (5'-GGAGCATATACGCCCGGAGT-3') and HPTR (5'-GTTTATCGGCACTTTGCATCG-3') as well as selection for hygromycin resistance.

### **References for Experimental Procedures**

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41. Elvik CG, et al. (2007) Creating a honey bee consensus gene set. *Genome Biol* 8(1):R13.
42. Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J* 6(2):271-282.

## Supplementary Tables

- **Supplementary Table 1** Details of sequencing and mapping data of each *LYP9* RIL and parents.
- **Supplementary Table 2** SNP markers distribution along each chromosome.
- **Supplementary Table 3** Distribution of recombinant breakpoints in genic regions along each chromosome.
- **Supplementary Table 4** Gene density in hotspot regions and heterozygous regions containing hotspots.
- **Supplementary Table 5** SNP density in hotspot regions on 12 chromosomes.
- **Supplementary Table 6** Summary of nr-KOME cDNAs and mRNAs excised from *japonica* (IRGSP) with complete alignments in *93-11* and *PA64s* assemblies. Columns show the percentage of nr-KOME cDNAs and mRNAs that are covered  $\geq 95\%$  of the total length using BLAT.
- **Supplementary Table 7** Primers used to evaluate gap filling methods.
- **Supplementary Table 8** Primers used to evaluate single base error correction methods.
- **Supplementary Table 9** Summary of structural variations in three comparisons.
- **Supplementary Table 10** Summary of evidence for the GLEAN gene models.
- **Supplementary Table 11** Summary of predicted protein-coding genes of *PA64s*.
- **Supplementary Table 12** Functional annotation using different databases.
- **Supplementary Table 13** Summary of predicted protein-coding genes of *93-11*.
- **Supplementary Table 14.** Twelve traits of *93-11* and *PA64s* observed in Hangzhou (HZ) and Hainan (HN).
- **Supplementary Table 15** Correlation analysis of 12 yield-associated traits in Hangzhou (HZ).
- **Supplementary Table 16** Correlation analysis of 12 yield-associated traits in Hainan (HN).
- **Supplementary Table 17** Additive QTLs for twelve traits detected in RIL population derived from *93-11* and *PA64s* in Hainan and Hangzhou and reported QTLs or genes.
- **Supplementary Table 18** Primers of SNP and InDel markers for fine mapping.
- **Supplementary Table 19** Primers used for real time PCR of candidate genes.
- **Supplementary Table 20** Phenotype of T<sub>1</sub> plants transformed with pCAMBIA1300-*DTH8*.
- **Supplementary Table 21** Regions shared by QTLs and recombinant hotspots.

**Supplementary Table 1** Details of sequencing and mapping data of each *LYP9* RIL and parents.

Sample ID	Reads			Bases					
	Total Reads	Mapped (%)	Unique Mapped (%)	Total Bases (Mb)	Mapping		Unique Mapping		Mis Match Bases (Mb)
					Mapped (%)	Mean Depth	Mapped (%)	Mean Depth	
<i>PA64s</i>	301704550	87.11	69.24	22544	83.34	60.90	66.50	48.52	146
9311	220731508	82.71	65.23	16598	79.72	45.07	63.02	35.63	142
BI10	13016758	77.05	60.69	976	75.75	1.99	59.65	1.56	6
BI100	37259138	74.36	59.23	3180	74.22	6.34	59.20	5.06	21
BI101	40644434	73.78	57.28	3478	73.60	6.88	57.19	5.35	25
BI109	44449918	73.16	57.10	3802	73.02	7.46	57.04	5.82	28
BI11	25047958	80.13	64.40	2068	79.42	4.42	63.86	3.55	13
BI112	39862786	73.97	57.96	3408	73.80	6.76	57.88	5.30	24
BI115	38938318	74.49	58.81	3329	74.34	6.64	58.76	5.25	23
BI116	37255504	73.23	57.70	3189	73.10	6.26	57.66	4.94	23
BI118	17001802	82.11	65.25	1275	80.85	2.77	64.24	2.20	7
BI119	14604564	79.34	61.87	1095	78.08	2.30	60.87	1.79	7
BI12	12884700	78.65	61.96	966	77.40	2.01	60.96	1.58	6
BI120	14092782	79.54	62.76	1057	78.30	2.22	61.77	1.75	7
BI121	14182188	78.48	62.79	1064	77.24	2.21	61.79	1.77	6
BI126	41132090	73.83	59.05	3522	73.71	6.97	59.01	5.58	24
BI127	31986464	73.82	58.29	2737	73.68	5.41	58.25	4.28	19
BI128	28512426	77.15	61.09	2383	76.39	4.89	60.53	3.88	16
BI132	22637472	77.64	62.08	1875	76.75	3.86	61.41	3.10	12

BI133	26332170	79.12	64.12	2198	78.28	4.62	63.48	3.75	14
BI134	15615598	81.02	63.84	1171	79.77	2.51	62.85	1.98	7
BI135	24964798	76.39	60.77	2058	75.44	4.17	60.05	3.32	14
BI136	13178418	79.22	62.60	988	77.44	2.06	61.18	1.62	6
BI138	15802226	79.73	62.99	1185	78.49	2.50	62.00	1.97	8
BI139	13498744	82.33	65.87	1012	81.07	2.21	64.86	1.76	6
BI140	13614306	79.18	62.08	1021	77.97	2.14	61.11	1.68	7
BI142	13444750	76.95	60.40	1008	75.76	2.05	59.46	1.61	7
BI143	25604752	79.16	62.62	2120	78.43	4.46	62.06	3.53	15
BI147	31453138	73.44	57.90	2680	73.31	5.28	57.86	4.17	19
BI148	25070888	77.59	60.97	2083	76.71	4.29	60.32	3.38	14
BI15	13563284	78.93	62.31	1017	77.68	2.12	61.31	1.68	7
BI150	12769698	78.91	61.64	958	77.13	1.98	60.24	1.55	6
BI153	23077016	77.82	61.24	1946	77.07	4.03	60.70	3.18	12
BI154	28863906	78.83	63.07	2407	77.98	5.04	62.44	4.04	16
BI155	24128720	79.20	61.74	1991	78.47	4.20	61.20	3.27	13
BI157	12899304	80.38	63.73	967	79.14	2.06	62.73	1.63	6
BI16	12993492	80.47	64.69	975	79.2	2.07	63.65	1.67	6
BI160	15116458	78.98	62.55	1134	77.74	2.37	61.55	1.87	7
BI161	12897030	78.31	60.6	967	77.07	2.00	59.62	1.55	6
BI162	12645306	79.98	63.19	948	78.74	2.01	62.20	1.58	6
BI163	16357026	80.45	64.74	1227	79.21	2.61	63.73	2.10	7
BI166	15204278	80.27	63.08	1140	79.03	2.42	62.10	1.90	7
BI167	12928450	78.87	62.73	970	77.64	2.02	61.74	1.61	6
BI168	13386300	79.92	63.25	1004	78.69	2.12	62.26	1.68	6
BI170	25219392	77.73	61.50	2092	77.01	4.33	60.96	3.43	13

BI172	27724940	71.67	55.91	2313	71.04	4.42	55.44	3.44	14
BI174	26846748	80.22	64.44	2231	79.52	4.76	63.91	3.83	14
BI177	15694798	80.72	63.62	1177	79.47	2.51	62.62	1.98	8
BI178	13868504	80.16	62.55	1040	78.93	2.21	61.57	1.72	6
BI18	12194036	72.96	56.65	915	71.77	1.76	55.71	1.37	6
BI181	12917948	78.99	62.15	969	77.76	2.02	61.17	1.59	6
BI185	14255186	79.16	63.06	1069	77.91	2.24	62.06	1.78	7
BI186	14093314	80.13	63.76	1057	78.86	2.24	62.73	1.78	6
BI19	14175900	77.47	60.77	1063	76.2	2.18	59.76	1.71	7
BI190	21502118	78.01	61.14	1764	77.2	3.66	60.53	2.87	12
BI191	13944856	77.84	61.16	1046	76.61	2.15	60.18	1.69	7
BI192	22854924	76.84	60.93	1888	76.09	3.86	60.37	3.07	12
BI194	14176730	78.21	60.02	1063	76.97	2.20	59.04	1.69	7
BI195	12824140	80.65	65.05	962	79.38	2.05	64.01	1.65	6
BI198	12687130	80.62	63.28	952	79.35	2.03	62.27	1.59	6
BI199	12778530	76.80	60.07	958	75.57	1.95	59.09	1.52	6
BI20	25795620	77.65	61.77	2175	77.09	4.50	61.37	3.59	15
BI203	24508406	78.27	61.72	2041	77.47	4.24	61.12	3.36	14
BI204	11762464	80.42	62.37	882	79.16	1.88	61.37	1.45	5
BI205	12931942	79.63	60.39	970	78.37	2.04	59.42	1.55	6
BI208	33550868	71.98	56.19	2871	72.01	5.56	56.28	4.34	20
BI209	42643806	73.76	58.94	3648	73.81	7.23	59.07	5.79	23
BI22	14240586	77.77	60.73	1068	76.5	2.19	59.72	1.71	7
BI221	27556376	75.44	58.37	2067	74.23	4.12	57.42	3.19	16
BI223	38012712	74.61	59.28	3252	74.64	6.52	59.38	5.19	21
BI225	35969372	73.83	58.99	3076	73.87	6.10	59.10	4.88	20



BI227	26887742	79.09	62.86	2242	78.37	4.73	62.33	3.75	15
BI228	26539070	79.96	63.72	2205	79.24	4.69	63.18	3.74	14
BI232	13010504	80.30	62.70	976	79.07	2.07	61.73	1.62	7
BI235	13098332	79.46	62.65	982	78.24	2.06	61.68	1.63	7
BI236	24809760	77.86	61.53	2053	77.14	4.25	61.00	3.36	14
BI238	15037220	80.77	63.98	1128	79.51	2.41	62.97	1.91	7
BI239	12947670	79.07	61.26	971	77.84	2.03	60.30	1.57	6
BI24	13452724	78.64	62.55	1009	77.38	2.10	61.54	1.67	7
BI241	11718342	78.63	60.17	879	77.4	1.83	59.22	1.40	6
BI246	12949396	79.49	61.86	971	78.26	2.04	60.89	1.59	6
BI248	27492264	81.28	65.75	2290	80.61	4.96	65.25	4.01	13
BI25	13587052	79.75	63.32	1019	78.49	2.15	62.30	1.71	6
BI251	13832110	78.22	58.86	1037	76.99	2.15	57.92	1.61	7
BI253	25726472	77.86	60.95	2129	77.16	4.42	60.44	3.45	14
BI255	26289704	79.70	63.16	2179	79.00	4.63	62.65	3.67	13
BI259	14356232	80.27	63.63	1077	79.02	2.29	62.63	1.81	7
BI264	12649454	81.10	63.98	949	79.85	2.04	62.98	1.61	6
BI265	14438326	81.31	64.40	1083	80.05	2.33	63.39	1.84	7
BI267	12641606	79.60	62.82	948	78.36	2.00	61.83	1.57	6
BI269	28695680	78.88	61.92	2392	78.08	5.01	61.33	3.94	16
BI271	13890146	80.11	63.01	1042	78.87	2.21	62.03	1.74	7
BI274	13500226	83.28	66.11	1013	82.01	2.23	65.09	1.77	5
BI277	11706286	77.89	60.15	878	76.67	1.81	59.20	1.40	6
BI283	18160614	79.89	61.39	1362	78.65	2.88	60.42	2.21	9
BI29	14396060	75.92	59.83	1080	74.70	2.17	58.86	1.71	7
BI30	14031800	78.55	60.70	1052	77.30	2.19	59.72	1.69	7

BI31	34163250	78.47	62.17	3007	78.15	6.32	61.91	5.00	20
BI32	22470064	80.95	65.79	1845	80.17	3.97	65.19	3.23	11
BI34	39832006	77.25	59.35	2987	76.02	6.10	58.40	4.69	21
BI35	16258484	78.05	60.08	1219	76.83	2.52	59.12	1.94	8
BI36	39997366	78.65	62.50	3504	78.29	7.37	62.21	5.86	23
BI37	35938198	78.71	63.55	3144	78.35	6.61	63.27	5.34	21
BI38	27901060	78.49	61.74	2345	77.85	4.90	61.26	3.86	16
BI40	12689014	80.83	64.83	952	79.57	2.03	63.81	1.63	6
BI41	27078076	78.77	63.05	2290	78.13	4.81	62.57	3.84	16
BI45	35744108	73.64	57.94	3056	73.39	6.03	57.81	4.74	22
BI47	37719676	73.54	58.26	3224	73.31	6.35	58.15	5.04	23
BI49	39172252	75.26	60.78	3347	75.02	6.75	60.65	5.45	24
BI5	13862524	78.01	59.85	1040	76.70	2.14	58.82	1.64	7
BI50	39904574	75.99	60.84	3409	75.75	6.93	60.72	5.56	22
BI51	13942982	80.37	63.86	1046	79.11	2.22	62.85	1.77	7
BI54	36957984	73.26	58.35	3160	73.34	6.23	58.52	4.97	21
BI59	38713958	72.12	57.42	3308	72.21	6.42	57.60	5.12	22
BI60	14364762	79.51	62.08	1077	78.28	2.27	61.10	1.77	7
BI61	12378582	80.19	63.91	928	78.93	1.97	62.89	1.57	6
BI62	16593298	79.98	62.99	1244	78.74	2.63	62.00	2.07	8
BI67	37833922	70.21	55.62	3233	70.28	6.11	55.78	4.85	22
BI68	24811340	76.85	60.48	2049	76.14	4.19	59.95	3.30	14
BI71	38993296	71.50	56.53	3330	71.61	6.41	56.71	5.07	24
BI74	35034872	75.01	59.48	2992	74.90	6.02	59.48	4.78	20
BI8	13487016	79.62	62.05	1012	78.38	2.13	61.07	1.66	7
BI83	29364700	76.03	60.03	2202	74.81	4.43	59.06	3.49	16

BI84	35887702	74.31	59.48	3063	74.21	6.11	59.47	4.90	22
BI89	38084532	72.50	57.63	3244	72.39	6.31	57.62	5.02	23
BI9	14999770	79.15	63.04	1125	77.83	2.35	61.96	1.87	7
BI90	12126842	81.77	66.22	910	80.51	1.97	65.19	1.59	5
BI92	34545320	72.68	57.78	2947	72.52	5.74	57.72	4.57	21
BI94	39371378	74.56	59.01	3358	74.42	6.71	58.97	5.32	23
BI95	13019820	78.58	61.65	976	77.32	2.03	60.65	1.59	6
BI97	13223270	76.19	58.94	992	74.46	1.98	57.60	1.53	7
BI98	17594250	79.84	62.35	1320	78.60	2.79	61.37	2.18	8

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**Supplementary Table 2** SNP markers distribution along each chromosome.

Chr.	Markers Distribution					Genes	
	Total	CDS	5' UTR	Gene	3' UTR	Number	Covered
1	20473	2367	349	7426	746	6714	1921
2	14608	1407	251	5171	550	5555	1467
3	12417	1224	283	4432	492	5693	1390
4	20305	2480	362	7449	627	5514	1597
5	11211	1122	218	3785	451	4704	1232
6	12480	1291	180	4245	428	4869	1327
7	21781	2164	361	7204	706	4600	1793
8	8217	783	140	2774	315	4327	861
9	10781	1123	148	3587	299	3513	931
10	13810	1718	190	4930	433	3672	1191
11	6691	1097	116	2614	214	4305	683
12	19073	2144	233	6266	649	4158	1264
All	171847	18920	2831	59883	5910	57624	15657

**Supplementary Table 3** Distribution of recombinant breakpoints in genic regions along each chromosome.

Chr.	Breakpoints	Recombinants	Gene	5' UTR	CDS	3' UTR
1	393	845	139	7	52	12
2	408	814	130	8	27	21
3	336	670	122	11	36	18
4	379	678	125	10	40	17
5	299	611	91	5	29	21
6	244	463	86	5	25	13
7	263	369	96	8	33	11
8	264	612	74	3	20	10
9	209	365	59	6	14	5
10	259	529	82	82	27	5
11	175	412	69	1	33	7
12	295	562	88	3	32	10
Total	3524	6930	1161	149	368	150

**Supplementary Table 4** Gene density in hotspot regions and heterozygous regions containing hotspots.

	Hots pot number	Hots pot contain gene	Gene loci number	Gene density (/kb)	Heterozygous region	Heterozygous region containing hot spot	Percent of Heterozygous regions with hot spots
Chr1	52	27	27	0.260	94	71	0.755
Chr2	32	18	20	0.313	121	70	0.578
Chr3	38	27	30	0.395	32	29	0.906
Chr4	38	23	25	0.329	120	99	0.825
Chr5	36	27	31	0.431	57	42	0.736
Chr6	26	17	19	0.365	13	10	0.769
Chr7	36	21	21	0.292	29	13	0.448
Chr8	26	15	16	0.308	44	34	0.772
Chr9	25	16	17	0.340	27	19	0.703
Chr10	24	14	14	0.292	81	62	0.765
Chr11	24	19	22	0.458	31	24	0.774
Chr12	29	11	11	0.190	139	116	0.834
Total	386	224	253	0.328	788	589	0.747
<i>Nipponbare</i> genome		370020kb	55986	0.151			

**Note:** Gene density was calculated by the ratio of gene loci number to the length of the related regions. Every hotspot is 2 kb in length. The total length of the hotspot regions of one chromosome is calculated by hotspot number  $\times$  2 kb. The *Nipponbare* gene density is calculated according to the TIGR database.

**Supplementary Table 5** SNP density in hotspot regions on 12 chromosomes.

<b>Chrs</b>	<b>SNPs in Hot spots</b>	<b>SNPs in chromosome</b>	<b>Chromosome length (k b)</b>	<b>SNP density in Hot spots(/kb)</b>	<b>SNP density in chromosome(/k b)</b>
Chr01	279	46656	43,268.88	2.68	1.08
Chr02	135	27995	35,930.38	2.11	0.78
Chr03	213	27930	36,406.69	2.80	0.77
Chr04	250	45091	35,278.23	3.29	1.28
Chr05	157	24239	29,894.79	2.18	0.81
Chr06	122	30293	31,246.79	2.35	0.97
Chr07	187	50519	29,696.63	2.60	1.70
Chr08	127	15658	28,439.31	2.44	0.55
Chr09	107	24735	23,011.24	2.14	1.07
Chr10	169	28540	23,134.76	3.52	1.23
Chr11	138	13139	28,512.67	2.88	0.46
Chr12	254	40114	27,497.21	4.38	1.46
Total	2138	374909	372,317.57	2.77	1.01

**Note:** Every hotspot is 2 kb in length.

**Supplementary Table 6** Summary of nr-KOME cDNAs and mRNAs excised from *japonica* (IRGSP) with complete alignments in *93-11* and *PA64s* assemblies. Columns show the percentage of nr-KOME cDNAs and mRNAs that are covered  $\geq 95\%$  of the total length using BLAST.

Assembly	Total (%)	95% Covered in Genome (%)		95% Covered in Chromosomes (%)		95% Covered in Scaffolds (%)		Total
	Found	All	Uniq	All	Uniq	All	Uniq	
nr-KOME vs. <i>93-11</i>	94.55	85.60	76.00	85.35	75.91	1.03	0.67	26278
nr-KOME vs. <i>PA64s</i>	93.92	82.90	75.82	82.81	75.79	0.40	0.23	26278
mRNAs vs. <i>93-11</i>	99.55	94.29	81.37	94.00	81.33	1.15	0.83	29389
mRNAs vs. <i>PA64s</i>	98.96	91.29	81.55	91.20	81.56	0.41	0.35	29389

**Supplementary Table 7** Primers used to evaluate gap filling methods.

Chr.	ID	Types	Direction	Primes	Product length (bp)	TM Value (°C)
01	C7264659	B	F	5'-CATGTCGTCGCTAAACCA-3'	457	52.7
			R	5'-CGCCTCCACAGTAAATCC-3'	457	53.2
01	C7276243	B	F	5'-AGGCA CGA GGT TATTGAG-3'	468	50.2
			R	5'-GGTTCTTA GCTGGGCA CT-3'	468	52.1
01	C7280601	A	F	5'-TTGGA GACTGAAAACGAA G-3'	462	50.5
			R	5'-ATTTGAGCTGGT GAAA GTG-3'	462	50.3
01	C7295595	C	F	5'-AGAGGATGGCAA CAA GCG-3'	479	56.4
			R	5'-ACCTGTCA GCCCATCTCA -3'	479	53.0
01	C7298599	A	F	5'-ATGATGCGGGTTGGTAGC-3'	459	56.2
			R	5'-GGGGCAA GTCAA GTAA GTC-3'	459	51.7
01	C7307443	C	F	5'-CGATTTTCA GTCCTACAGAGT-3'	548	51.3
			R	5'-CCTGTCTTATGAGGTGCTTT-3'	548	51.9
01	C7309097	C	F	5'-AGCTGGATATGGACAACTGA-3'	408	52.5
			R	5'-GGAA CCGCTTCTGGATTT-3'	408	54.6
01	C7314353	A	F	5'-GCA CGAATCTTAAAGCAATC-3'	425	53.0
			R	5'-TTCCGTCCTTCCACCTCT-3'	425	54.5
02	C7182779	B	F	5'-GGCTGAACATGCTCTATTG-3'	422	50.9
			R	5'-GGTTA GGT TTA GGGTTGTCT-3'	422	50.4
02	C7198337	B	F	5'-ACTGCGGTGACAGGTGGAA-3'	474	59.0
			R	5'-TTAGGACGGA GGGAGTAGGC-3'	474	58.5
02	C7219941	C	F	5'-AAGCGGCTTCCCTCTACT-3'	486	58.2
			R	5'-ATCTGCCCTCCTGTCGTT-3'	486	59.0



02	C7261389	A	F	5'-GGAA GCAAAACAGCA CGAA-3'	445	57.0
			R	5'-TCATCCCCCTCCCCAACAT-3'	445	57.5
03	C7254331	A	F	5'-TCATTCCCCTTGT CGTGC-3'	423	56.7
			R	5'-CAA GCCGTCA GGTCTCAA-3'	423	54.3
03	C7291631	C	F	5'-TGCAA GAGGCTTAATACGG-3'	499	54.1
			R	5'-AGGGCACTTGACTGACCGA-3'	499	58.3
04	C7207755	A	F	5'-GAAAATAACGCTCGGTAGA-3'	475	50.7
			R	5'-AGAGGGTTAGTGTAAGTGGG-3'	475	50.9
04	C7245175	B	F	5'-ACGGCACGAA GAGGAATG-3'	405	56.2
			R	5'-TCGGATGCTTGTGGGAT-3'	405	56.4
04	C7248103	C	F	5'-AAAGGTGGCTAAAAGTGGA-3'	447	52.4
			R	5'-TAGGGAAA GTGGCATCAA-3'	447	51.4
04	C7279271	C	F	5'-GACCAAAGATGGC GAGTA-3'	452	50.8
			R	5'-AGGGTCGGTATGATTGTG-3'	452	50.2
05	C7223687	C	F	5'-GAAACTATGCGATGCTATGC-3'	444	53.7
			R	5'-AAA ACTGAGAA GCCACGA-3'	444	50.6
05	C7227091	C	F	5'-GGGAAATCGTGAAATCGT-3'	403	52.5
			R	5'-CAAAA GGTCAATGGCAAAC-3'	403	53.7
05	C7235105	A	F	5'-CGGTTTGGGGA GATAGAG-3'	435	52.3
			R	5'-CATA GGGAA GAAA GCGATA-3'	435	50.5
06	C7211853	B	F	5'-AACTTGTA GCCTATGCCTTGT-3'	440	53.9
			R	5'-TACCCATCCGCA CCTTTC-3'	440	56.1
06	C7218799	B	F	5'-AGAGGAGCCAATAACCAAA-3'	429	52.2
			R	5'-CAAGCTAACCAACCAAGT-3'	429	50.2
06	C7234203	C	F	5'-CTGTTCCATGCCCTCGTCT-3'	471	57.9
			R	5'-TCTATTTTATCTCCCCAAGC-3'	471	51.7

06	C7247971	A	F	5'-ATGGA CTCCCA CAGGTTAT-3'	499	50.9
			R	5'-TGTGGTAATGCAACTCCTC-3'	499	50.8
07	C7167625	B	F	5'-ACCTCTGGTTTCGCAATG-3'	482	53.5
			R	5'-GCAACTTCA GCCTATTCTATCT-3'	482	53.2
07	C7212223	C	F	5'-CA GGTCCCTTCCAAATGT-3'	498	52.2
			R	5'-TGTTTAGTTCCCTCCGTTT-3'	498	52.2
07	C7213359	A	F	5'-AAATCATA GCCA GA CCA CA-3'	445	50.5
			R	5'-GATA CCATTCAACGA CAACA-3'	445	51.0
07	C7238465	B	F	5'-TGA CGCATA CGA CTA CGG-3'	489	52.8
			R	5'-GGTTGTTCCACCTTGAC-3'	489	52.2
07	C7250609	C	F	5'-GGTTTCTTTGCTTGATGG-3'	448	50.6
			R	5'-ACTAAATTGGTGTCTGGGA-3'	448	50.5
07	C7270079	C	F	5'-TTTCCA GCATCACTATCA CA-3'	404	51.6
			R	5'-AAGCCTTCAGCTAGGCC-3'	404	51.9
08	C7268071	B	F	5'-ACTGGTAA GTGGGCATTG-3'	495	50.6
			R	5'-CTTTGTTCCCTTCTGTTC-3'	495	51.7
08	C7299327	B	F	5'-TGA GCGGCA GTGGTAA GT-3'	478	54.2
			R	5'-CA GAAATCGGA GGCAA GA-3'	478	53.8
08	C7310585	C	F	5'-CA GA TCA GTCGAAAACGG-3'	461	51.2
			R	5'-TCCAA GTCCCA GAAATACC-3'	461	51.9
08	C7321337	C	F	5'-GTTTTGCCTCGTAGACCG-3'	479	53.6
			R	5'-CTCCGTGACTGAGTAATGAAGA-3'	479	54.6
09	C7198411	A	F	5'-CATTTGGCAATA GGA GGT-3'	403	50.2
			R	5'-TGACTGGATGTTGGAA GG-3'	403	50.8
09	C7206849	C	F	5'-TTTGGACGGA GGGAGTAT-3'	468	52.1
			R	5'-ACGAAAA GCGA CCGA GGA-3'	468	58.7

09	C7224685	C	F	5'-GA GGTGGATGGA GGATGA-3'	419	52.2
			R	5'-AAGGA GCGA GGA GAA GAA-3'	419	51.8
09	C7249941	C	F	5'-AATGGAA GCAAATGAA GGAG-3'	419	54.5
			R	5'-CGGCTAATACCCCAATACAG-3'	419	55.3
10	C7243181	B	F	5'-GA GGC GGGTA GTGA GAA CA-3'	506	55.5
			R	5'-TGCGATGCGAA GCTGAAA-3'	506	59.2
10	C7243877	B	F	5'-CGAAGGATAAAA GTGGGA-3'	466	50.4
			R	5'-CGGTGCTTGA CCTGA GAA-3'	466	54.3
11	C7314909	C	F	5'-GTTA GTGGACCAAATGCG-3'	530	51.4
			R	5'-TGCTTACCTTGGAAATCGT-3'	530	50.7
12	C7134351	C	F	5'-ATTCCATA CTCATCA GACATCG-3'	489	54.2
			R	5'-CCTGGTATCTGGTTTTGC-3'	489	50.5
12	C7150330	B	F	5'-GCCGGTAGGATTTCA CTC-3'	465	52.2
			R	5'-CCCGAAGAACATCAACAC-3'	465	50.6
12	C7202949	C	F	5'-AAAGGCTACATA GGTTCTGTG-3'	406	52.4
			R	5'-AAGCAA GTGGATGGGAAA-3'	406	53.1
12	C7224329	A	F	5'-ATGGATTCCTGCTGA GTT-3'	439	51.5
			R	5'-AAGCCCTTCATCCTTAGTG-3'	439	51.7

Note:

1, F: forward; R: reverse.

2, Red background indicates the PCR amplification was failed, and the yellow background indicates the PCR amplification was non-specific.

3, Different types, A represents the forward primer located in the new introduced sequence in the gap, while the reverse primer locates on the original scaffolds; B represents the reverse primer located in the new introduced sequence in the gap, while the forward primer locates on the original scaffolds; C represents the gap was filled completely, and the PCR product covered the gap.

**Supplementary Table 8** Primers used to evaluate single base error correction methods.

Chr.	ID	Result	Direction	Primes	Product length (bp)	TM Value (°C)
01	Chr01_31515688	Y	F	5'-GCA GCATTCAAAGTA GTCC-3'	403	50.2
			R	5'-CAAACCGTGGCAAAATAGTA-3'	403	54.2
01	Chr01_520442	Y	F	5'-TCTTTGACCCTTGGTGATG-3'	471	53.5
			R	5'-GGCAAACGCCAAGAAAAT-3'	471	56.0
02	Chr02_21966715	Y	F	5'-GTAGTCCTGGATGATTTTGA G-3'	489	51.3
			R	5'-GACGATTGCTTAGGGTATG-3'	489	50.4
02	Chr02_514372	Y	F	5'-CATCATCGCATAAGTTGGC-3'	434	54.5
			R	5'-GGATTGGAA GGGATTA GA GTA-3'	434	53.1
02	Chr02_11342411	Y	F	5'-TAGCCATACATTTTGTCCAG-3'	485	51.2
			R	5'-ATACTTGTTGAGCCTTTGC-3'	485	50.1
03	Chr03_441478	Y	F	5'-AAATCGTTTCGGAGCAA G-3'	431	53.4
			R	5'-CAGTCATACAACCAA GCATC-3'	431	50.7
03	Chr03_2528131	Y	F	5'-GATTCCATCCAA CCATAAGC-3'	455	54.4
			R	5'-CTCGGCATCCTCCTACAA-3'	455	53.6
03	Chr03_4824809	Y	F	5'-ATGGGTATCCA GTGTTGC-3'	495	50.1
			R	5'-TAATGGATTGCTTGCCTC-3'	495	51.3
03	Chr03_23356087	N	F	5'-CCTCATCCTGCTTTCGTG-3'	421	54.0
			R	5'-TTCATCGGCTCCTCCTCA-3'	421	56.5
04	Chr04_1558863	Y	F	5'-CACCTGACCCAA GCCATT-3'	438	55.8
			R	5'-TTCCCGACCCAAATCAAC-3'	438	55.9
04	Chr04_2187887	Y	F	5'-GAGTGA GAA GCCTGAGACG-3'	439	52.2
			R	5'-GTATGGGCTGACATTGGA-3'	439	51.0

05	Chr05_1591959	Y	F	5'-TGATTA GTGCCA GTCTCCA-3'	447	51.2
			R	5'-AATCA GAACA GCAA CAACG-3'	447	51.2
05	Chr05_2148100	Y	F	5'-TACTTTGCTGGCTGCTCT-3'	455	51.2
			R	5'-AAGTCAAACA GTGGGGTATT-3'	455	51.1
05	Chr05_3683921	Y	F	5'-TA GCCTCCA GTGGTTCAA-3'	492	51.2
			R	5'-TGATGTA GCCATTTGTGA GA-3'	492	51.6
05	Chr05_5371264	Y	F	5'-CAAAACATCAAAACCTCCCA-3'	452	55.8
			R	5'-TTCCCCAACA GCCTTCTT-3'	452	55.0
05	Chr05_22124925	Y	F	5'-GAA GCA CA GTTCCA GGTTG-3'	412	53.1
			R	5'-TCTCAATGGAA GGCAA GG-3'	412	53.6
06	Chr06_15963277	Y	F	5'-TTGGCGAA CA GGGTA GTA-3'	471	51.7
			R	5'-GATAATGGA GGA GAA GGCT-3'	471	50.6
06	Chr06_21241690	Y	F	5'-TTGTTACTA CCTCTGTCCCATA-3'	473	52.1
			R	5'-ACCA GTCCCTA CACTCCGT-3'	473	53.6
07	Chr07_4847390	Y	F	5'-AGTTGTTTGGCTATCCTTGG-3'	431	54.5
			R	5'-CATTGTTTGATTGTGGCAGT-3'	431	53.9
07	Chr07_6645423	Y	F	5'-TGACTTGGACATA GGGCTTGA-3'	483	58.0
			R	5'-AGA GGCTTCGGGTCGTTAC-3'	483	56.7
07	Chr07_12953515	Y	F	5'-ATGGAA GGCAGCATTAGTT-3'	461	52.2
			R	5'-AATGCGTTGCTCGTGTCA-3'	461	55.8
08	Chr08_97825	Y	F	5'-GTA GAGTCATCGTCATCCTTC-3'	461	50.9
			R	5'-ACCATCCAACATTTCCCT-3'	461	51.5
08	Chr08_265350	Y	F	5'-TGCGACGCTTCCTCATA G-3'	496	55.0
			R	5'-TCCTTCATACCTA CCTACGG-3'	496	52.0
08	Chr08_19940031	Y	F	5'-GCCAAA ACTCCA CTTCTCC-3'	487	54.3
			R	5'-GCAACAACA ACCTTCCAATA-3'	487	53.6

09	Chr09_66838	Y	F	5'-ATCCCTATCTGTTGACGCC-3'	444	54.5
			R	5'-ATTCTGATTCCCCATTCC-3'	444	51.2
09	Chr09_485749	Y	F	5'-CGAAGTGGCTACTATGGC-3'	469	51.0
			R	5'-CACTCAGAGGACTACCCAAC-3'	469	51.3
10	Chr10_10089666	Y	F	5'-GCACCGAATCCAGCCCTAT-3'	472	59.7
			R	5'-TTCCGCCACCCTTCTACTC-3'	472	57.4
10	Chr10_10301587	Y	F	5'-ATCCTCGCTCGTATCCGTT-3'	431	57.0
			R	5'-AAGAAA GCCCGTTGTTGG-3'	431	55.8
11	Chr11_6999575	Y	F	5'-GAATAAGGTGCGAGGAACA-3'	410	53.2
			R	5'-GCAAGCAGAGCAAGAAAGT-3'	410	52.9

**Note:** Y indicated the amplification was successful, N indicated the sequence which we amplified wasn't nearby the site we selected.

**Supplementary Table 9** Summary of structural variations in three comparisons.

		<b>No. of SVs</b>	<b>Total length (kb)</b>	<b>Average length (bp)</b>
<i>9311_TIGR</i>	Insertion	200935	1593	NA
	Deletion	202285	1660	NA
	Inversion	260	427	1643
<i>PA64s_TIGR</i>	Insertion	164570	1223	NA
	Deletion	161161	1321	NA
	Inversion	176	266	1514
<i>PA64s_9311</i>	Insertion	156397	1062	NA
	Deletion	149567	1018	NA
	Inversion	323	561	1738

**Note:** We aligned the updated *93-11* genome sequence and the newly assembly of *PA64s* against the reference sequence *Nipponbare*, and also aligned the *PA64s* genome against *93-11*.

**Supplementary Table 10** Summary of evidence for the GLEAN gene models. P: *ab initio* prediction; H: homology-based; C: cDNA/EST expressed genes. According to number of gene sources support, the evidence was further separated into single (with one gene source) and more (with two or more gene sources). The overlap threshold is relative to the CDS region of GLEAN genes.

	≥20% overlap		≥50% overlap		≥80% overlap	
	Number	Percent (%)	Number	Percent (%)	Number	Percent (%)
P (single)	80	0.22	709	1.92	3722	10.08
P (more)	7851	21.27	8989	24.35	9612	26.04
H (single)	1	0	15	0.04	100	0.27
H (more)	2	0.01	18	0.05	141	0.38
P+H	28972	78.5	27152	73.56	23048	62.45

**Supplementary Table 11** Summary of predicted protein-coding genes of *PA64s*.

Gene set		Number	Average transcript length (bp)	Average CDS length (bp)	Average exons number	Average exon length (bp)	Average intron length (bp)
<i>De novo</i> prediction	Augustus	38,079	2381.33	897.93	3.28	273.7	650.43
	Fgenesh	37,049	2716.2	1171.37	4.64	252.37	424.24
	Genscan	43,548	3627.82	712.3	2.97	239.73	1478.98
Homolog prediction	Maize	45,243	1654.59	790.87	2.95	267.88	442.39
	Rice	78,492	1141.91	585.29	2.5	234.08	371
	Sorghum	34,052	2293.47	989.38	3.69	268.19	484.96
Glean		36,909	2605.07	1110.2	4.1	270.55	481.67



**Supplementary Table 12** Functional annotation for *PA64s* genome based on different databases.

	<b>Number</b>	<b>Percent (%)</b>
Total	36909	-
Annotated	35845	97.12
Swissprot	23007	62.33
TrEMBL	35838	97.1
InterPro	25021	67.79
KEGG	17849	48.36
GO	18978	51.42
Unannotated	1064	2.88

**Supplementary Table 13** Summary of predicted protein-coding genes of *93-11*.

Gene set		Number	Average Transcript Length (bp)	Average CDS Length (bp)	Average Exons per Gene	Average Exon Length (bp)	Average Intron Length (bp)
<i>De novo</i> prediction	Augustus	41,991	2269.39	864.25	3.11	278.21	667.06
	Genscan	47,383	3606.9	711.96	2.99	238.23	1455.81
	Fgenesh	39,792	2644.45	1146.92	4.49	255.39	429
Homolog prediction	<i>O.sativa</i>	105,569	1126.62	583.92	2.38	245.44	393.54
	<i>Z.mays</i>	58,547	1698.25	824.81	2.81	293.91	483.55
	<i>S.bicolor</i>	40,514	2459.59	1036.77	3.58	289.75	551.86
EST prediction	<i>O.sativa</i>	51,401	12597.69	770.83	3.12	246.88	5572.68
GLEAN		40,464	2494.96	1070.08	3.98	269.18	478.9

**Supplementary Table 14** Twelve traits of *93-11* and *PA64s* observed in Hangzhou (HZ) and Hainan (HN).

Trait	<i>93-11</i> -HZ	<i>PA64s</i> -HZ	<i>93-11</i> -HN	<i>PA64s</i> -HN
HD (d)	92	85	100	95
PH (cm)	130.0 ± 3.0**	72.0 ± 2.0	122.2 ± 3.5**	69.5 ± 3.0
ETN	9.0 ± 1.7	12.3 ± 1.5	10.4 ± 1.0	12.9 ± 1.2
SN	214.0 ± 28.0**	193.0 ± 7.1	214.3 ± 21.9**	177.3 ± 17.0
PL (cm)	26.1 ± 0.1*	24.8 ± 0.6	25.6 ± 1.2*	24.0 ± 0.5
PPB	10.7 ± 0.6	11.0 ± 1.0	11.7 ± 0.6	12.0 ± 1.1
SPB	66.5 ± 2.1**	43.5 ± 2.1	55.3 ± 5.9**	39.5 ± 4.0
SS (%)	88.9 ± 2.9**	0.7 ± 0.4	95.8 ± 0.7**	4.1 ± 5.8
TGW (g)	30.80 ± 0.30**	3.41 ± 0.07	28.17 ± 0.12**	21.83 ± 0.40
GL (mm)	9.74 ± 0.28**	8.40 ± 0.22	9.46 ± 0.23**	8.75 ± 0.06
GW (mm)	2.64 ± 0.05**	2.44 ± 0.09	3.05 ± 0.07**	2.65 ± 0.12
PY (g)	29.53 ± 0.22**	0.00 ± 0.00	19.13 ± 0.50**	4.30 ± 0.42

Mean ±SD. (n=6).

\* and \*\* indicate the least significant difference at 0.05 and 0.01 probability level compared with *PA64s* in HZ or HN, respectively.

**Supplementary Table 15** Correlation analysis of 12 yield-associated traits in Hangzhou (HZ).

	HD-HZ	PH-HZ	ETN-HZ	SN-HZ	PL-HZ	PPB-HZ	SPB-HZ	SS-HZ	TGW-HZ	GL-HZ	GW-HZ
PH-HZ	<b>0.4656</b> (0.0001)										
ETN-HZ	-0.1598 (0.0683)	<b>-0.3001</b> (0.0005)									
SN-HZ	0.1883 (0.0313)	<b>0.4586</b> (0.0001)	-0.2265 (0.0093)								
PL-HZ	0.0203 (0.8181)	<b>0.5611</b> (0.0001)	<b>-0.3433</b> (0.0001)	<b>0.5056</b> (0.0001)							
PPB-HZ	<b>0.3816</b> (0.0001)	<b>0.2977</b> (0.0006)	-0.2112 (0.0155)	<b>0.5155</b> (0.0001)	<b>0.3349</b> (0.0001)						
SPB-HZ	0.1138 (0.1955)	<b>0.3460</b> (0.0001)	-0.1232 (0.1609)	<b>0.8436</b> (0.0001)	<b>0.4121</b> (0.0001)	<b>0.3847</b> (0.0001)					
SS-HZ	0.0436 (0.6211)	<b>0.4051</b> (0.0001)	-0.0343 (0.6975)	0.0679 (0.4392)	0.0955 (0.2762)	-0.0609 (0.4882)	0.0519 (0.5548)				
TGW-HZ	0.1154 (0.1893)	<b>0.3571</b> (0.0001)	-0.1664 (0.0575)	-0.1305 (0.1357)	0.2315 (0.0076)	0.0200 (0.8201)	-0.0733 (0.4037)	<b>0.2724</b> (0.0016)			
GL-HZ	-0.1166 (0.1847)	0.1871 (0.0323)	-0.1991 (0.0226)	-0.0852 (0.3315)	<b>0.3584</b> (0.0001)	-0.0541 (0.5382)	-0.0801 (0.3615)	0.0037 (0.9666)	<b>0.4086</b> (0.0001)		
GW-HZ	<b>0.3303</b> (0.0001)	0.1417 (0.1065)	-0.2049 (0.0189)	-0.0571 (0.5157)	-0.0191 (0.8278)	0.0506 (0.5646)	-0.0574 (0.5130)	-0.1185 (0.1760)	<b>0.4071</b> (0.0001)	-0.0760 (0.3868)	
PY-HZ	0.0691 (0.4331)	<b>0.5373</b> (0.0001)	0.0309 (0.7263)	<b>0.3844</b> (0.0001)	<b>0.2784</b> (0.0012)	0.0845 (0.3354)	<b>0.3521</b> (0.0001)	<b>0.6100</b> (0.0001)	<b>0.2506</b> (0.0038)	0.0456 (0.6034)	-0.0821 (0.3493)

**Note:** Pearson correlation coefficients (Prob > |R| under Ho:Rho=0/number of observations) with Prob > 0.005 are shown in bold.

**Supplementary Table 16** Correlation analysis of 12 yield-associated traits in Hainan (HN).

	HD-HN	PH-HN	ETN-HN	SN-HN	PL-HN	PPB-HN	SPB-HN	SS-HN	TGW-HN	GL-HN	GW-HN
PH-HN	-0.0276 (0.7639)										
ETN-HN	0.0693 (0.4717)	0.1683 (0.0761)									
SN-HN	0.2359 (0.0095)	0.2114 (0.0222)	0.1529 (0.1176)								
PL-HN	0.1259 (0.1707)	<b>0.2645</b> <b>(0.0040)</b>	0.1666 (0.0879)	<b>0.4976</b> <b>(0.0001)</b>							
PPB-HN	<b>0.4980</b> <b>(0.0001)</b>	0.0021 (0.9820)	0.0575 (0.5584)	<b>0.4305</b> <b>(0.0001)</b>	0.1699 (0.0636)						
SPB-HN	0.2379 (0.0089)	0.2368 (0.0102)	0.0419 (0.6697)	<b>0.6751</b> <b>(0.0001)</b>	<b>0.3364</b> <b>(0.0002)</b>	<b>0.4151</b> <b>(0.0001)</b>					
SS-HN	0.0176 (0.8664)	0.1291 (0.2052)	-0.0207 (0.8490)	0.0374 (0.7248)	0.2221 (0.0343)	-0.0457 (0.6674)	0.0885 (0.4040)				
TGW-HN	0.1330 (0.1476)	0.1560 (0.0931)	-0.0232 (0.8133)	-0.1281 (0.1652)	0.0823 (0.3739)	-0.0030 (0.9743)	-0.0880 (0.3411)	0.0608 (0.5670)			
GL-HN	-0.0159 (0.8614)	<b>0.2792</b> <b>(0.0014)</b>	-0.0005 (0.9954)	0.0109 (0.9062)	0.1955 (0.0332)	-0.1352 (0.1428)	-0.1033 (0.2635)	-0.0367 (0.7158)	<b>0.3757</b> <b>(0.0001)</b>		
GW-HN	0.0103 (0.9096)	0.1794 (0.0427)	-0.0562 (0.5524)	0.1093 (0.2369)	-0.0097 (0.9164)	0.0539 (0.5606)	0.0136 (0.8835)	0.0429 (0.6704)	0.1221 (0.1858)	0.0113 (0.8979)	
PY-HN	-0.0538 (0.5933)	0.2070 (0.0350)	-0.1338 (0.2062)	-0.0063 (0.9509)	0.0105 (0.9186)	-0.1399 (0.1717)	0.0291 (0.7775)	0.1618 (0.1517)	0.1068 (0.2977)	0.2600 (0.0074)	0.0817 (0.4075)

**Note:** Pearson correlation coefficients (Prob > |R| under Ho:Rho=0/number of observations) with Prob > 0.005 are shown in bold.

**Supplementary Table 17** Additive QTLs for twelve traits detected in RIL population derived from *93-11* and *PA64s* in Hainan and Hangzhou and reported QTLs or genes.

Trait	Site	QTL	Chr.	Pos (cM)	LOD	P value	PVE	SE	Reference/Gene
HD	HZ	<i>qHD8</i>	8	22.53-25.99	14.24	0.01	0.349	9.209	<i>DTH8</i>
HD	HN	<i>qHD8</i>	8	22.53-28.88	3.62	0.01	0.127	3.450	<i>DTH8</i>
HD	RD	<i>Qhd8</i>	8	7.91-27.53					43
HD	HZ	<i>qHD10</i>	10	22.9-36.15	4.73	0.01	0.096	4.828	
PH	HZ	<i>qPH2</i>	2	56.44-62.61	9.35	0.00	0.112	10.120	
PH	RD	<i>Ph2-1</i>	2	38.85-60.67					44
PH	HN	<i>qPH3</i>	3	30.84-38.16	5.07	0.01	0.158	7.144	
PH	RD	<i>qPHT-3</i>	3	25.47-51.21					45
PH	HZ	<i>qPH5</i>	5	89.61-100.48	4.89	0.00	0.065	7.737	<i>EU11</i>
PH	HZ	<i>qPH6</i>	6	55.66-61.48	4.66	0.01	0.096	9.217	
PH	RD	<i>qph6f</i>	6	55.47-66.62					46
PH	HZ	<i>qPH8</i>	8	28.88-32.95	13.58	0.01	0.268	15.357	
PH	HZ	<i>qPH11</i>	11	72.84-81.97	4.87	0.01	0.079	-8.360	
PH	HZ	<i>qPH12</i>	12	70.84-91.33	3.80	0.01	0.085	8.634	<i>NRL1</i>
PH	RD	<i>ph12.1</i>	12	67.36-105.43					47
ETN	HZ	<i>qETN4</i>	4	93.92-107.92	2.82	0.01	0.134	-1.613	<i>HTD1</i>
PL	HZ	<i>qPL2</i>	2	55.86-63.00	4.43	0.01	0.081	1.456	
PL	HZ	<i>qPL4</i>	4	107.15-113.50	5.10	0.01	0.093	1.557	
PL	RD	<i>qPLT4-1</i>	4	100.82-124.27					48
PL	HZ	<i>qPL5</i>	5	73.09-82.60	5.27	0.01	0.099	1.610	
PL	RD	<i>unnamed</i>	5	48.21-74.05					49
PL	HZ	<i>qPL11</i>	11	77.05-82.93	7.55	0.01	0.158	-2.026	
PL	RD	<i>unnamed</i>	11	66.67-82.16					50
PPB	HZ	<i>qPPB3</i>	3	2.55-12.60	4.00	0.01	0.094	-0.986	

PPB	HZ	<i>qPPB8</i>	8	23.10-28.30	6.03	0.01	0.153	1.258	
SPB	HZ	<i>qSPB1</i>	1	115.40-125.75	2.70	0.01	0.100	3.489	<i>LAX1</i>
SPB	RD	<i>qNSB1-1</i>	1	119.99-168.93					51
SN	HZ	<i>qSN8</i>	8	23.10-30.45	3.97	0.01	0.136	35.533	<i>DTH8</i>
SN	RD	<i>spp8a</i>	8	26.18-37.23					52
SN	HZ	<i>qSN9</i>	9	30.43-38.53	3.75	0.01	0.118	33.190	
SS	HZ	<i>qSS1</i>	1	152.06-163.73	3.23	0.01	0.248	0.176	
SS	RD	<i>qBSSR1</i>	1	132.47-175.17					53
SS	HZ	<i>qSS6</i>	6	61.67-67.40	3.08	0.03	0.091	0.107	
SS	HN	<i>qSS12</i>	12	75.69-90.37	3.36	0.01	0.252	0.159	<i>P/TMS12-1</i>
SS	RD	<i>qSSR12-1</i>	12	88.24-105.43					54
GL	HZ	<i>qGL1</i>	1	15.99-20.64	4.27	0.01	0.097	0.331	
GL	HN	<i>qGL1</i>	1	16.98-22.60	3.74	0.01	0.104	0.296	
GL	HZ	<i>qGL3</i>	3	47.67-55.91	4.98	0.01	0.113	0.358	<i>GS3</i>
GL	HN	<i>qGL3</i>	3	49.26-57.64	4.36	0.01	0.147	0.353	<i>GS3</i>
GL	RD	<i>qGL-3</i>	3	55.72-57.45					55
GL	HZ	<i>qGL6</i>	6	3.86-12.56	3.80	0.01	0.082	0.287	
GL	HN	<i>qGL6</i>	6	0.00-13.51	4.83	0.00	0.112	0.280	
GL	HZ	<i>qGL11</i>	11	74.18-82.16	4.30	0.01	0.096	-0.330	
GW	HZ	<i>qGW2</i>	2	137.85-142.71	3.83	0.01	0.124	0.108	
GW	HN	<i>qGW2</i>	2	136.89-142.71	5.40	0.01	0.140	0.123	
GW	RD	<i>qGW-2-3</i>	2	132.60-138.24					56
GW	HZ	<i>qGW4</i>	4	128.71-134.12	5.39	0.01	0.071	0.087	
GW	HN	<i>qGW4</i>	4	127.72-134.12	6.21	0.01	0.161	0.132	
GW	HZ	<i>qGW8</i>	8	16.27-28.49	5.41	0.01	0.072	0.088	
GW	HN	<i>qGW12</i>	12	97.16-104.66	3.00	0.01	0.082	-0.094	
TGW	HN	<i>qTGW1</i>	1	19.49-28.08	5.09	0.00	0.115	1.208	
TGW	RD	<i>gw1a</i>	1	17.74-26.90					57

TGW	HZ	<i>qTGW2</i>	2	137.85-145.60	3.51	0.01	0.085	1.799	
TGW	RD	<i>qTGW2-2</i>	2	138.24-156.90					58
TGW	HZ	<i>qTGW4</i>	4	130.43-134.12	6.41	0.01	0.163	2.487	
TGW	RD	<i>tgwt4</i>	4	124.27-134.12					59
PY	HZ	<i>qPY4</i>	4	12.95-22.97	5.05	0.01	0.108	5.846	
PY	HZ	<i>qPY6</i>	6	55.85-64.33	4.57	0.01	0.125	6.290	
PY	RD	<i>unnamed</i>	6	56.62-66.62					60
PY	HZ	<i>qPY8</i>	8	25.99-31.61	4.53	0.01	0.096	5.519	
PY	HZ	<i>qPY12</i>	12	72.76-91.33	3.05	0.01	0.084	5.152	

**Note:** PVE is the percentage of phenotypic variation explained by the detected QTL; SE is substitution effect.

#### References for Supplementary Table 17

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**Supplementary Table 18** Primers of SNP and InDel markers for fine mapping.

Marker	Forward primer	Reverse primer	Tm (°C)	Product size (bp)
SNP8-45	5'-TTGTA GTC TTTTATA GTTAGG TTC-3'	5'-TAA GTGATGAAA GAA CA GTCG-3'	50	543
SNP8-47	5'-TCCTACA CACA CCAATAGACGGG-3'	5'-TGATGGAA GAAAAATCTGGCA TC-3'	60	594
SNP1	5'-AAGTCTCCGCTCTAATTGAAAC-3'	5'-TCTGTAGGAGTTCGGTTGTG-3'	53	218
IND1	5'-ACTTGGTTGTTTGCATTACATG-3'	5'-ACAA GTGTATCTTTATTTATTTGTGC-3'	55	83/75*
SNP1-308	5'-TATCAA CTGGCTGACCACTG-3'	5'-ATACATCAAATTTTGCTTGGATTC-3'	58	532
SNP1-309	5'-TCGCTATCATTGGCTGTCATC-3'	5'-TTGACTTCA CAATATCTCCAA GAG-3'	55	660
SNP2	5'-ACCCTGGTCTTAGGTGGGTTG-3'	5'-ACAA GACGAATCA CCGCAAAAG-3'	60	401
SNP3	5'-ATATCATCTGAAATCTGAACTTG-3'	5'-TACGTGAGCTACCATCCTTG-3'	53	365
IND2	5'-TCCGCTTTGTCCCTGAGTGTTTC-3'	5'-ACCCTTGCAAAAAGCGTTCCA G-3'	65	98/106*

\*93-11/PA64s

**Supplementary Table 19** Primers used for real time PCR of candidate genes.

Marker	Forward primer	Reverse primer	Tm (°C)	Product size (bp)
<i>GS3</i>	5'-ATGCTCGTGCCGCTGCA CC-3'	5'-ACAAGCA GGGGGGCA GCAAC-3'	60	110
<i>EU11</i>	5'-ACGGTGGGGATGGTGGTGC-3'	5'-ACGAACA GGTA GGTGCCCTTGG-3'	60	125
<i>DTH8</i>	5'-TCGAGGGTGATGGAGTTCG-3'	5'-TTCCTCTT TAGTCTAAACCATTGC-3'	58	122
<i>HTDI</i>	5'-TGGTGCTGGATGCAAA GAAGATAG-3'	5'-ACCATGGAATCCCATTGGAAAAG-3'	55	95
<i>LAX1</i>	5'-TCCTCAAGGC GCA GGT CAC-3'	5'-GCCATCTCCA GCGTCGTC-3'	58	177
<i>NRL1</i>	5'-AAGAGGGACTTCCTCAAGAACAAG-3'	5'-TCGTA CT CGCGCTTCA CCTT-3'	60	74
<i>OsACTIN1</i>	5'-ACCCCTGCTATGTACGTCGCCATC-3'	5'-ACCATCACCA GA GTCCAACACAATACC-3'	60	90

**Supplementary Table 20** Phenotype of T<sub>1</sub> plants transformed with pCAMBIA1300-*DTH8*.

Trait	PA64s	T <sub>1</sub> -1	T <sub>1</sub> -2	T <sub>1</sub> -3
HD (d)	85	94	99	94
PH (cm)	72.0 ± 2.0	90.0 ± 7.5	122.0 ± 1.4	124.3 ± 2.1
SN	193.0 ± 7.1	210.0 ± 8.2	233.7 ± 12.5	225.7 ± 7.8

Mean ±SD. (n=6).

**Supplementary Table 21** Regions shared by QTLs and recombinant hotspots.

Site	QTL/recombinant hot spot	Chr.	Pos (cM)
HZ	<i>qSPB1</i>	1	115.40-125.75
	RH1-27	1	
	RH1-28	1	
	RH1-29	1	
	RH1-30	1	
HZ	<i>qPPB3</i>	3	2.55-12.60
	RH3-1	3	
	RH3-2	3	
HZ	<i>qGL3</i>	3	47.67-55.91
HN	<i>qGL3</i>	3	49.26-57.64
	RH3-13	3	
	RH3-14	3	
	RH3-15	3	
HZ	<i>qPY4</i>	4	12.95-22.97
	RH4-7	4	
	RH4-8	4	
	RH4-9	4	
	RH4-10	4	
	RH4-11	4	
HZ	<i>qPL4</i>	4	107.15-113.50
	RH4-26	4	
	RH4-27	4	
HN	<i>qGW4</i>	4	127.72-134.12
HZ	<i>qGW4</i>	4	128.71-134.12
	RH4-31	4	
	RH4-32	4	
	RH4-33	4	
HZ	<i>qPH5</i>	5	89.61-100.48
	RH5-28	5	
	RH5-29	5	
HN	<i>qGL6</i>	6	0.00-13.51
HZ	<i>qGL6</i>	6	3.86-12.56
	RH6-1	6	
	RH6-2	6	
	RH6-3	6	
	RH6-4	6	
HZ	<i>qPH6</i>	6	55.66-61.48
HZ	<i>qPY6</i>	6	55.85-64.33
	RH6-19	6	
	RH6-20	6	

HZ	<i>qSS6</i>	6	61.67-67.40
	RH6-21	6	
HZ	<i>qGW7</i>	7	13.86-18.46
	RH7-2	7	
HZ	<i>qPH8</i>	8	28.88-32.95
	RH8-3	8	
HZ	<i>qSN9</i>	9	30.43-38.53
HZ	<i>qSPB9</i>	9	31.01-38.34
	RH9-12	9	
HZ	<i>qHD10</i>	10	22.9-36.15
	RH10-6	10	
	RH10-7	10	
	RH10-8	10	
	RH10-9	10	
	RH10-10	10	
	RH10-11	10	
HZ	<i>qPH11</i>	11	72.84-81.97
	RH11-21	11	
	RH11-22	11	
HZ	<i>qPY11</i>	11	74.18-81.97
HZ	<i>qGL11</i>	11	74.18-82.16
HN	<i>qGL11</i>	11	75.90-82.35
HZ	<i>qPL11</i>	11	77.05-82.93
	RH11-23	11	
	RH11-24	11	
HN	<i>qPH12-1</i>	12	40.14-54.05
	RH12-6	12	
	RH12-7	12	
	RH12-8	12	
HZ	<i>qPH12-2</i>	12	70.84-91.33
HZ	<i>qPY12</i>	12	72.76-91.33
HN	<i>qSS12</i>	12	75.69-90.37
HZ	<i>qSS12</i>	12	78.16-90.37
	RH12-13	12	
	RH12-14	12	

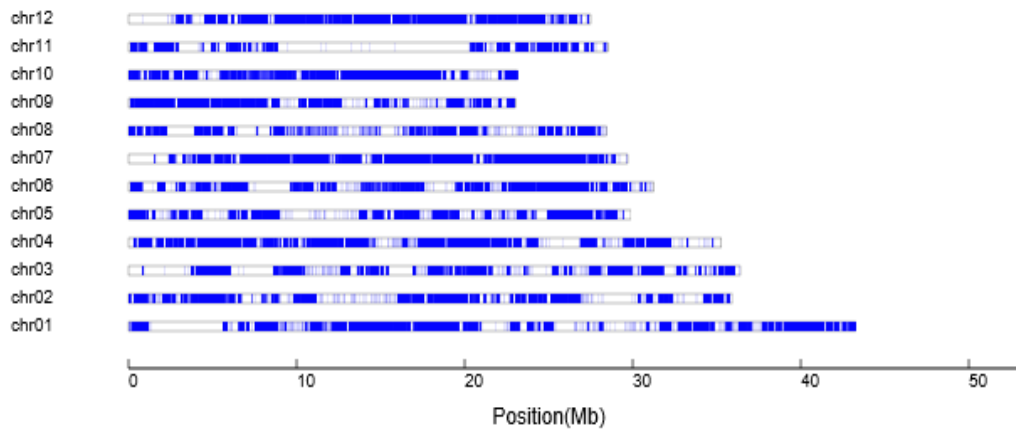
RH: Recombinant hotspots.

## Supplementary Figures

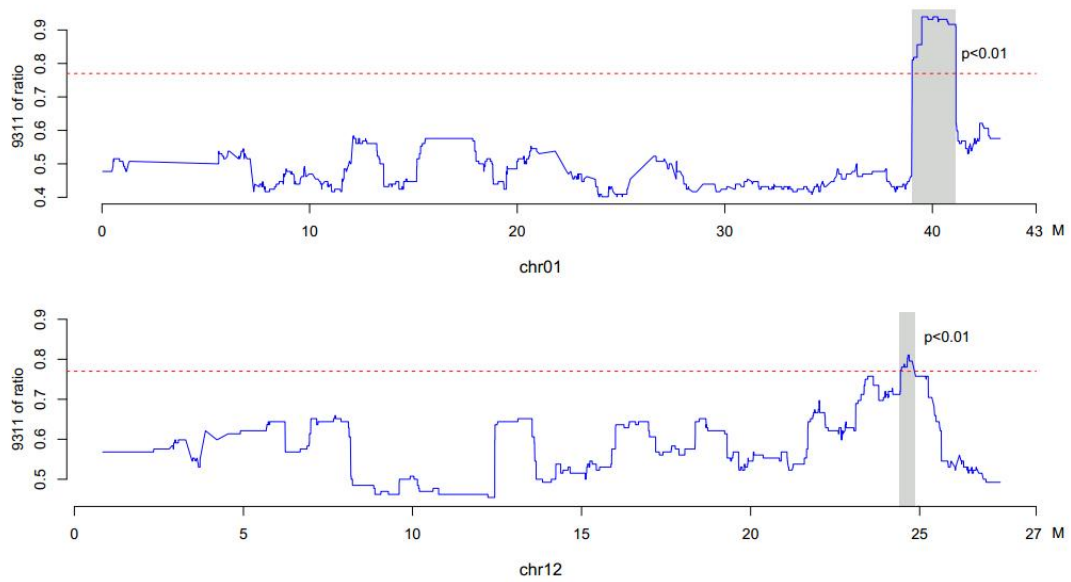
- **Supplementary Figure 1** Physical positions of SNP markers on each chromosome.
- **Supplementary Figure 2** Two major distortion regions on chromosome 1 and chromosome 12.
- **Supplementary Figure 3** Distribution of recombinant hotspots across 12 chromosomes in the RILs.
- **Supplementary Figure 4** Top enriched DNA motifs identified in sequences surround hotspots.
- **Supplementary Figure 5** Genetic versus physical distance maps of 12 chromosomes. The genetic distance was calculated depending on physical distance and recombination rate using *Kosambi* function. Red triangles point to the positions of centromere on each chromosome.
- **Supplementary Figure 6** An example of falsely anchored scaffolds revealed by linkage map and syntenic analysis. There the region of falsely anchored scaffolds disturbed the normal linkage relationships in the graphic map of genotypes and was not supported by normal syntenic analysis with the reference.
- **Supplementary Figure 7** Synteny analysis among the three genomes: *PA64*, *93-11* and *Nipponbare*: (a) *93-11* vs. *PA64s* (b) *Nipponbare* vs. *PA64s* (c) *93-11* vs. *Nipponbare*.
- **Supplementary Figure 8** Comparative analysis of InDels among three genomes.
- **Supplementary Figure 9** The Venn diagram shows common and unique gene families in *93-11*, *PA64s* and *Nipponbare*. The number of genes belonging to the corresponding families is presented in brackets under the number of families.
- **Supplementary Figure 10** KEGG enrichment of special genes in *93-11* and *PA64s*.
- **Supplementary Figure 11** Distribution of twelve yield component traits of RIL derived from *93-11* and *PA64s* in Hangzhou (HZ) and Hainan (HN). Arrow head represents the trait value of *93-11* or *PA64s*.
- **Supplementary Figure 12** Seven yield-associated traits and five QTL genotypes of four RIL lines and two parents. Abbreviations are indicated as following: heading date (HD), spikelet number per panicle (SN), plant height (PH), effective tiller number (ETN), seed set (SS), 1000-grain weight (TGW), and yield per plant (PY). Bar represents S.D. Five QTLs (*qHD8/qSN8*, *qPH5*, *qETN4*, *qSS12*) cover the loci of cloned candidate genes (*DTH8*, *EU11*, *HTD1*, *P/TMS12-1*), respectively. P represents *PA64s* genotype and 9 for *93-11* genotype.
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positions of candidate genes.

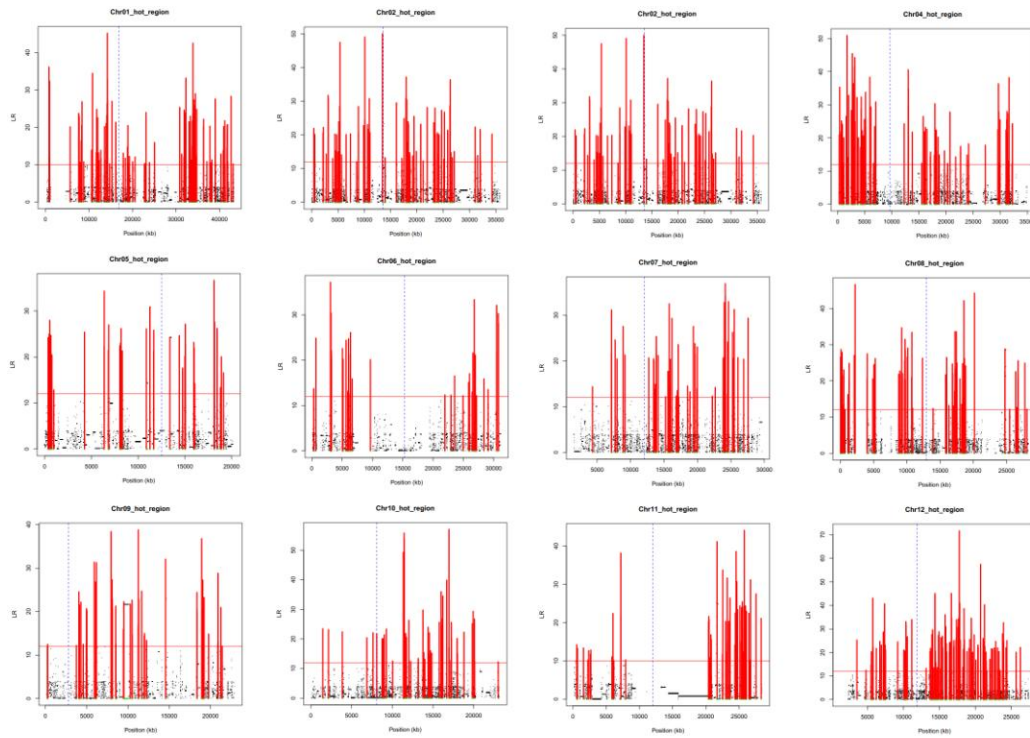
- **Supplementary Figure 14** Expression analysis of candidate genes in *93-11* and *PA64s*. Total RNA was isolated from panicles (for *GS3*, *DTH8*, and *LAX1*) and first internodes (for *EU11* and *NRL1*) in the booting stage, and tiller buds (for *HTD1*) at the tillering stage. The transcript levels of these genes were normalized against those of *OsACTIN1* gene. Values are the mean  $\pm$  S.D. of three replicates.



**Supplementary Figure 1** Physical positions of SNP markers on each chromosome.



**Supplementary Figure 2** Two major distortion regions on chromosome 1 and chromosome 12.

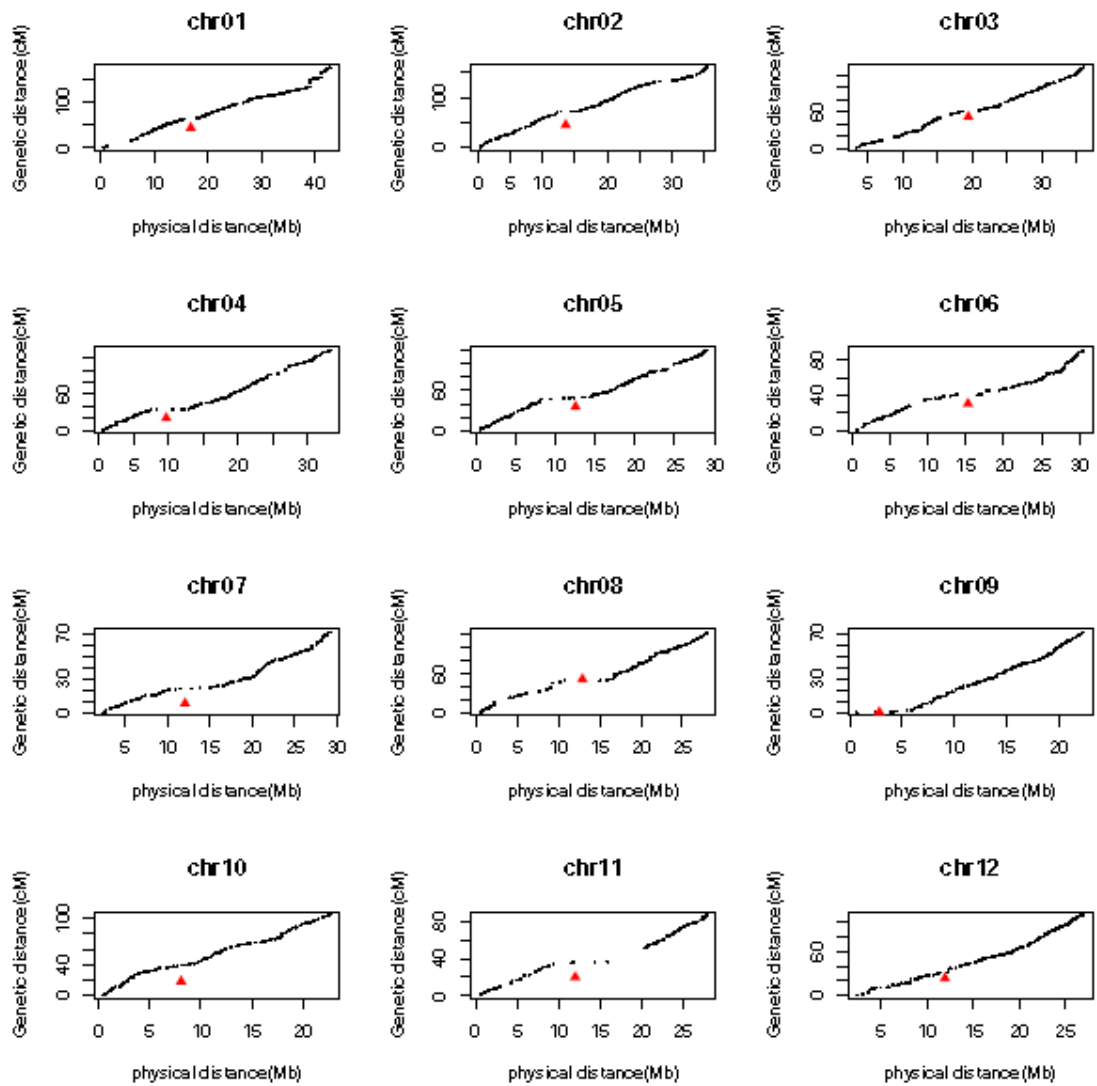


**Supplementary Figure 3** Distribution of recombinant hotspots across 12 chromosomes in the RILs.

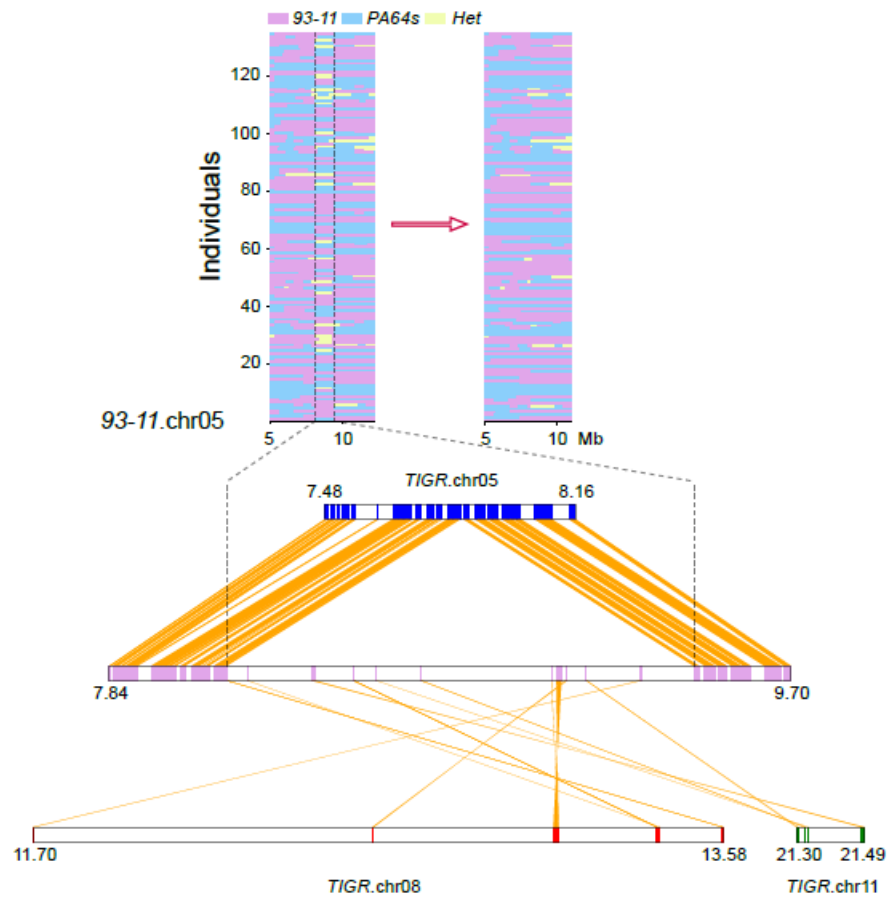
Sequence	E-value	Presence	Sequence	E-value	Presence
	1.1e-590	210		4.8e-362	150
	1.8e-354	153		4.8e-298	257
	1.4e-296	250		3.8e-275	263
	7.3e-239	211		9.5e-217	269
	7.3e-207	217		4.7e-190	60

**Supplementary Figure 4** Top enriched DNA motifs identified in sequences surround hotspots.

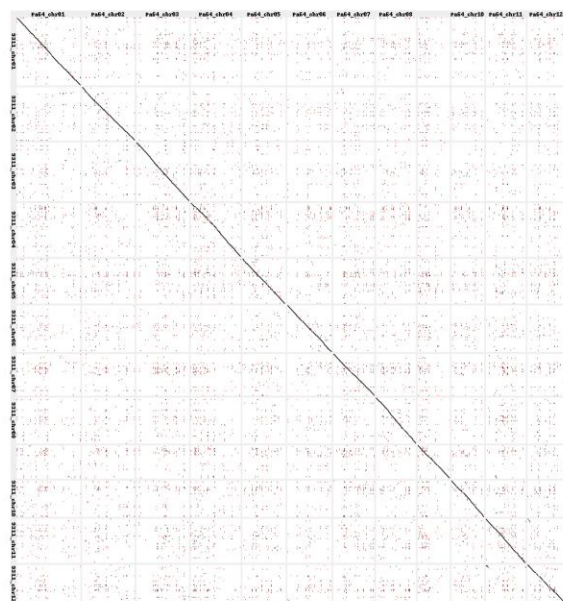




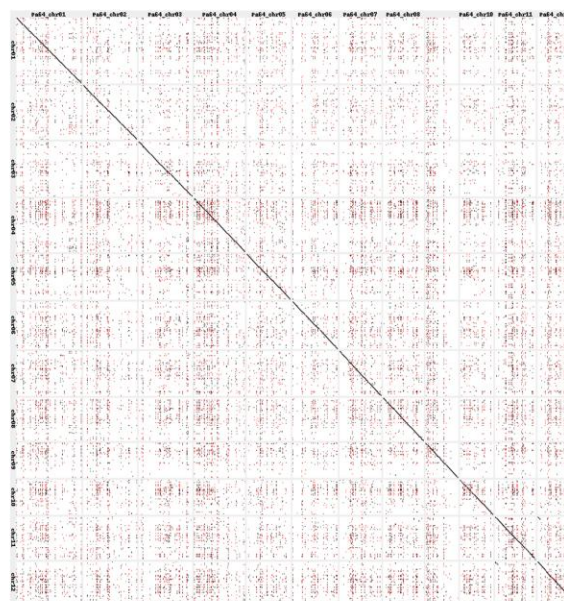
**Supplementary Figure 5** Genetic versus physical distance maps of 12 chromosomes. The genetic distance was calculated depending on physical distance and recombination rate using *Kosambi* function. Red triangles point to the positions of centromere on each chromosome.



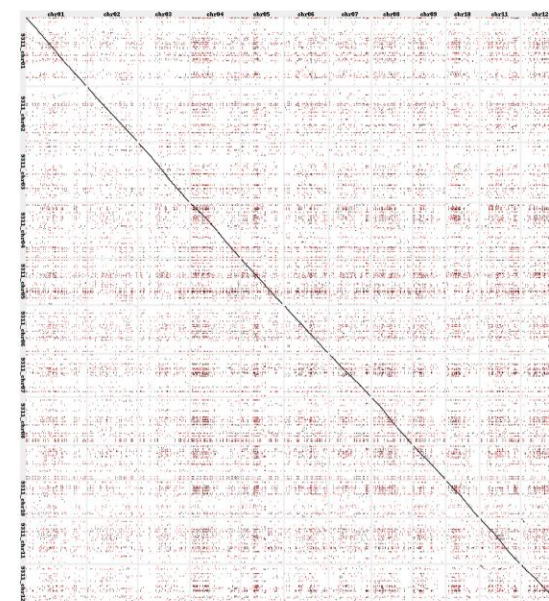
**Supplementary Figure 6** An example of falsely anchored scaffolds revealed by linkage map and syntenic analysis. The region of falsely anchored scaffolds disturbed the normal linkage relationships in the graphic map of genotypes and was not supported by normal syntenic analysis with the reference.



(a)

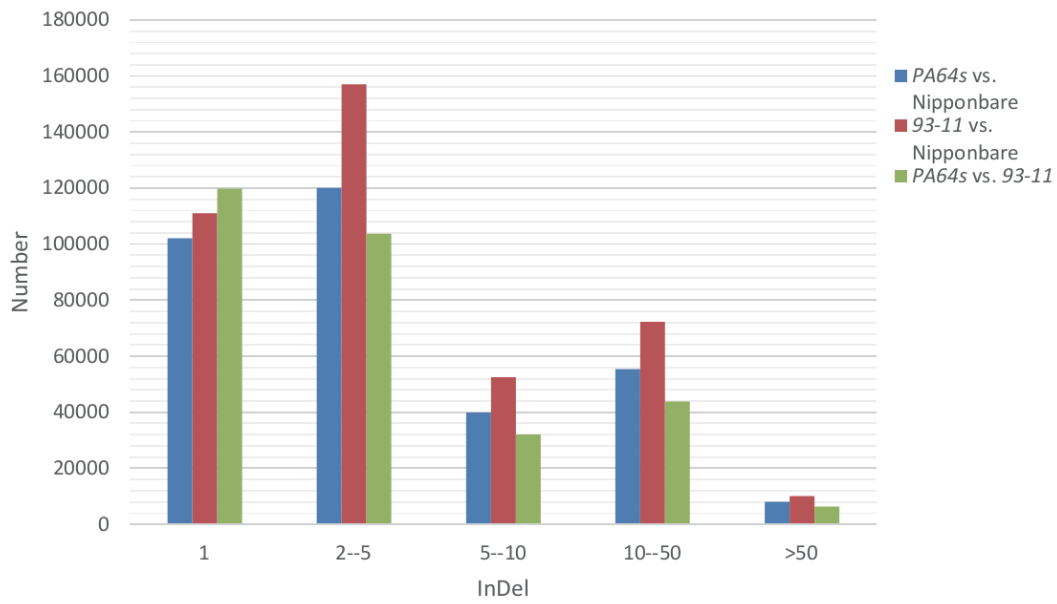


(b)

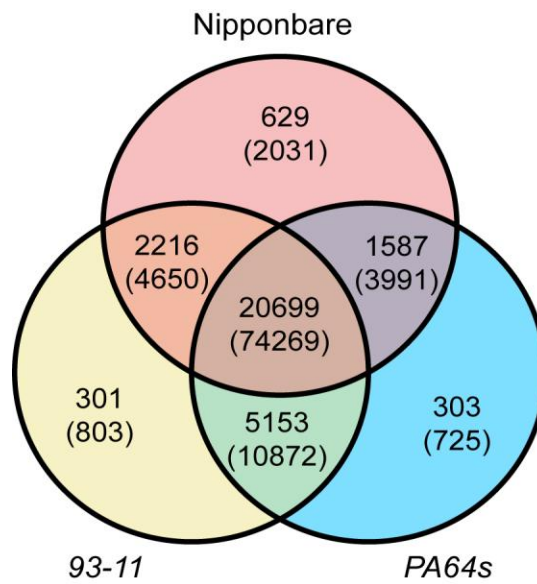


(c)

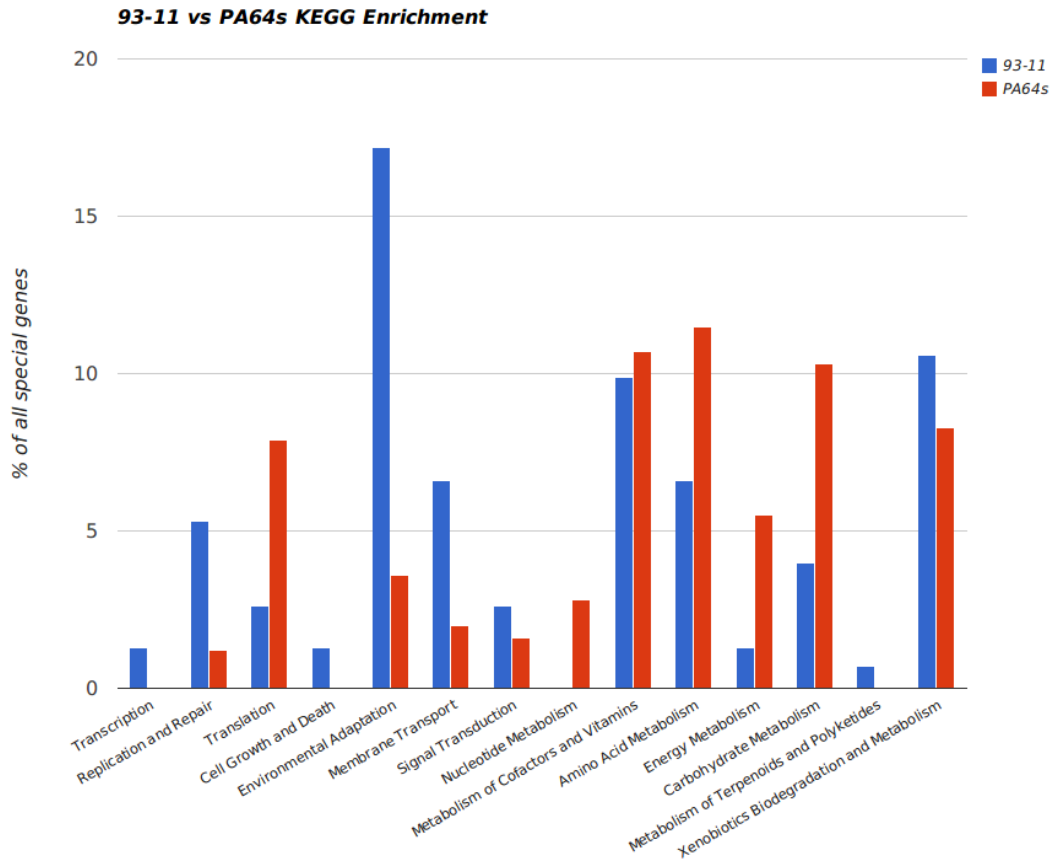
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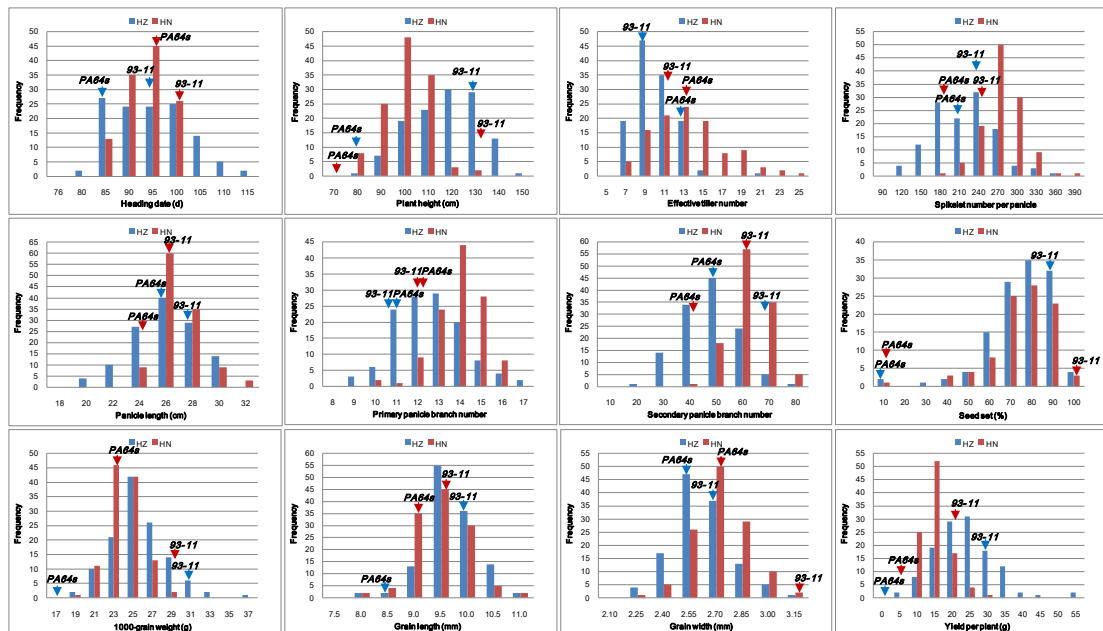
**Supplementary Figure 8** Comparative analysis of InDels among three genomes.



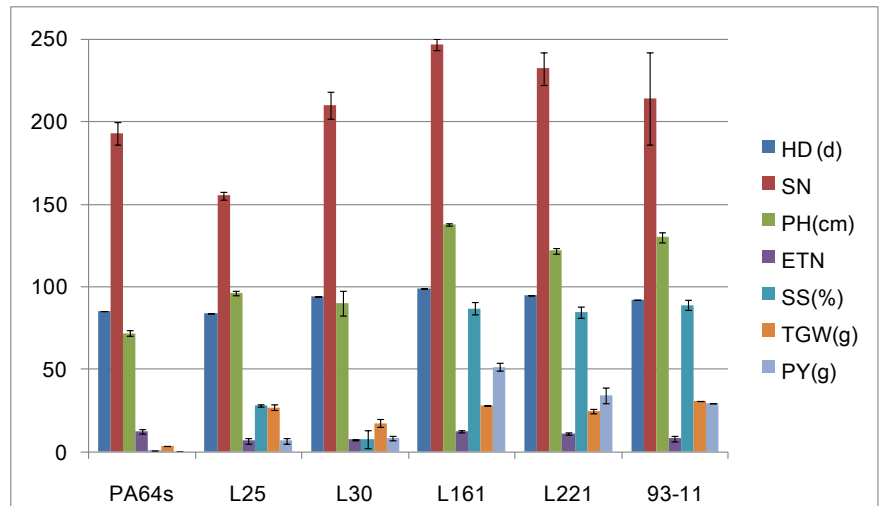
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Supplementary Figure 10 KEGG enrichment of special genes in 93-11 and PA64s.

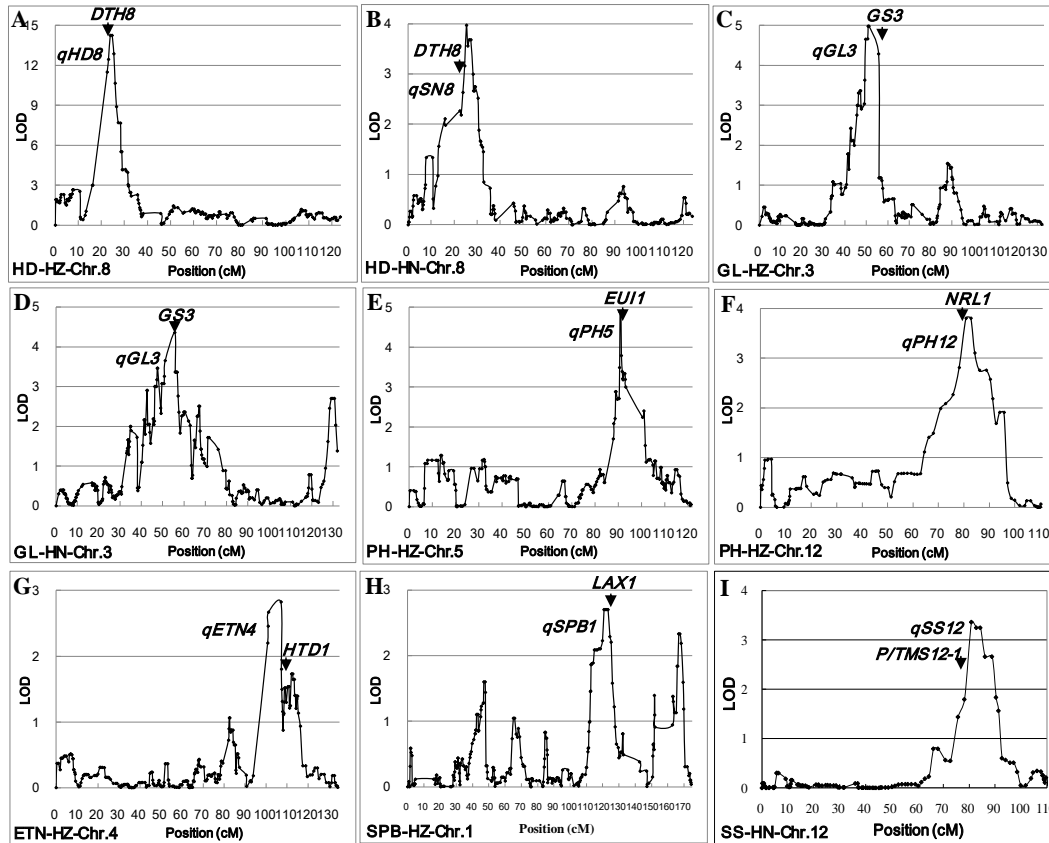


Supplementary Figure 11 Distribution of twelve yield component traits of RIL derived from 93-11 and PA64s in Hangzhou (HZ) and Hainan (HN). Arrow head represents the trait value of 93-11 or PA64s.

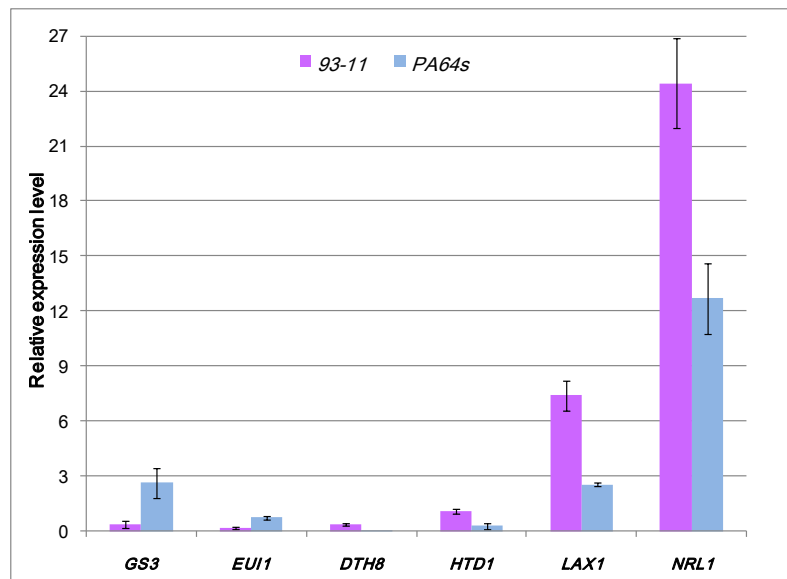


QTL	PA64s	L25	L30	L161	L221	93-11	Candidate gene
<i>qHD8/qSN8</i>	P	P	9	9	9	9	<i>DTH8</i>
<i>qPH5</i>	P	9	P	9	P	9	<i>EUI1</i>
<i>qETN4</i>	P	P	9	P	P	9	<i>HTD1</i>
<i>qSS12</i>	P	P	P	9	9	9	<i>P/TMS12-1</i>
<i>qTGW4</i>	P	9	P	9	9	9	

**Supplementary Figure 12** Seven yield-associated traits and five QTL genotypes of four RIL lines and two parents. Abbreviations are indicated as following: heading date (HD), spikelet number per panicle (SN), plant height (PH), effective tiller number (ETN), seed set (SS), 1000-grain weight (TGW), and yield per plant (PY). Bar represents S.D. Five QTLs (*qHD8/qSN8*, *qPH5*, *qETN4*, *qSS12*) cover the loci of cloned candidate genes (*DTH8*, *EUI1*, *HTD1*, *P/TMS12-1*), respectively. P represents *PA64s* genotype and 9 for *93-11* genotype.



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