Rice Gene Index (RGI): a comprehensive pan-genome database for comparative and functional genomics of Asian rice

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Abstract

To integrate the genomic information of the rice pan-genome, we performed comparative analyses and established a user-friendly Rice Gene Index (RGI, https://riceome.hzau.edu.cn) platform with 16 platinum standard reference genomes and supplementary transcriptome data. To logically organize and scientifically the index of 744,233 genes among rice accessions, we detected 112,658 Ortholog Gene Indices, and provide 'GeneCard' pages to query genomic, transcriptomic, and homology information for each gene. The RGI allows users to search for relationships and comprehensive information of genes in keyword-based, sequence-based, and relationship-based ways. Furthermore, users can visualize these relationships at local and global scales corresponding to 'Microcollinearity' and 'Macrocollinearity' modules.

Keywords

Rice; Pan-genome; Homologs; Index; Visualization

Background

Asian rice (*Oryza sativa*) is the staple food for half the world and is a model crop that has been extensively studied. It contributes ~20% of calories to the human diet (Stein et al., 2018). With the increase in global population and rapid changes in climate, rice breeders need to develop new and sustainable cultivars with higher yields, healthier grains, and reduced environmental footprints (Wing et al., 2018). Since the first gold standard reference genome of rice variety Nipponbare has been published (International Rice Genome Sequencing Project., 2005), an increasing number of rice accessions have been sequenced, assembled, and annotated with global efforts. Nowadays, a single reference genome is obviously insufficient to perform the genetic difference analysis for rice accessions (Huang et al., 2021). Therefore, the pan-genome has been proposed as a solution, which allows the discovery of more presence-absence variants, as compared with single reference genome-based studies (Zhao et al., 2018). Over the past years, several databases, such as RAP-db (https://rapdb.dna.affrc.go.jp), RGAP (http://rice.uga.edu), and Gramene (https://www.gramene.org), have long-term served rice genomic research by providing information based on one or a series of individual reference genomes. To integrate and utilize the genomic information of multiple accessions, we performed comparative analyses and established a user-friendly Rice Gene Index (RGI, https://riceome.hzau.edu.cn) platform. RGI is the first gene-based pan-genome database for rice.

Data collection

To set up a solid foundation for this database, we selected 16 platinum standard reference genomes of rice accessions that represent the major Asian rice subpopulations when K=15 (Song et al., 2021; Stein *et al.*, 2018; Zhou et al., 2020), (Figure 1A). Starting with a set of unified *de novo* annotations performed by Gramene (unpublished) of 14 genomes and 4 published annotations including Minghui 63 (MH63), Zhenshan 97 (ZS97), and Nipponbare (RGAP and RAP-db) (Kawahara et al., 2013; Sakai et al., 2013)), we incrementally integrated the genes and transcripts identified by newly sequenced Iso-Seq data into the Gramene annotation results, as the basics to build homology relationships between 18 annotations. (Supplemental Table 1) In addition, a series of Iso-Seq and RNA-Seq data of multiple tissues from selected accessions (Supplemental Table 2) were collected and fully presented as baseline information in RGI, which included gene expression, full-length transcripts, and alternative splicing (AS) events. Details on data processing are described in supplemental methods.

Result

As the primary datasets in RGI, the genome annotations of 16 rice accessions contained an average of 41,346 genes, of which an average of 1,178 genes are supplemented by Iso-Seq data (Supplementary Table 3). The GeneTribe pipeline (Chen et al., 2020) identified an average of 33,350 gene pairs between annotations (Supplemental Figure 2), which classified ‘reciprocal best hits’ (RBHs), ‘single-side best hits’ (SBHs), ‘1-to-many’ hits or ‘singleton’ hits. By counting unique homolog gene groups, a total of 119,783 non-redundant gene groups were determined to represent the whole Asian rice gene set. To further unify the gene groups in *Oryza sativa*, we defined a unified and sustainable number — Ortholog Gene Index (OGI), which is a homolog group clustered by connected graph methods based on RBH relationships, with an updatable score that indicates its representativeness in all accessions. Of the 112,658 Ortholog Gene Indices, we classified them into 21,418 OGI core genes (19.01% of OGI) appearing in all rice accessions, 40,141 OGI dispensable genes, and 51,099 OGI accession-specific genes (Supplemental Figure 1A). And we found that the specific genes are younger and shorter (T-test, $p = 2e\text{-}16$) than core genes (Supplemental Information 1).

Utility

The first objective of RGI is to logically organize and scientifically index all genes among rice accessions. RGI provides ‘GeneCard’ to show comprehensive information for individual genes with convenient links to other modules and outside databases on one page (Figure 1C). By entering a gene ID of rice, through the search box on the homepage, users may browse the ‘GeneCard’ page on 3 sections: 1) Basic information includes sequence, gene function, gene expression, links for accessing various modules and other databases, etc. (Supplemental Figure 4A). 2) ‘Transcripts’ exhibits graph and table of transcripts structure. In addition to the baseline expression analysis of all genes, 116,640 AS events at the transcriptome level were extensively revealed by the analysis of different groups (Supplemental Figure 4B, Supplemental Table 4). For example, two AS events were detected for *OsNiR* (OsNip_01g0357100), a critical gene that encodes nitrite reductase in nitrogen assimilation (Yu et al., 2021) (Figure 1D). Additionally, ‘Homologues’ lists all associated homologs of a gene across annotations through links graph and table ways. This section also shows the phylogenetic tree. Furthermore, RGI provides informative pages to show the association graph of genes in each Ortholog Gene Index (Supplemental Figure 4C).

Second, RGI provides three ways to search for relationships and comprehensive information for genes.

(1) Through keyword-based searches, users can easily search OGI#, gene ID, gene symbol, Gene Ontology, or functional terms in the query box. If users search

the famous gene *SD1* in RGI, 306 items will be returned with basic information, which could link to other modules or databases.

(2) In the way of sequence-based searches, the classical 'BLAST' tool allows users to query amino acid or nucleotide sequences in sequence databases of the whole genome and protein. To easily access other modules, the tool returns gene ID linking to 'GeneCard' or chromosome location linking to 'JBrowse' when using the protein or nucleotide database, respectively.

(3) For association-based searches, the 'Homologues' module allows users to query and connect the homologous genes through a given gene ID, which may obtain the homology relationship among annotations then build a homologous gene tree and display gene structures (Figure 1F), as well as multiple sequence alignments and positions on the chromosome of these homologs. For example, *OsTPP7* (LOC_Os09g20390), an anaerobic germination tolerance gene, was found to be absent in IR64 but present in other accessions by 'Homologues' (Supplemental Table 5) and manually verified the results. It indicates that IR64 has less tolerance to anaerobic germination (Yang et al., 2019).

Third, RGI can visualize the relationship of these annotated genes across accessions at local and global scales corresponding to two modules as follows:

(1) At the local scale, the 'MicroCollinearity' module enables users to demonstrate genomic collinearities of a gene and its flanking genes in selected accessions (Figure 1E). The homologous relations among genomes help to investigate gene-based variations in the local regions of multiple accessions. Many genes encoding nucleotide-binding site leucine-rich repeat (NLR) proteins are found in the region close to the end of rice chromosome 11 long arm (Supplemental Figure 5) (Song *et al.*, 2021), the collinearity comparison results detected by this module show that these NLR genes are significantly more abundant in MH63 than in other accessions, which potentially contribute to MH63's superior resistance to rice diseases.

(2) At the global scale, 'MacroCollinearity' helps users to explore collinearity between accessions and study rearrangements of rice genome at the whole-

chromosome level. With this module, structure variations may be easily detected and the interactive tool 'Dot Plot' was embedded to show the collinearity details and links to associated genome loci on 'JBrowse'. (Figure 1G). A useful module 'GenePair' is provided to visualize collinearity comparisons of ortholog gene pairs between two accessions on both global and local scales.

All information mentioned above is logically organized and seamlessly integrated by modules and tools in RGI. Four extra modules ('JBrowse' (Figure 1I), 'GOEnrichment' (Figure 1H), 'GeneDescription', and 'Download') were additionally integrated to enhance RGI's serviceability (Supplemental Information 2). The technical details on RGI construction of RGI are described in Supplemental Information 3.

Discussion

Although more than 100 chromosomal-level genomes of Asian rice have been published, most of the relevant databases focus on single genomes for specific domains (e.g., LncRNA, epigenomic, etc.) (Copetti et al., 2015; Xie et al., 2021; Zhang et al., 2021). Two 'pan-genome' databases have been published (i.e. RPAN (https://cgm.sjtu.edu.cn/3kricedb/index.php) provides data on individual rice accessions, and Rice RC (http://ricerc.sicau.edu.cn/RiceRC) has a focus on structure variants), while our RGI comprehensively creates and focuses on gene-level relationships across representative Asian rice accessions, establishes a standardized gene index for Asian rice, and provides richer search and visualization capabilities for the whole rice research community.

DECLARATIONS

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in https://riceome.hzau.edu.cn/. PacBio Iso-Seq raw data are available from NCBI under BioProject PRJNA760839. The RNA-Seq raw data are available from NCBI under BioProject PRJNA659864 and PRJNA5970706.

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Authors' contributions

R.A.W., W.G. and J.Z. designed and conceived the research. K.L.M. provided SSD seed, extracted DNA and RNA for genome and transcript sequencing (both by PacBio and Illumina) for 12 Asian rice. Z.Y., Y.Z., YU.Z. and M.L. performed the homologues and transcriptome analysis. Z.Y. and Y.C. built the database and managed the computing platforms. Z.Y., Y.C., Y.Z., Y.O., D.C., R.M., H.Z., W.X., K.L.M., R.A.W., W.G. and J.Z. wrote and edited the paper. All authors read and approved the final manuscript.

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Supplemental Information

See the Supplementary Information.

Figures

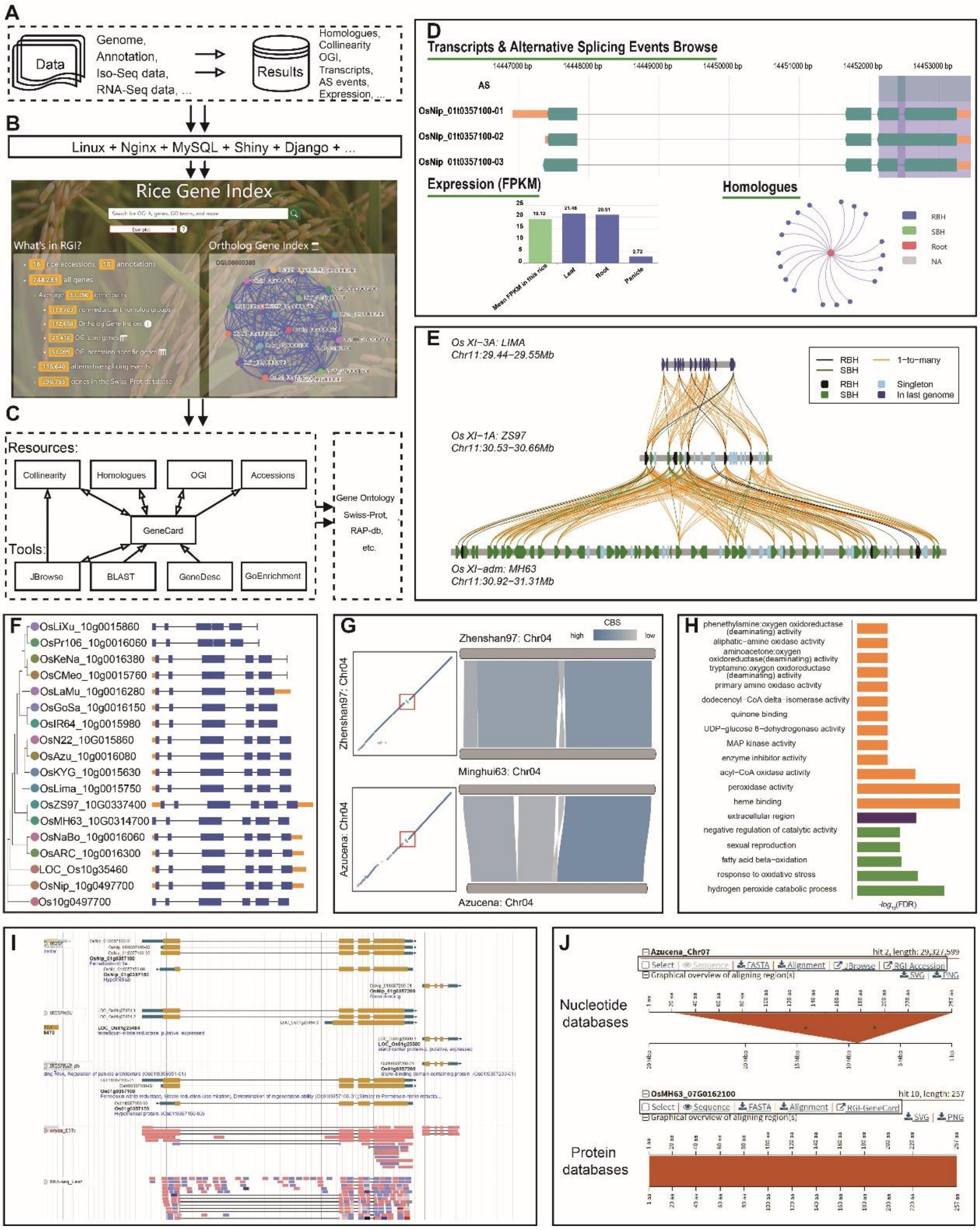
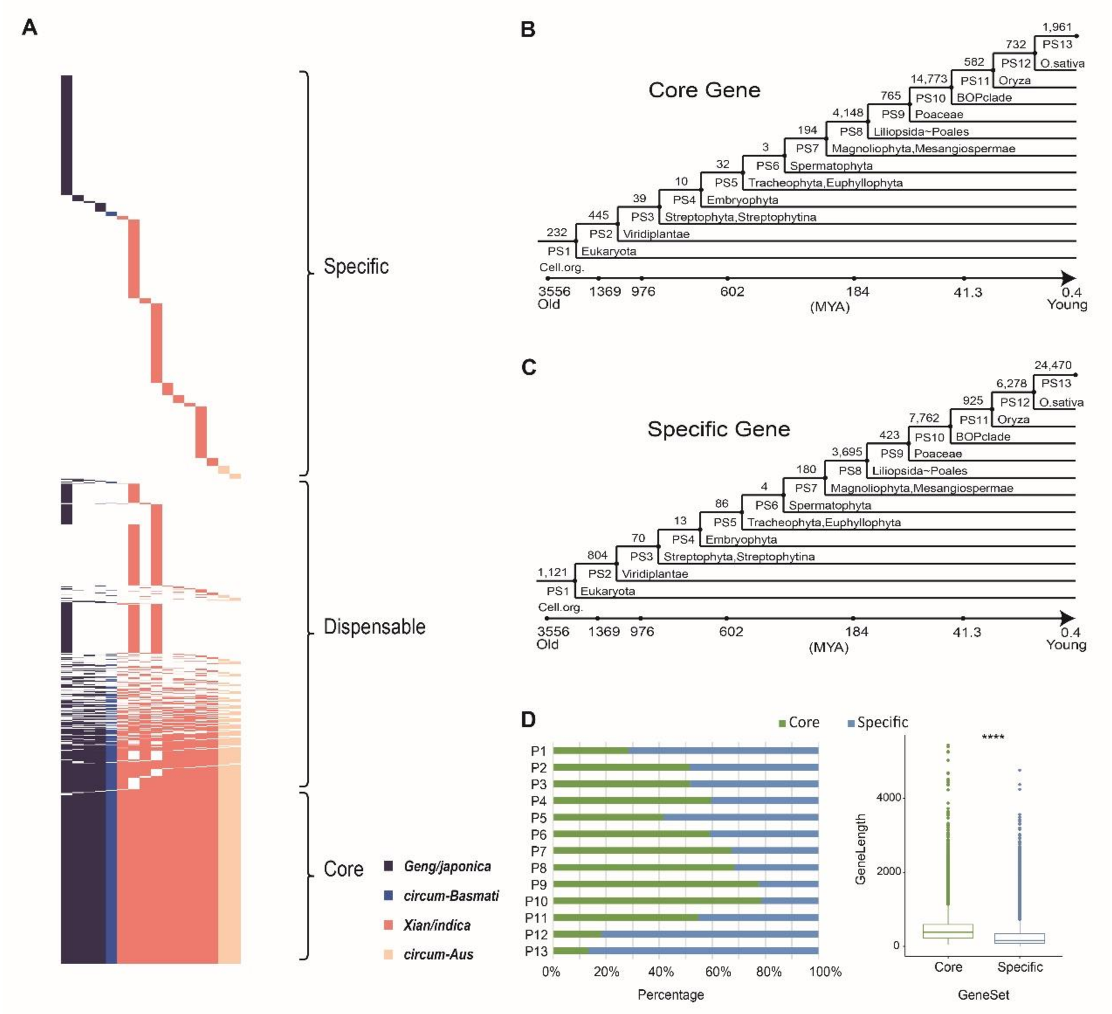
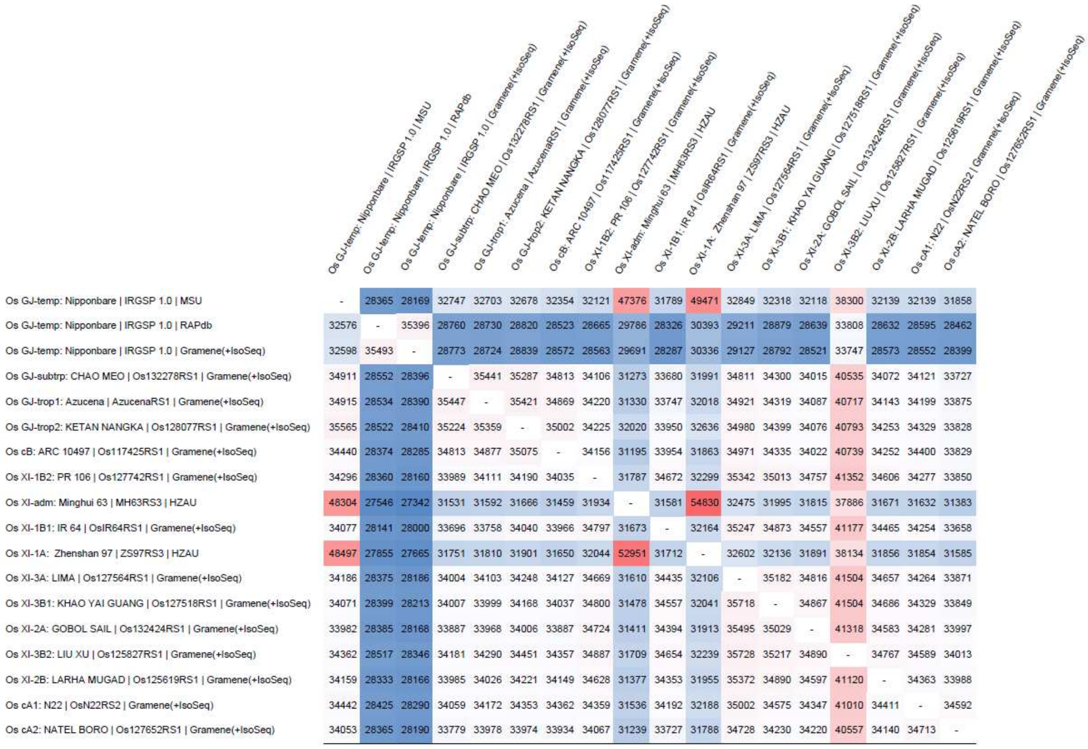


Figure 1. Design and online output example of RGI.

(A-C) Overview of the construction of the Rice Gene Index (RGI). (A) The data layer demonstrates the source data and results provided in RGI. (B) The middleware layer shows the tools used to build the RGI website. (C) The architecture of RGI shows functions and tools. 'GeneCard' is a central module in RGI, which links to other modules via a network. (D) 'GeneCard' page shows *OsNiR*'s transcripts structure, AS events, Gene expression, and homologs. (E) 'MicroColinearity' module shows homologous relationships of a disease-resistant gene cluster on the local scale between the MH63 and ZS97 genomes. The black, green, and yellow lines represent RBH, SBH, and 1-to-many relationships, respectively. For genes, black, green, blue, and yellow represent genes with RBH, SBH, singleton, and 1-to-may relationships, respectively. (F) Gene tree in the 'Homologues' module. (G) 'MacroCollinearity' module shows the collinear blocks in chromosome 4 between ZS97 and MH63, and chromosome 4 between MH63 and Azucena. Colors indicate the collinear block scores. Inversions were highlighted by red triangle. (H) GO enrichment analysis. (I) *OsNiR*'s location in JBrowse. (J) The partial result of searching *GHD7*'s sequence in sequence databases of the whole genome (from 16 assemblies) and protein (from 18 annotations) by the 'BLAST' tool. The black triangles show the functions and links to other pages.

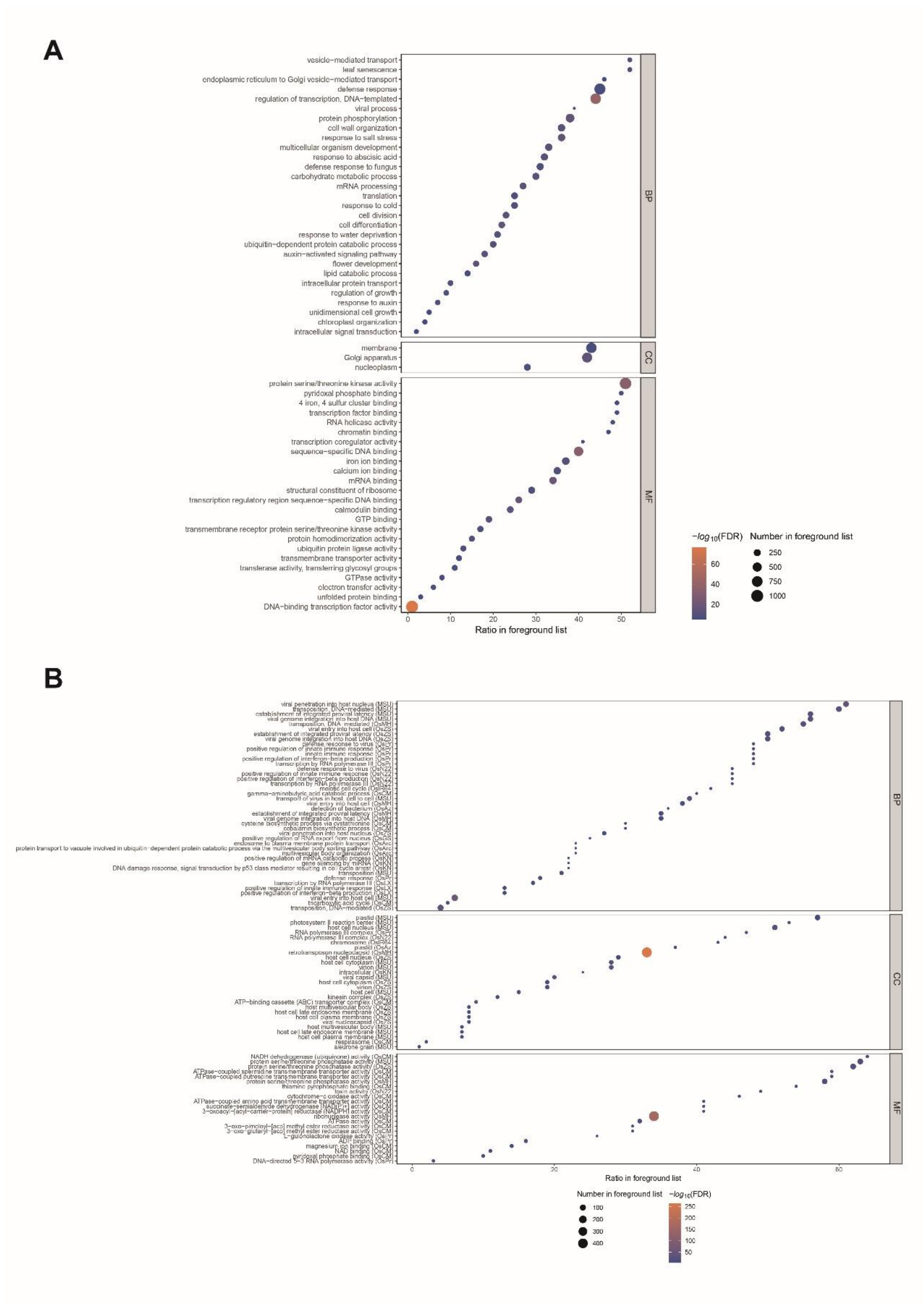


Supplemental Figure 1. Gene presence/absence variations in 16 Asian rice accessions. (A) The core genes are present in all accessions, the dispensable genes are present in <16 of accessions, and the accession-specific genes are only present in one accession. The presence of genes is colorful (4 subpopulations in Asian rice: *Geng/japonica*, *Xian/indica*, *circum-Aus*, and *circum-Basmati*), and the absence of genes is white. (B and C) The numbers of core genes (B) and accession-specific genes (C) that emerged at different evolutionary times, from PS1 (single-cell organisms) to PS13 (*O. sativa*). (D) The age distribution (left) and gene length (right) of the core and genes. (See Methods)

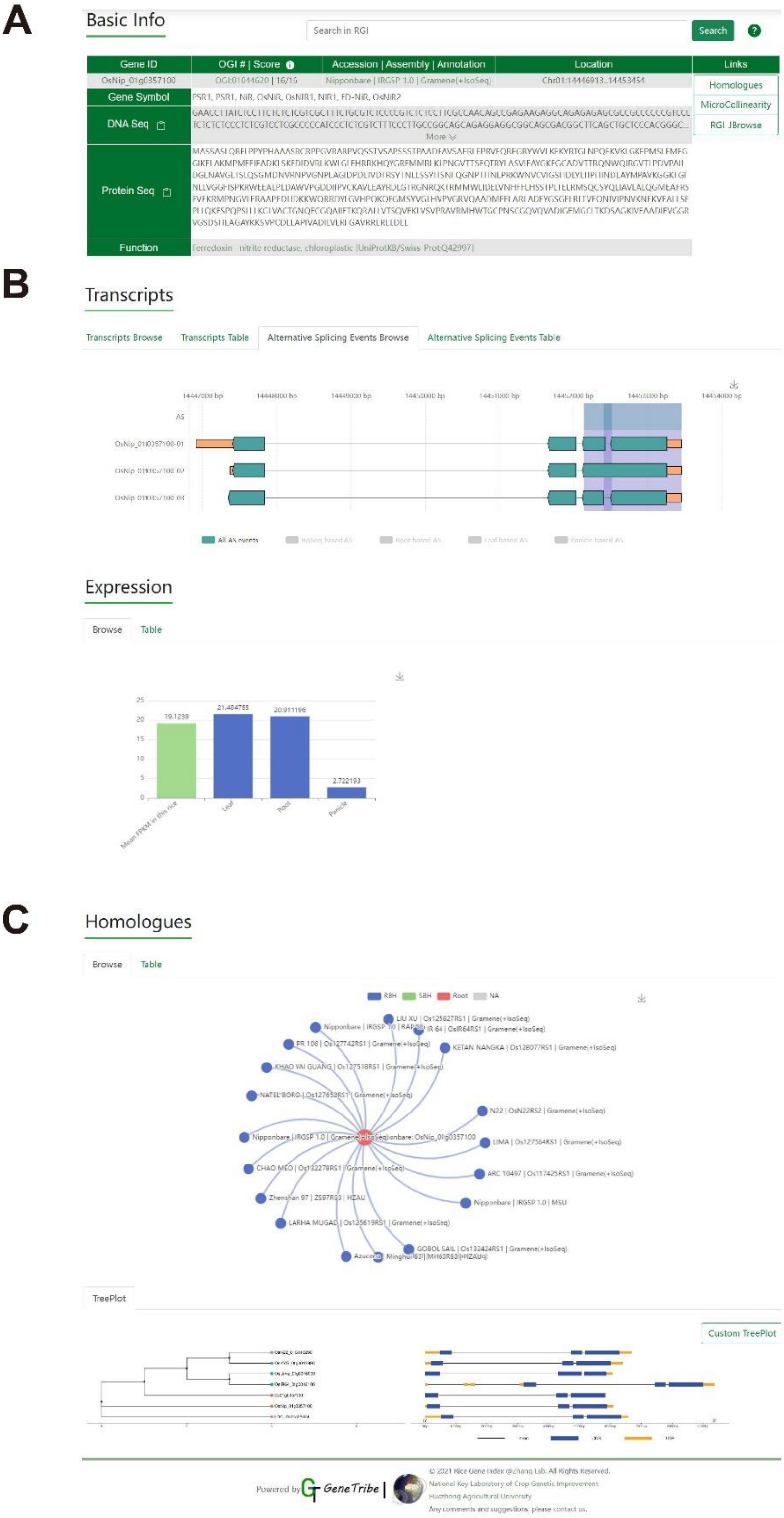


	Os GJ-temp: Nipponbare | IRGSP 1.0 | MSU	Os GJ-temp: Nipponbare | IRGSP 1.0 | RAPdb	Os GJ-temp: Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	Os GJ-subtrp: CHAO MEO | Os132278RS1 | Gramene(+IsoSeq)	Os GJ-trop1: Azucena | AzucenaRS1 | Gramene(+IsoSeq)	Os GJ-trop2: KETAN NANGKA | Os128077RS1 | Gramene(+IsoSeq)	Os cB: ARC 10497 | Os117425RS1 | Gramene(+IsoSeq)	Os XI-1B2: PR 106 | Os127742RS1 | Gramene(+IsoSeq)	Os XI-adm: Minghui 63 | MH63RS3 | HZAU	Os XI-1B1: IR 64 | OsIR64RS1 | Gramene(+IsoSeq)	Os XI-1A: Zhenshan 97 | ZS97RS3 | HZAU	Os XI-3A: LIMA | Os127564RS1 | Gramene(+IsoSeq)	Os XI-3B1: KHAO YAI GUANG | Os127518RS1 | Gramene(+IsoSeq)	Os XI-2A: GOBOL SAIL | Os132424RS1 | Gramene(+IsoSeq)	Os XI-3B2: LIU XU | Os125827RS1 | Gramene(+IsoSeq)	Os XI-2B: LARHA MUGAD | Os125619RS1 | Gramene(+IsoSeq)	Os cA1: N22 | OsN22RS2 | Gramene(+IsoSeq)	Os cA2: NATEL BORO | Os127652RS1 | Gramene(+IsoSeq)
Os GJ-temp: Nipponbare | IRGSP 1.0 | MSU	-	28365	28169	32747	32703	32678	32354	32121	47376	31789	49471	32849	32318	32118	38300	32139	32139	31858
Os GJ-temp: Nipponbare | IRGSP 1.0 | RAPdb	32576	-	35396	28760	28730	28820	28523	28665	29786	28326	30393	29211	28879	28639	33808	28632	28595	28462
Os GJ-temp: Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	32598	35493	-	28773	28724	28839	28572	28563	29691	28287	30336	29127	28792	28521	33747	28573	28552	28399
Os GJ-subtrp: CHAO MEO | Os132278RS1 | Gramene(+IsoSeq)	34911	28552	28396	-	35441	35287	34813	34106	31273	33680	31991	34811	34300	34015	40535	34072	34121	33727
Os GJ-trop1: Azucena | AzucenaRS1 | Gramene(+IsoSeq)	34915	28534	28390	35447	-	35421	34869	34220	31330	33747	32018	34921	34319	34087	40717	34143	34199	33875
Os GJ-trop2: KETAN NANGKA | Os128077RS1 | Gramene(+IsoSeq)	35565	28522	28410	35224	35359	-	35002	34225	32020	33950	32636	34980	34399	34076	40793	34253	34329	33828
Os cB: ARC 10497 | Os117425RS1 | Gramene(+IsoSeq)	34440	28374	28285	34813	34877	35075	-	34156	31195	33954	31863	34971	34335	34022	40739	34252	34400	33829
Os XI-1B2: PR 106 | Os127742RS1 | Gramene(+IsoSeq)	34296	28360	28160	33989	34111	34190	34035	-	31787	34672	32299	35342	35013	34757	41352	34606	34277	33850
Os XI-adm: Minghui 63 | MH63RS3 | HZAU	48304	27546	27342	31531	31592	31666	31459	31934	-	31581	54830	32475	31995	31815	37886	31671	31632	31383
Os XI-1B1: IR 64 | OsIR64RS1 | Gramene(+IsoSeq)	34077	28141	28000	33696	33758	34040	33966	34797	31673	-	32164	35247	34873	34557	41177	34465	34254	33658
Os XI-1A: Zhenshan 97 | ZS97RS3 | HZAU	48497	27855	27665	31751	31810	31901	31650	32044	52951	31712	-	32602	32136	31891	38134	31856	31854	31585
Os XI-3A: LIMA | Os127564RS1 | Gramene(+IsoSeq)	34186	28375	28186	34004	34103	34248	34127	34669	31610	34435	32106	-	35182	34816	41504	34657	34264	33871
Os XI-3B1: KHAO YAI GUANG | Os127518RS1 | Gramene(+IsoSeq)	34071	28399	28213	34007	33999	34168	34037	34800	31478	34557	32041	35718	-	34867	41504	34686	34329	33849
Os XI-2A: GOBOL SAIL | Os132424RS1 | Gramene(+IsoSeq)	33982	28385	28168	33887	33968	34006	33887	34724	31411	34394	31913	35495	35029	-	41318	34583	34281	33997
Os XI-3B2: LIU XU | Os125827RS1 | Gramene(+IsoSeq)	34362	28517	28346	34181	34290	34451	34357	34887	31709	34654	32239	35728	35217	34890	-	34767	34589	34013
Os XI-2B: LARHA MUGAD | Os125619RS1 | Gramene(+IsoSeq)	34159	28333	28166	33985	34026	34221	34149	34628	31377	34353	31955	35372	34890	34597	41120	-	34363	33988
Os cA1: N22 | OsN22RS2 | Gramene(+IsoSeq)	34442	28425	28290	34059	34172	34353	34362	34359	31536	34192	32188	35002	34575	34347	41010	34411	-	34592
Os cA2: NATEL BORO | Os127652RS1 | Gramene(+IsoSeq)	34053	28365	28190	33779	33978	33974	33934	34067	31239	33727	31788	34728	34230	34220	40557	34140	34713	-

Supplemental Figure 2. The 18 × 18 gene annotation matrix, which shows the number of 1-to-1 homologs, and demonstrates the homologous gene pairs. The color corresponds to the number of homologs. Red represents higher gene pairs number and blue represents lower gene pairs number.

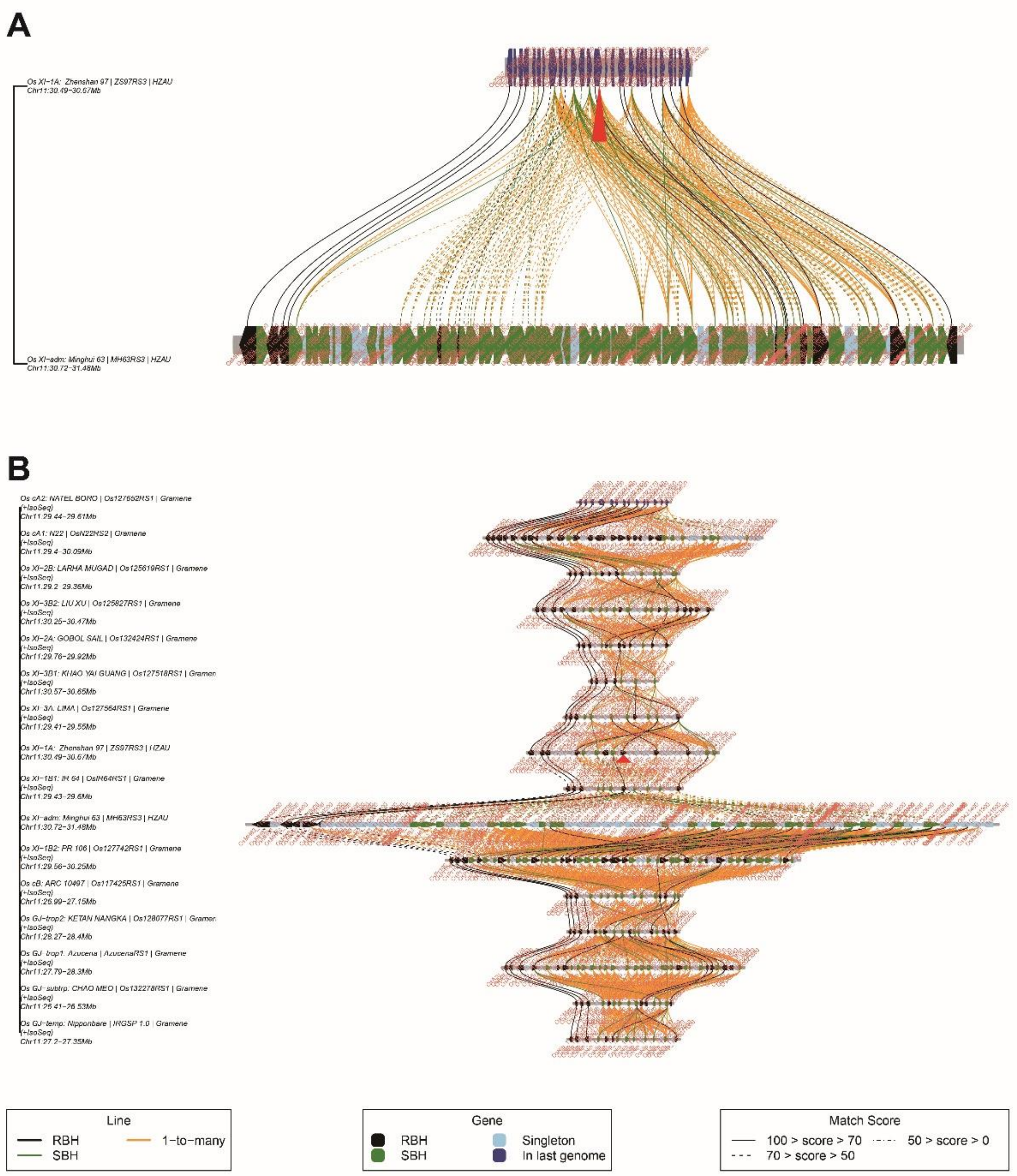


Supplemental Figure 3. The bubble plot of the Gene Ontology enrichment analysis in (A) the core gene set and (B) the accession-specific gene set.



Gene ID	OGI # | Score	Accession | Assembly | Annotation	Location	Links
OsNip_01g0357100	OGI:01044620 | 16/16	Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	Chr01:14446913..14453454	Homologues MicroCollinearity RGI JBrowse
Gene Symbol	PSR1, PSR1, NiR, OsNiR, OsNIR1, NIR1, FD-NiR, OsNiR2			
DNA Seq	More			
Protein Seq				
Function	Ferredoxin--nitrite reductase, chloroplastic [UniProtKB/Swiss-Prot:Q42997]			

Supplemental Figure 4. The ‘GeneCard’ page.



Supplemental Figure 5. In previous studies, NLR genes were found to be highly duplicated in chromosome 11 of MH63 compared with other genomes. (A) displays the micro-collinearity of NLR genes in MH63 and ZS97 by searching NLR gene *OsZS97_11G0430400* in the 'MicroCollinearity' module. (B) displays the microcollinearity of NLR genes in 16 varieties by searching NLR gene *OsZS97_11G0430400* in the 'MicroCollinearity' module. (A) and (B) show *OsZS97_11G0430400*'s 15 flanking genes.

Supplementary Tables

Supplemental Table 1. Summary of RGI data composition.

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Accession Name	Subpopulations	Assembly	Annotation[a]	Locus Tag Prefix
Os GJ-temp: Nipponbare	Geng-japonica-temp	IRGSP1.0	Gramene (+Isoseq)	OsNip_
			MSU	LOC_Os
			RAP-db	Os
Os GJ-subtrp: CHAO MEO	Geng-japonica-trop1	AzucenaRS1	Gramene (+Isoseq)	OsAzu_
Os GJ-trop1: Azucena	Geng-japonica-trop2	Os128077RS1	Gramene (+Isoseq)	OsKeNa_
Os GJ-trop2: KETAN NANGKA	Geng-japonica-subtrp	Os132278RS1	Gramene (+Isoseq)	OsCMeo_
Os cB: ARC 10497	circum-Basmati	Os117425RS1	Gramene (+Isoseq)	OsARC_
Os XI-1B2: PR 106	Xian-indica-1B2	Os127742RS1	Gramene (+Isoseq)	OsPr106_
Os XI-adm: MH63	Xian-indica-adm	MH63RS3	HZAU	OsMH63_
Os XI-1B1: IR 64	Xian-indica-1B1	OsIR64RS1	Gramene (+Isoseq)	OsIR64_
Os XI-1A: ZS97	Xian-indica-1A	ZS97RS3	HZAU	OsZS97_
Os XI-3A: LIMA	Xian-indica-3A	Os127564RS1	Gramene (+Isoseq)	OsLima_
Os XI-3B1: KHAO YAI GUANG	Xian-indica-3B1	Os127518RS1	Gramene (+Isoseq)	OsKYG_
Os XI-2A: GOBOL SAIL	Xian-indica-2A	Os132424RS1	Gramene (+Isoseq)	OsGoSa_
Os XI-3B2: LIU XU	Xian-indica-3B2	Os125827RS1	Gramene (+Isoseq)	OsLiXu_
Os XI-2B: LARHA MUGAD	Xian-indica-2B	Os125619RS1	Gramene (+Isoseq)	OsLaMu_
Os cA1: N22	circum-Aus1	OsN22RS2	Gramene (+Isoseq)	OsN22_
Os cA2: NATEL BORO	circum-Aus2	Os127652RS1	Gramene (+Isoseq)	OsNaBo_

a. this row is the annotation source, Gramene (+Isoseq) means *de novo* annotation attached transcripts from Iso-Seq data

Supplemental Table 2. Iso-Seq and RNA-Seq data from multiple tissues (i.e., leaves, roots, and immature panicles) of 16 rice accessions.

Supplemental Table 2A. Iso-Seq data of 16 rice accessions

Accessions	SMRT CELL[a]			HQ FLNC Reads			HQ Transcripts			
	Leaf	Panicle	Root	Leaf	Panicle	Root	Leaf	Panicle	Root	Merged
Os GJ-temp: Nipponbare	1	1	1	19,101	26,760	34,261	14,751	20,883	27,415	40,644
Os GJ-subtrp: CHAO MEO	1	1	1	26,589	28,501	26,100	22,916	17,237	22,084	40,025
Os GJ-trop1: Azucena	1	1	1	29,209	33,939	32,440	24,908	25,060	23,557	45,578
Os GJ-trop2: KETAN NANGKA	1	1	2	17,148	53,122	28,529	9,905	25,429	24,123	37,942
Os cB: ARC 10497	1	-	1	25,932		25,265	19,225	-	19,584	29,880
Os XI-1B2: PR 106	1	1	1	31,088	44,374	26,093	19,008	21,528	21,720	38,785
Os XI-adm: MH63	-	-	-	-	-	-	-	-	-	-
Os XI-1B1: IR 64	1	1	1	19,168	31,556	31,937	14,951	24,625	21,376	39,818
Os XI-1A: ZS97	-	-	-	-	-	-	-	-	-	-
Os XI-3A: LIMA	1	-	1	32,067	-	26,603	17,771	-	14,487	23,989
Os XI-3B1: KHAO YAI GUANG	1	1	1	31,036	-	43,031	19,756	-	22,178	30,559
Os XI-2A: GOBOL SAIL	2	-	1	31,967	-	42,680	15,857	-	21,485	26,869
Os XI-3B2: LIU XU	1	1	1	23,628	31,519	23,625	14,308	16,786	19,427	32,223
Os XI-2B: LARHA MUGAD	1	-	1	29,618	-	38,265	15,838	-	19,437	25,832
Os cA1: N22	1	1	1	43,709	50,872	29,457	26,290	24,509	21,098	43,742
Os cA2: NATEL BORO	1	1	1	37,614	41,834	28,826	17,928	20,225	21,157	35,923

a. this row shows the number of PacBio SMRT cells

Supplemental Table 2B. RNA-Seq data of 16 rice accessions

Accessions	Biological Replication[a]			Alignment Rate		
	Leaf	Panicle	Root	Leaf	Panicle	Root
Os GJ-temp: Nipponbare	1	1	1	99.54%	99.29%	99.57%
Os GJ-subtrp: CHAO MEO	1	1	1	96.14%	93.87%	92.04%
Os GJ-trop1: Azucena	1	1	1	99.50%	99.41%	98.48%
Os GJ-trop2: KETAN NANGKA	1	1	1	71.11%	37.23%	17.79%
Os cB: ARC 10497	1	-	1	98.54%	-	94.72%
Os XI-1B2: PR 106	1	1	1	62.25%	29.27%	20.68%
Os XI-adm: MH63	2	2	2	95.12%	89.89%	85.26%
Os XI-1B1: IR 64	1	1	1	99.24%	99.22%	98.29%
Os XI-1A: ZS97	2	2	2	88.25%	88.36%	60.60%
Os XI-3A: LIMA	1	-	1	98.56%	-	96.37%
Os XI-3B1: KHAO YAI GUANG	1	1	1	94.99%	-	99.45%
Os XI-2A: GOBOL SAIL	1	-	1	96.66%	-	93.84%
Os XI-3B2: LIU XU	1	1	1	99.51%	99.29%	99.42%
Os XI-2B: LARHA MUGAD	1	-	1	86.39%	-	78.36%
Os cA1: N22	1	1	1	99.60%	99.73%	98.58%
Os cA2: NATEL BORO	1	1	1	96.37%	86.24%	86.43%

a. this row shows the number of biological replication

Supplemental Table 3. Numbers of annotated genes in 16 rice accessions.

Supplemental Table 3. Gene number of 16 rice varieties with 18 gene annotation

Accession|Assembly|Annotation	Annotated Genes	Genes from Iso-Seq Data
Os GJ-temp: Nipponbare | IRGSP 1.0 | MSU	55,801	-
Os GJ-temp: Nipponbare | IRGSP 1.0 | RAPdb	37,859	-
Os GJ-temp: Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	38,404	733
Os GJ-subtrp: CHAO MEO | Os132278RS1 | Gramene(+IsoSeq)	37,240	1,054
Os GJ-trop1: Azucena | AzucenaRS1 | Gramene(+IsoSeq)	36,882	1,079
Os GJ-trop2: KETAN NANGKA | Os128077RS1 | Gramene(+IsoSeq)	37,994	1,388
Os cB: ARC 10497 | Os117425RS1 | Gramene(+IsoSeq)	37,181	844
Os XI-1B2: PR 106 | Os127742RS1 | Gramene(+IsoSeq)	36,720	1,255
Os XI-adm: Minghui 63 | MH63RS3 | HZAU	59,903	-
Os XI-1B1: IR 64 | OsIR64RS1 | Gramene(+IsoSeq)	36,925	987
Os XI-1A: Zhenshan 97 | ZS97RS3 | HZAU	60,935	-
Os XI-3A: LIMA | Os127564RS1 | Gramene(+IsoSeq)	37,994	973
Os XI-3B1: KHAO YAI GUANG | Os127518RS1 | Gramene(+IsoSeq)	37,522	1,105
Os XI-2A: GOBOL SAIL | Os132424RS1 | Gramene(+IsoSeq)	36,467	1,810
Os XI-3B2: LIU XU | Os125827RS1 | Gramene(+IsoSeq)	44,942	1,183
Os XI-2B: LARHA MUGAD | Os125619RS1 | Gramene(+IsoSeq)	37,474	1,244
Os cA1: N22 | OsN22RS2 | Gramene(+IsoSeq)	37,598	1,280
Os cA2: NATEL BORO | Os127652RS1 | Gramene(+IsoSeq)	36,392	1,664

Supplemental Table 4. Summary of alternative splicing events in 16 rice accessions.

Supplemental Table 4. Summary of alternative splicing events in 16 rice accessions

Accession|Assembly|Annotation	A3	A5	AF	AL	MX	RI	SE	Total
Os GJ-temp: Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	1,250	634	237	91	2	1,181	326	3,721
Os GJ-subtrp: CHAO MEO | Os132278RS1 | Gramene(+IsoSeq)	2,761	2,574	530	182	113	2,228	1,715	10,103
Os GJ-trop1: Azucena | AzucenaRS1 | Gramene(+IsoSeq)	2,735	2,583	575	187	100	2,257	1,719	10,156
Os GJ-trop2: KETAN NANGKA | Os128077RS1 | Gramene(+IsoSeq)	2,484	2,350	509	167	101	2,072	1,581	9,264
Os cB: ARC 10497 | Os117425RS1 | Gramene(+IsoSeq)	2,253	2,009	451	151	91	1,912	1,396	8,263
Os XI-1B2: PR 106 | Os127742RS1 | Gramene(+IsoSeq)	2,530	2,415	519	169	116	2,153	1,637	9,539
Os XI-adm: Minghui 63 | MH63RS3 | HZAU	-	-	-	-	-	-	-	-
Os XI-1B1: IR 64 | OsIR64RS1 | Gramene(+IsoSeq)	2,634	2,434	546	208	96	2,177	1,646	9,741
Os XI-1A: Zhenshan 97 | ZS97RS3 | HZAU	-	-	-	-	-	-	-	-
Os XI-3A: LIMA | Os127564RS1 | Gramene(+IsoSeq)	1,841	1,670	367	127	62	1,791	1,182	7,040
Os XI-3B1: KHAO YAI GUANG | Os127518RS1 | Gramene(+IsoSeq)	1,986	1,770	403	149	60	1,792	1,236	7,396
Os XI-2A: GOBOL SAIL | Os132424RS1 | Gramene(+IsoSeq)	1,563	1,355	351	115	45	1,450	940	5,819
Os XI-3B2: LIU XU | Os125827RS1 | Gramene(+IsoSeq)	2,537	2,424	493	168	97	1,981	1,683	9,383
Os XI-2B: LARHA MUGAD | Os125619RS1 | Gramene(+IsoSeq)	1,803	1,588	377	154	57	1,611	1,129	6,719
Os cA1: N22 | OsN22RS2 | Gramene(+IsoSeq)	2,742	2,548	514	937	108	2,464	1,705	11,018
Os cA2: NATEL BORO | Os127652RS1 | Gramene(+IsoSeq)	2,281	2,198	474	156	104	1,796	1,469	8,478

Supplemental Table 5. Homologues of gene *LOC_Os11g29290* in 16 accessions. The result was produced by the 'Homologues' module and checked manually.

Supplemental Table 5. Homologs of *OsTPP7* (LOC_Os09g20390) in 16 accessions

Accession|Assembly|Annotation	Homologous	Type
Os GJ-temp: Nipponbare | IRGSP 1.0 | MSU	LOC_Os11g29290	RBH
Os GJ-temp: Nipponbare | IRGSP 1.0 | RAPdb	Os11g0483000	RBH
Os GJ-temp: Nipponbare | IRGSP 1.0 | Gramene(+IsoSeq)	OsNip_11g0483000	RBH
Os GJ-subtrp: CHAO MEO | Os132278RS1 | Gramene(+IsoSeq)	OsCMeo_11g0013950	RBH
Os GJ-trop1: Azucena | AzucenaRS1 | Gramene(+IsoSeq)	OsAzu_11g0014030	RBH
Os GJ-trop2: KETAN NANGKA | Os128077RS1 | Gramene(+IsoSeq)	OsKeNa_11g0013990	RBH
Os cB: ARC 10497 | Os117425RS1 | Gramene(+IsoSeq)	OsARC_11g0013770	RBH
Os XI-1B2: PR 106 | Os127742RS1 | Gramene(+IsoSeq)	OsPr106_11g0014050	RBH
Os XI-adm: Minghui 63 | MH63RS3 | HZAU	OsMH63_11G0261700	RBH
Os XI-1B1: IR 64 | OsIR64RS1 | Gramene(+IsoSeq)	NA	NA
Os XI-1A: Zhenshan 97 | ZS97RS3 | HZAU	OsZS97_11G0273800	RBH
Os XI-3A: LIMA | Os127564RS1 | Gramene(+IsoSeq)	OsLima_11g0014120	RBH
Os XI-3B1: KHAO YAI GUANG | Os127518RS1 | Gramene(+IsoSeq)	OsKYG_11g0014170	RBH
Os XI-2A: GOBOL SAIL | Os132424RS1 | Gramene(+IsoSeq)	OsGoSa_11g0013820	RBH
Os XI-3B2: LIU XU | Os125827RS1 | Gramene(+IsoSeq)	OsLiXu_11g0013660	RBH
Os XI-2B: LARHA MUGAD | Os125619RS1 | Gramene(+IsoSeq)	OsLaMu_11g0013960	RBH
Os cA1: N22 | OsN22RS2 | Gramene(+IsoSeq)	OsN22_11G013780	RBH
Os cA2: NATEL BORO | Os127652RS1 | Gramene(+IsoSeq)	OsNaBo_11g0014000	RBH

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