Title:
Investigation of the genetic diversity of a Core Collection of Japanese Rice Landraces (JRC) using whole-genome sequencing.

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Title and running head:
Investigation of the genetic diversity of a Core Collection of Japanese Rice Landraces (JRC) using whole-genome sequencing.

Genetic diversity of JRC based on WGS

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Abstract

The Rice Core Collection of Japanese Landraces (JRC) consisting of 50 accessions was developed by the genebank at the National Agriculture and Food Research Organization (NARO) in 2008. As a Japanese landrace core collection, the JRC has been used for many research projects, including screening for different phenotypes and allele mining for target genes. To understand the genetic diversity of Japanese Landraces, we performed whole genome re-sequencing of these 50 accessions, and obtained a total of 2,145,095 SNPs and 317,832 insertion-deletions (indels) by mapping against the Oryza sativa ssp. japonica Nipponbare genome. A JRC phylogenetic tree based on 1,394 representative SNPs showed that JRC accessions were divided into two major groups and one small group. We used the multiple genome browser, TASUKE+ to examine haplotypes of flowering genes and detected new mutations in these genes. Finally, we performed GWAS for agronomical traits using the JRC and another core collection, the world rice core collection (WRC), comprising of 69 accessions also provided by the NARO genebank. In leaf blade width a strong peak close to NAL1, a key gene for the regulation of leaf width, and in heading date, a peak near HESO1, involved in flowering regulation were observed in GWAS using the JRC. They were also detected in GWAS using the combined JRC+WRC. Thus, JRC and JRC+WRC are suitable populations for GWAS in particular traits.

Introduction

Using exotic materials for breeding can be used to produce advanced rice cultivars, exploiting the variation contained with them to breed for improved performance. To avoid the evaluation of large numbers of samples in large genebank collections of
genetic resources, rice core collections have been developed around the world (Kojima et al., 2005; Ebana et al., 2008; Agrama et al., 2009; Zhang et al., 2011). Among the 2,000 Japanese accessions stored in NARO genebank, 236 accessions were selected based on their passport data. The Rice Core Collection of Japanese Landraces (JRC) comprising of 50 accessions was selected to retain 87.5% of the alleles in these 236 accessions based on 32 genome-wide SSR markers (Ebana et al., 2008). The JRC was developed by the NARO genebank as a suitable population for understanding rice adaptation in northern areas, such as Japan (Ebana et al., 2008).

The JRC and the World Rice Core Collection (WRC), which was also developed by the NARO genebank, have been used for many research projects, in screening for different phenotypes, and allele mining for target genes (Suzaki et al., 2009; Ochiai et al., 2011; Fujino et al., 2013; Iijima et al., 2019; Taguchi-Shiobara et al., 2013; Ueno et al., 2009). Because the JRC and WRC include only 50 and 69 accessions, respectively, they are compact and manageable populations for use in these research projects. Although genetic diversity of JRC seems smaller than that of WRC, significant variations in genes have been identified in the JRC. For example, to identify genes important for the adaptation of rice cultivars to northern latitudes, the JRC accessions were used for the investigation of allelic variations in \( H_d5 \) (Fujino et al., 2013). A 19-bp deletion causing a loss-of-function of \( H_d5 \) was found in JRC accessions, and it was likely selected as the mutation which causes earlier heading, thus adapting rice to cultivation at higher latitudes. JRC accessions were also used for the evaluation of endosperm enzyme activity to improve the texture and eating quality of rice (Iijima et al., 2019). Evaluation of eating-quality and endosperm enzyme activities in a large set of Japanese rice cultivars, including the JRC revealed that low levels of endosperm enzyme activity are associated with high eating-quality in rice.
varieties bred in Japan. BORON EXCESS TOLERANT1 (BET1), responsible for borontoxicity, was isolated by observation of cultivar differences among JRC accessions, especially Wataribune (JRC19) (Ochiai et al., 2011). A major Cadmium (Cd) transporter, OsHMA3 was isolated from WRC30 (Anjana Dhan) through measurements of Cd concentration in shoots of the WRC and JRC (Ueno et al., 2010).

To date, to identify causal genes from these core collections, it has been necessary to perform QTL analyses on F2 populations derived from crosses between divergent cultivars. High-resolution whole genome sequencing data of core collections enables efficient QTL analysis and gene isolation from cultivars in core collections, however, the JRC whole genome sequence (WGS) data has not been published so far.

Recently, genome wide association studies (GWAS) have become a common tool in rice, especially because of the reduction in cost of Next Generation Sequencing (NGS) analysis. Although GWAS enables us to omit the crossing process for the isolation of causal genes (Yano et al., 2016, 2019), there are a number of factors to consider in effective GWAS analysis. GWAS populations usually consist of large numbers of landraces and cultivars, for example 517 landraces in (Huang et al., 2010), 373 indica accessions in (Huang et al., 2012) and 529 cultivated lines in (Yang et al., 2018). Phenotyping of such large populations is time consuming. Previously, Tanaka et al., 2020 reported that several peaks related to seed traits were detected by using the WRC core collection in GWAS and these peaks were close to well-known genes, such as Rc, waxy and GS3. Although the WRC represents a highly structured population, for some traits, this core collection that consists of only 69 rice cultivars could match the performance of larger GWAS population (Tanaka et al., 2020).

In this report, we evaluated the genetic diversity of the JRC using WGS data and
performed haplotype analysis of different genes using TASUKE+ to detect new alleles in JRC accessions. We also performed GWAS for normally distributed agronomical traits using the JRC and WRC accessions, and detected significant peaks close to well-known genes.

Results

Classification of the Rice Core Collection of Japanese Landraces (JRC) based on whole-genome sequencing data

The JRC comprises 50 accessions (Supplemental Table 1) and is provided by the genebank at NARO as a suitable population for understanding rice adaptation in northern areas, such as Japan. The NARO World Rice Core Collection (WRC) was also developed by the genebank at NARO (Kojima et al., 2005) and both core collections have been distributed to more than 300 research groups for different purposes. To promote effective use of the JRC as a genetic and breeding resource, we obtained whole-genome sequencing (WGS) data of the 50 accessions and mapped the reads to the *Oryza sativa* ssp. *japonica* Nipponbare genome (Os-Nipponbare-Reference-IRGSP-1.0) (Kawahara et al., 2013) using bwa (Li and Durbin, 2009). The average depth was 28.8, ranging from 17.4 to 39.9; the average number of SNPs and insertion-deletions (indels) per accession were 523,212 and 103,299, respectively (Supplemental Table 1). When we simultaneously used all 50 JRC accessions, we obtained a total of 2,145,095 SNPs and 317,832 indels by mapping against the *Oryza sativa* ssp. *japonica* Nipponbare genome. Based on 1,394 representative SNPs selected to be in approximate linkage equilibrium (using the Snphylo pipeline; Lee et al., 2014), we constructed a phylogenetic tree to classify the 50 accessions (Figure 1A). JRC accessions were divided into three major groups; two *japonica* groups
(J-1, J-2) and one small indica group (I-1). J-2 corresponded to tropical japonica varieties, and J-1 was a group of temperate japonica. The number of SNPs detected in these accessions is J-1 < J-2 < I-1 reflecting their genetic relationship with the temperate japonica reference genome.

Next, we performed joint genotyping of the JRC and WRC accessions. We used a total of 119 accessions and obtained 2,595,145 SNPs and 321,694 indels by mapping against the Oryza sativa ssp. japonica Nipponbare genome (Kawahara et al., 2013). Then, we constructed a phylogenetic tree using both the JRC and the WRC accessions based on 2,004 representative SNPs (Figure 1B). In a previous report (Ebana et al., 2008), JRC40, 41, 42, 43 and 44 were classified into the indica group based on both 32 SSR markers and their color reaction with phenol; however, JRC21, 41, 42, 43 and 44 were classified into distant group based on WGS data in this study, while JRC40 was classified as japonica (Figure 1A, B).

**Haplotype analysis of flowering time genes in the JRC**

Heading date is one of the most important agronomical traits, and diversity of heading date has contributed to the development of rice cultivation in a wider range of latitudes (Khush, 1997). To clarify the functional diversity of the flowering-time genes in JRC, we exhibited sequence variation in JRC using a multiple genome browser, TASUKE+ (Kumagai et al., 2019, 2013), and performed haplotype analysis for flowering genes (Figure 2, Table 1).

We detected three kinds of deletions in Hd1 among JRC accessions. JRC20, 49, and 50 (Hosogara, Rikutou Rikuu 2 and Himenomochi) carried a 43-bp deletion in exon
1 and JRC14 and 18 carried a 2-bp deletion in exon 2 of *Hd1* (Yano et al., 2000). JRC07 (Iruma Nishiki) carried a 4-bp deletion in exon 1 of *Hd1* and this deletion has not been previously reported (Table 1). A nonsynonymous substitution at amino acid 24 resulting in a premature stop codon was detected in the *Hd1* gene in JRC22 (Mansaku). A nonsynonymous substitution at amino acid 57 resulting in a premature stop codon was also detected in the *Hd1* gene in JRC38 (Nagoya Shiro), and these mutations have also not been previously reported.

JRC44 (Karahoushi) has an 8-bp deletion in the 7th exon of *Hd2*, and JRC41 (Akamai) carries a variant which changes Tyr to His at amino acid 704 in Hd2 CCT domain resulting in a non-functional protein (Koo et al., 2013) (Table 1). JRC21 (Akamai), 42 (Touboshi) and 43 (Akamai) classified into *indica* group have a previously unreported 1-bp deletion in *Hd2* (Table 1). These results indicate that haplotype analysis of flowering genes in JRC with TASUKE+ is an efficient way to discover new mutations.

Both *Hd6* and *Hd16*, encoding casein kinase proteins, are involved in photoperiod sensitivity, which is known to be the main determinant of heading date (Hori et al., 2013; Takahashi et al., 2001). In *Hd6*, a nonsynonymous substitution at amino acid 146 resulting in a premature stop codon was observed in JRC23, 27, 30, 50 (Table 1) and the same SNP was also reported in Nipponbare (WRC01) (Takahashi et al., 2001). Only JRC50 carries a functional substitution from Ala to Thr at amino acid 331 of Hd16 which was identified in a commercial Japanese cultivar, Koshihikari (Hori et al., 2013) (Table 1). A SNP which changes a Gln to a stop codon at amino acid 53 of Ghd7 (Xue et al., 2008) was only observed in JRC17 (Table 1). Matsubara et al., 2012 reported a functional substitution from Leu to Ser at amino acid 558 of Hd17 and this substitution was observed in 27 accessions in the JRC (Table 1). JRC21, 42, and 43 have a functional mutation
which changes Glu to Lys at amino acid 105 in RFT1 (Ogiso-Tanaka et al., 2013) and JRC21, 43, and 44 carry a 1-bp deletion in DTH8 (Table 1). JRC21, 42, 43, and 44 were categorized as indica in the phylogenetic tree based on WGS data (Figure 1), and these two mutations were also detected only from indica accessions in the WRC (Tanaka et al., 2020). We concluded that these mutations occurred after haplogroup differentiation.

**Genome-wide Association Studies (GWAS)**

We performed GWAS for different agronomical traits: amylose content in seed, leaf blade width and heading date, using the JRC and WRC accessions. We compared the utility of the JRC or WRC populations alone, or combined as one JRC+WRC population as a resource for GWAS (Figure 3-5, Supplemental Figure1-3). Previously, GWAS with WRC only detected high impact QTLs, such as Rc, waxy and GS3 (Tanaka et al., 2020).

First, we used amylose content of JRC and JRC+WRC seeds for GWAS (Figure 3, Supplemental Figure 1). The distribution of seed amylose content was trimodal (Figure 3A, Supplemental Figure 1A). As with GWAS using the WRC, a significant peak associated with amylose content was observed close to the waxy (Os06g0133000) gene using the JRC and JRC+WRC populations (Figure 3B, Supplemental Figure 1B). As an outlier as shown in Figure 3D, two accessions, JRC22 (Mansaku) and JRC50 (Himenomochi) showed significantly lower amylose content although they do not have a 23-bp insertion in waxy (Figure 3C, D, Supplemental Table 1). When we investigated the details of the genome sequence of waxy in JRC22 and JRC50, we detected 67-bp deletion in exon 1 of waxy in JRC22, and the insertion of a 7.8kb retrotransposon-like sequence in the waxy sequence of JRC50 (Hori et al., 2007). To our knowledge, the 67-bp deletion in the waxy gene seen in JRC22 has not been reported before.
Next, we performed GWAS for leaf blade width using JRC only, WRC only and JRC+WRC (Figure 4, Supplemental Figure 2). No significant peak was observed in the result of GWAS with WRC (Figure 4B). JRC and WRC accessions exhibited a normal distribution for leaf width (Figure 4A, Supplemental Figure 2A), and a high peak was observed on chromosome 4 in GWAS using the JRC (Figure 4B). When we used JRC+WRC as the GWAS population, the peak on chromosome 4 was higher than a significance threshold (Supplemental Figure 2B). The detected peaks were close to NARROW LEAF1 (NAL1), Os04g061500, known as a major regulator of leaf width (Fujita et al., 2013; Jiang et al., 2015). JRC and WRC accessions carrying haplotype A in NAL1 had wider leaves than those with haplotype B (Figure 4C, D, Supplemental Figure 2C, D).

Compared with GWAS using the WRC, we improved the detectability of known genes associated with normally distributed phenotypes, such as leaf width, using the JRC only or JRC+WRC as the GWAS population (Figure 4B, Supplemental Figure 2B). Next, we performed GWAS for heading date with JRC only, WRC only and JRC+WRC, respectively (Figure 5, Supplemental Figure 3). Because heading date was affected by a large number of genes (Tsuji et al., 2011), no significant peak was detected by GWAS with WRC only (Figure 5B). On the other hand, relatively high peaks were detected on chromosome 1, 3 and 11 in the results of GWAS with JRC only (Figure 5B). When we used JRC+WRC as a GWAS population for heading date, these peaks were beyond a P-Value threshold (Supplemental Figure 3B). On chromosome 1, a peak was located near to the homolog of Arabidopsis thaliana HEN1 suppressor 1 (HESO1) gene, Os01g0846450, recently reported as a new gene for the regulation of days to heading (Yano et al., 2016). JRC and WRC accessions carrying haplotype A in HESO1 showed
earlier heading dates than those with haplotype B (Figure 5D, Supplemental Figure 3D). These results indicate that the JRC and the combined JRC and WRC will be suitable populations for GWAS in particular traits, such as leaf width and heading date.

Discussion

Genetic diversity of a Core Collection of Japanese Rice Landraces (JRC)

In this study, we evaluated the genetic diversity of the JRC using whole genome sequencing (WRC) data. Additionally, we performed haplotype analysis of flowering genes in the JRC using TASUKE+ system and also developed GWAS pipelines using the JRC and WRC accessions that were able to detect known genes for agronomic traits.

Our genetic study revealed that the JRC was divided into three subgroups, J-1, J-2 and I-1 (Figure 1). In passport data recorded when the accessions were collected, JRC01 to JRC14 were upland rice varieties and were also categorized as *tropical japonica*. These 12 accessions in the JRC were included in J-2 group in our results (Figure 1, supplemental table). These results indicated that classification of the JRC using WGS accurately reflects their passport data.

Four accessions in the JRC, JRC21, JRC40, JRC41, JRC43 have the same accession name, Akamai. When we used WGS data of the JRC, the classification of JRC21 and JRC40 were opposite from previous report using SSR markers i.e. JRC21 was indica, not *japonica*, and JRC40 was *japonica*, not *indica*. One advantage of using WGS data to characterize genetic resources is that whole genome genotype information allows us to understand their genetic diversity exactly, avoiding errors based on small numbers of markers, and correcting any errors of identification that may have occurred during collection or propagation.
Haplotype analysis of the waxy gene in JRC and WRC accessions

Amylose content in the endosperm is known as an important determinant of rice eating quality because it reflects the functionality of the rice grain’s starch. The waxy (Wx) gene (Os06g0133000) encodes granule-bound starch synthase (GBSS) which controls amylose synthesis in rice endosperm. The differentiation of glutinous and non-glutinous rice is determined by alleles of the waxy locus. Rice cultivars carrying Wxa have a high (approximately 25~30%) amylose content and those carrying Wxb, with a G to T substitution at the 5’ UTR splicing site, show decreased expression of waxy, associated with intermediate (approximately, 15~20%) amylose concentration (Hirano et al., 1998) (Supplemental table 1). Glutinous rice varieties generally result from loss of function mutations in the waxy gene, result in very low amylose concentrations. In the JRC and WRC, most glutinous rice cultivars containing little seed amylose have a 23-bp insertion or a retrotransposon-like sequence in waxy (Hori et al., 2007; Wanchana et al., 2003). Only JRC22 was a glutinous rice without a known allele of waxy (Figure 3, supplemental table 1). We detected a new allele of waxy from this JRC accession, suggesting that JRC, although a small population is still useful material for understanding rice breeding.

Oryza sativa was derived from an ancestral wild rice, O. rufipogon. O. rufipogon carries the Wxa allele and the G to T substitution at the 5’ UTR splicing site was selected during the domestication of O. sativa japonica as the Wxb allele (Hirano et al., 1998) resulting in intermediate amylose concentrations, and sticky, but non-glutinous rice. In the JRC and WRC, all glutinous rice accessions carried the Wxb allele at the 5’ UTR splicing site, and in addition, the 23-bp insertion, the transposon insertion or the 67-bp deletion inactivating the waxy gene and resulting in the glutinous phenotype.
(Supplemental table 1). These results suggested that glutinous rice was selected from among *japonica* cultivars that were domesticated in east Asia, wherein the *Wx*<b> </b> allele had become fixed in the population based on the resulting stickiness of the cooked rice. Most Japanese glutinous rice varieties in the JRC obtained the same 23-bp insertion in *waxy*, except for the 67-bp deletion and transposon insertion (Figure 3). These results suggest that the desirability of the glutinous rice phenotype led to the wide distribution of the mutations in the local region.

**Genetic diversity of NAL genes in the JRC and WRC**

In figure 4, we detected the *NAL1* locus associated with leaf width using the JRC as a GWAS population, or using the combined JRC+WRC, when the peak was higher than a significance threshold. Haplotype B of *NAL1* was present in almost half of the JRC and WRC accessions, however, no significant peak was observed in GWAS using the WRC alone (Figure 4B). To determine the reason for the difference in GWAS results between the JRC and the WRC, we investigated the relationship between the *NAL1* genotypes and leaf width in the WRC (Supplemental Figure 4). In the WRC, accessions carrying haplotype A in *NAL1* also showed wider leaves than those with haplotype B (Supplemental Figure 4A). These results indicate that population structure and/or kinship relatedness in the WRC might cause lower P-value in *NAL1* locus. Until now, four genes, *NAL1*, *NAL2*, *NAL3* and *NAL7* have been reported as major regulators of leaf width (Fujino et al., 2008; Ishiwata et al., 2013; Jiang et al., 2015). In the JRC and WRC accessions, we did not find any SNPs in *NAL2* or *NAL3*. In the *NAL7* gene, several moderate-impact nonsynonymous SNPs causing amino acid substitutions were observed in WRC accessions. Conversely only three accessions categorized into the *indica* group
carried a moderate-impact SNP in the JRC (Supplemental figure 4B). These results suggest that the genetic diversity of NAL7 gene in the WRC masked the effect of the NAL1 gene for leaf width. Although the peak was observed in the JRC alone, it became significant using JRC+WRC due to the increased statistical power obtained when using a greater number of accessions.

GWAS for heading date using the JRC and WRC

Although we detected HESO1 for heading date using the JRC and WRC as GWAS population, peaks close to strong QTLs for heading date, such as Hd1 and Hd2 were not observed (Figure 5, Supplemental Figure 3). In Hd1, except for the 2-bp deletion in exon 2 (Yano et al., 2000), widely distributed DNA polymorphisms with high impact, such as indels were not observed in the JRC using TASUKE+ (Figure 2, Table 1). In GWAS with JRC+WRC, DNA polymorphisms with minor allele frequency (MAF) less than 0.05 among the 119 accessions were removed from the variant dataset. These rare DNA polymorphisms in Hd1 were not used in the GWAS procedure, so a significant peak close to Hd1 was not detected in our experiments.

An 8-bp deletion on exon 7 in Hd2 was commonly observed both in the JRC and WRC, however, it was only distributed in accessions categorized into indica group both in JRC and WRC (Table 1) (Tanaka et al., 2020). Because of the problem of population structure, the effect of 8-bp deletion in Hd2 might be ignored in calculation of GWAS by linear mixed model (Korte et al., 2012).

Using the JRC and WRC as GWAS population, a significant peak associated with heading date was detected on chromosome 1 close to HESO1. HESO1 was originally
isolated by GWAS with *japonica* cultivars (Yano et al., 2016), and Haplotypes A and B were almost equally distributed in the JRC. On the other hand, only WRC01 (Nipponbare, *japonica*) and WRC43 (Dianyu 1, *japonica*) carried the Haplotype A allele of the *HESO1* gene among the WRC accessions (Figure 5). In this report, the *japonica* allelic frequency of *HESO1* was sufficient to allow the detection of the *HESO1* locus associated with heading date.

Although JRC contains only 50 accessions, the detectability of GWAS with the JRC for particular traits was higher than with WRC. In addition, the combination of JRC and WRC includes 119 accessions, so it is still possible to collect high-quality phenotype data with low cost, but also to improve the statistical power of the detection. For many traits, phenotyping is labor-intensive, and thus it is important to select the minimum size of population adequate for the detection of significant association peaks. The JRC is a small population, without a high degree of population structure, excepting the five *indica* varieties. In some cases, it could out-perform the WRC in GWAS. However, a better statistical power could be achieved by using the JRC+WRC population if it is possible to acquire phenotype data for all accessions.

**Materials and Methods**

**Plant materials and whole-genome sequencing**

We used JRC accessions maintained at the NARO gene bank (Supplementary Table S 1). Total DNA was extracted from leaves from one plant of each variety using the DNeasy Plant Mini Kit (Qiagen). The DNA libraries were sequenced using Illumina Hiseq 2000 or Hiseq X instruments (Illumina Co, Ltd.), and paired-end reads were obtained. All reads were mapped against Os-Nipponbare-Reference-IRGSP-1.0 (Kawahara et al., 2013)...
pseudomolecules using bwa mem (Li and Durbin, 2009) and duplicates were removed using picard MarkDuplicates (http://broadinstitute.github.io/picard/).

**Variant calling**

Variants were called essentially following the GATK Best practices for germline SNP/Indel discovery (Auwera et al., 2013). Variants were first called on a by-sample basis using GATK HaplotypeCaller, then variants were consolidated in a joint calling step with GenotypeGVCFs (Poplin et al., 2018). GATK version 4.0.11.0 was employed for all steps. Variants were then filtered using bcftools view (Li, 2011) with the parameters ‘-m2 -M2 -g hom --output-type z --exclude-uncalled -e "MAF < 0.05 || N MISSING > 0 || QD < 5.0 || FS > 50.0 || SOR > 3.0 || MQ < 50.0 || MQRankSum < -2.5 || ReadPosRankSum < -1.0 || ReadPosRankSum > 3.5"’, resulting in a set of variants where no position had missing data or a minor allele frequency less than 0.05. All nucleotide polymorphisms were categorized for their potential effects using SnpEff 4.3t (Cingolani et al., 2012) with the Oryza_sativa database.

**Phylogenetic tree and Population structure**

The Snphylo pipeline (Lee et al., 2014) was used to create a maximum likelihood phylogenetic tree based on representative genomic SNPs. The pipeline was employed using default parameters and 100 bootstrap replicates to create the bootstrapped maximum likelihood tree.

**GWAS**
For GWAS, we used MLM models (Yu et al., 2006). Association studies were performed using R (R Core Team, 2018) using modified scripts from the MVP (https://rdrr.io/github/XiaoleiLiuBio/MVP/), GAPIT (Lipka et al., 2012) and GENESIS (Gogarten et al., 2019) packages. Visualization used scripts from MVP, GAPIT and from the R package qqman (Turner, 2014). We removed variants with MAF<5% from the relevant dataset in GWAS when using the WRC, JRC or JRC+WRC populations.

**Visualization of genotypes using TASUKE+**

Variants were genotyped by sample up to the GATK HaplotypeCaller (Poplin et al., 2018) step in the variant calling section as above, and filtered using bcftools (Li, 2011) with the condition -e “QD < 5.0 || FS > 50.0 || SOR > 3.0 || MQ < 50.0 || MQRankSum < -2.5 || ReadPosRankSum < -1.0 || ReadPosRankSum > 3.5”. Variants for each accession were displayed using the TASUKE+ genome browser (Kumagai et al., 2019). TASUKE+ is a web browser-based visualization system for whole-genome variant data. The input files of WRC for TASUKE were created using a custom data analysis pipeline, using the same mapping and variant calls as for the GWAS variant dataset, but without filtering for minor allele frequency or allele number. These datasets are accessible at https://ricegenome-corecollection.dna.affrc.go.jp and users can choose a specific region and/or gene in which they are interested.

**Accession codes**

The sequence data has been deposited in DNA Data Bank of Japan Sequence Read Archive (DRA). The accession numbers are from DRR240814 to DRR240859 and DRR095338, DRR095344, DRR095348, DRR095346 (Supplementary Table S1).
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Figure legends
Figure 1 Phylogenetic tree of JRC (50 accessions) and WRC (69 accessions).
(A) Unrooted Maximum Likelihood tree of the JRC based on 1,394 SNPs called by mapping JRC resequencing data against the O. sativa ssp. japonica Nipponbare genome. Five accessions, JRC21, 41, 42, 43 and 44 were classified into the indica group (red).
(B) Unrooted Maximum Likelihood tree of JRC and WRC based on 2,004 SNPs. JRC and WRC were divided into japonica (blue), indica (red) and aus (magenta). WRC01 (Nipponbare), WRC02 (Kasalath) and WRC05 (Naba) are shown in green color as representative cultivars for each group. JRC accessions are shown in black.

Figure 2 Screen shot of Os06g0275000 (Hd1) genotypes in JRC accessions displayed using the TASUKE+ system. Each color represents the effect of sequence changes scored by SnpEff. Blue: modifier. Yellow: low. Orange: moderate. Red: high.
Figure 3 GWAS for amylose content using linear mixed model

(A) Histogram of amylose content (mass %) in JRC. (B) Manhattan plot for amylose content using JRC (top) and WRC (bottom) accessions. Arrows indicate the \textit{waxy} locus. (C) Gene structure of and DNA polymorphism in the \textit{waxy} gene. The red arrowhead indicates the position of the 23-bp insertion. The blue arrowhead indicates the position of the 67-bp deletion in JRC22. The black arrowhead indicates the position of the transposon insertion in JRC50. (D) Box-plot showing amylose content for JRC accessions bearing Haplotype A or Haplotype B at the \textit{waxy} gene.

Figure 4 GWAS for leaf blade width using linear mixed model

(A) Histogram of leaf blade width in JRC. (B) Manhattan plot for leaf blade width in JRC (top) and WRC (bottom). The arrow indicates the \textit{NAL1} locus. (C) Gene structure of and DNA polymorphism in the \textit{NAL1} gene. The red arrowhead indicates the position of the A → G substitution. (D) Box-plot showing leaf blade width for JRC accessions bearing Haplotype A or Haplotype B at the \textit{NAL1} gene.

Figure 5 GWAS for days to heading using linear mixed model

(A) Histogram of days to heading (days) in JRC. (B) Manhattan plot for days to heading in JRC (top) and WRC (bottom). The arrow indicates the \textit{HESO1} locus. (C) Gene structure of and DNA polymorphism in the \textit{HESO1} gene. The red arrowhead indicates the position of the G → A substitution. (D) Box-plot showing days to heading for JRC accessions bearing Haplotype A or Haplotype B at the \textit{HESO1} gene.

Supplemental figure 1 GWAS for amylose content with JRC+WRC and identification of
the candidate gene for peak.

(A) Histogram of seed amylose content (mass %). (B) Manhattan and QQ plots generated using linear mixed model. Arrow indicates \textit{waxy} locus. (C) Gene structure of \textit{waxy} and DNA polymorphism in that gene. The red arrowhead indicates the position of the 23-bp insertion. (D) Box-plot for amylose content based on the haplotypes for \textit{waxy} gene.

Supplemental figure 2 GWAS for leaf blade width with JRC+WRC and identification of the candidate gene for peak.

(A) Histogram of leaf blade width (cm) in JRC+WRC. (B) Manhattan and QQ plots generated using linear mixed model. Arrow indicates \textit{NAL1} locus. (C) Gene structure of \textit{NAL1} and DNA polymorphism in that gene. The red arrowhead indicates the position of the A \rightarrow G substitution. (D) Box-plot for leaf blade width based on the haplotypes for \textit{NAL1}.

Supplemental Figure 3 GWAS for days to heading with JRC+WRC and identification of the candidate gene for peak.

(A) Histogram of days to heading in JRC+WRC. (B) Manhattan and QQ plots generated using linear mixed model. Arrow indicates \textit{HESO1} locus. (C) Gene structure of \textit{HESO1} and DNA polymorphism in that gene. The red arrowhead indicates the position of the G \rightarrow A substitution. (D) Box-plot for days to heading based on the haplotypes for \textit{HESO1}.

Supplemental Figure 4 Haplotypes of \textit{NAL7} (\textit{Os03g0162000}) gene.

(A) Box-plot for leaf blade width based on the haplotypes for \textit{NAL7} in WRC. (B) Screen shot of \textit{NAL7} genotypes in WRC and JRC using TASUKE+.
Table 1 Distribution of functional mutations in JRC previously identified in the flowering-time genes. fs: frame shift. *: Stop codon. Newly identified mutations are shown in blue text.

References

Agrama, H.A., Yan, W., Lee, F., Fjellstrom, R., Chen, M.-H., Jia, M., et al. (2009) Genetic Assessment of a Mini-Core Subset Developed from the USDA Rice Genebank. *Crop Science*. 49: 1336–1346.

Auwera, G.A.V. der, Carneiro, M.O., Hartl, C., Poplin, R., Angel, G. del, Levy-Moonshine, A., et al. (2013) From FastQ Data to High-Confidence Variant Calls: The Genome Analysis Toolkit Best Practices Pipeline. *Current Protocols in Bioinformatics*. 43: 11.10.1-11.10.33.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., et al. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly*. 6: 80–92.

Ebana, K., Kojima, Y., Fukuoka, S., Nagamine, T., and Kawase, M. (2008) Development of mini core collection of Japanese rice landrace. *Breed Sci*. 58: 281–291.

Fujino, K., Matsuda, Y., Ozawa, K., Nishimura, T., Koshiba, T., Fraaije, M.W., et al. (2008) NARROW LEAF 7 controls leaf shape mediated by auxin in rice. *Mol Genet
Fujino, K., Yamanouchi, U., and Yano, M. (2013) Roles of the Hd5 gene controlling heading date for adaptation to the northern limits of rice cultivation. *Theor Appl Genet.* 126: 611–618.

Fujita, D., Trijatmiko, K.R., Tagle, A.G., Sapasap, M.V., Koide, Y., Sasaki, K., et al. (2013) NAL1 allele from a rice landrace greatly increases yield in modern indica cultivars. *PNAS.* 110: 20431–20436.

Gogarten, S.M., Sofer, T., Chen, H., Yu, C., Brody, J.A., Thornton, T.A., et al. (2019) Genetic association testing using the GENESIS R/Bioconductor package. *Bioinformatics.* 35: 5346–5348.

Hirano, H.Y., Eiguchi, M., and Sano, Y. (1998) A single base change altered the regulation of the Waxy gene at the posttranscriptional level during the domestication of rice. *Mol Biol Evol.* 15: 978–987.

Hori, K., Ogiso-Tanaka, E., Matsubara, K., Yamanouchi, U., Ebana, K., and Yano, M. (2013) Hd16, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. *The Plant Journal.* 76: 36–46.

Hori, Y., Fujimoto, R., Sato, Y., and Nishio, T. (2007) A novel wx mutation caused by insertion of a retrotransposon-like sequence in a glutinous cultivar of rice (*Oryza*
sativa). Theor Appl Genet. 115: 217–224.

Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., et al. (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics. 42: 961–967.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, A., Zhao, Q., et al. (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nature Genetics. 44: 32–39.

Iijima, K., Suzuki, K., Hori, K., Ebana, K., Kimura, K., Tsujii, Y., et al. (2019) Endosperm enzyme activity is responsible for texture and eating quality of cooked rice grains in Japanese cultivars. Bioscience, Biotechnology, and Biochemistry. 83: 502–510.

Ishiwata, A., Ozawa, M., Nagasaki, H., Kato, M., Noda, Y., Yamaguchi, T., et al. (2013) Two WUSCHEL-related homebox Genes, narrow leaf2 and narrow leaf3, Control Leaf Width in Rice. Plant Cell Physiol. 54: 779–792.

Jiang, D., Fang, J., Lou, L., Zhao, J., Yuan, S., Yin, L., et al. (2015) Characterization of a Null Allelic Mutant of the Rice NAL1 Gene Reveals Its Role in Regulating Cell Division. PLOS ONE. 10: e0118169.

Kawahara, Y., de la Bastide, M., Hamilton, J.P., Kanamori, H., McCombie, W.R., Khush, G.S. (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol.
Kojima, Y., Ebana, K., Fukuoka, S., Nagamine, T., and Kawase, M. (2005)
Development of an RFLP-based Rice Diversity Research Set of Germplasm. *Breed Sci.*
55: 431–440.

Koo, B.-H., Yoo, S.-C., Park, J.-W., Kwon, C.-T., Lee, B.-D., An, G., et al. (2013)
Natural Variation in OsPRR37 Regulates Heading Date and Contributes to Rice
Cultivation at a Wide Range of Latitudes. *Molecular Plant.* 6: 1877–1888.

Korte, A., Vilhjálmsson, B.J., Segura, V., Platt, A., Long, Q., and Nordborg, M. (2012)
A mixed-model approach for genome-wide association studies of correlated traits in
structured populations. *Nat Genet.* 44: 1066–1071.

Kumagai, M., Kim, J., Itoh, R., and Itoh, T. (2013) Tasuke: a web-based visualization
program for large-scale resequencing data. *Bioinformatics.* 29: 1806–1808.

Kumagai, M., Nishikawa, D., Kawahara, Y., Wakimoto, H., Itoh, R., Tabei, N., et al.
(2019) TASUKE+: a web-based platform for exploring GWAS results and large-scale
resequencing data. *DNA Res.* 26: 445–452.

Lee, T.-H., Guo, H., Wang, X., Kim, C., and Paterson, A.H. (2014) SNPhylo: a pipeline
to construct a phylogenetic tree from huge SNP data. *BMC Genomics.* 15: 162.
Li, H. (2011) A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*. 27: 2987–2993.

Li, H., and Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*. 25: 1754–1760.

Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., et al. (2012) GAPIT: genome association and prediction integrated tool. *Bioinformatics*. 28: 2397–2399.

Matsubara, K., Ogiso-Tanaka, E., Hori, K., Ebana, K., Ando, T., and Yano, M. (2012) Natural Variation in Hd17, a Homolog of Arabidopsis ELF3 That is Involved in Rice Photoperiodic Flowering. *Plant Cell Physiol*. 53: 709–716.

Ochiai, K., Shimizu, A., Okumoto, Y., Fujiwara, T., and Matoh, T. (2011) Suppression of a NAC-Like Transcription Factor Gene Improves Boron-Toxicity Tolerance in Rice. *Plant Physiology*. 156: 1457–1463.

Ogiso-Tanaka, E., Matsubara, K., Yamamoto, S., Nonoue, Y., Wu, J., Fujisawa, H., et al. (2013) Natural Variation of the RICE FLOWERING LOCUS T 1 Contributes to Flowering Time Divergence in Rice. *PLOS ONE*. 8: e75959.

Poplin, R., Ruano-Rubio, V., DePristo, M.A., Fennell, T.J., Carneiro, M.O., Auwera, G.A.V. der, et al. (2018) Scaling accurate genetic variant discovery to tens of thousands
of samples. *bioRxiv*. 201178.

R Core Team (2018) R: A language and environment for statistical computing.

Suzaki, T., Ohneda, M., Toriba, T., Yoshida, A., and Hirano, H.-Y. (2009) FON2 SPARE1 Redundantly Regulates Floral Meristem Maintenance with FLORAL ORGAN NUMBER2 in Rice. *PLOS Genetics*. 5: e1000693.

Taguchi-Shiobara, F., Ozaki, H., Sato, H., Maeda, H., Kojima, Y., Ebitani, T., et al. (2013) Mapping and validation of QTLs for rice sheath blight resistance. *Breeding Science*. 63: 301–308.

Takahashi, Y., Shomura, A., Sasaki, T., and Yano, M. (2001) Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subunit of protein kinase CK2. *PNAS*. 98: 7922–7927.

Tanaka, N., Shenton, M., Kawahara, Y., Kumagai, M., Sakai, H., Kanamori, H., et al. (2020) Whole-Genome Sequencing of the NARO World Rice Core Collection (WRC) as the Basis for Diversity and Association Studies. *Plant Cell Physiol*. 61: 922–932.

Tsuji, H., Taoka, K., and Shimamoto, K. (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. *Current Opinion in Plant Biology*. 14: 45–52.
Turner, S.D. (2014) qman: an R package for visualizing GWAS results using Q-Q and manhattan plots. *bioRxiv*. 005165.

Ueno, D., Kono, I., Yokosho, K., Ando, T., Yano, M., and Ma, J.F. (2009) A major quantitative trait locus controlling cadmium translocation in rice (Oryza sativa). *New Phytologist*. 182: 644–653.

Ueno, D., Yamaji, N., Kono, I., Huang, C.F., Ando, T., Yano, M., et al. (2010) Gene limiting cadmium accumulation in rice. *PNAS*. 107: 16500–16505.

Wanchana, S., Toojinda, T., Tragoonrung, S., and Vanavichit, A. (2003) Duplicated coding sequence in the waxy allele of tropical glutinous rice (Oryza sativa L.). *Plant Science*. 165: 1193–1199.

Xue, W., Xing, Y., Weng, X., Zhao, Y., Tang, W., Wang, L., et al. (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nature Genetics*. 40: 761–767.

Yang, M., Lu, K., Zhao, F.-J., Xie, W., Ramakrishna, P., Wang, Guangyuan, et al. (2018) Genome-Wide Association Studies Reveal the Genetic Basis of Ionomic Variation in Rice. *The Plant Cell*. 30: 2720–2740.

Yano, K., Yamamoto, E., Aya, K., Takeuchi, H., Lo, P., Hu, L., et al. (2016) Genome-wide association study using whole-genome sequencing rapidly identifies new genes
influencing agronomic traits in rice. *Nature Genetics*. 48: 927–934.

Yano, K., Morinaka, Y., Wang, F., Huang, P., Takehara, S., Hirai, T., et al. (2019) GWAS with principal component analysis identifies a gene comprehensively controlling rice architecture. *PNAS*. 116: 21262–21267.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., et al. (2000) Hd1, a Major Photoperiod Sensitivity Quantitative Trait Locus in Rice, Is Closely Related to the Arabidopsis Flowering Time Gene CONSTANS. *Plant Cell*. 12: 2473–2483.

Yu, J., Pressoir, G., Briggs, W.H., Vroh Bi, I., Yamasaki, M., Doebley, J.F., et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*. 38: 203–208.

Zhang, P., Li, J., Li, X., Liu, X., Zhao, X., and Lu, Y. (2011) Population Structure and Genetic Diversity in a Rice Core Collection (Oryza sativa L.) Investigated with SSR Markers. *PLOS ONE*. 6: e27565.
Figure 1 Phylogenetic tree of JRC (50 accessions) and WRC (69 accessions).

(A) Unrooted Maximum Likelihood tree of the JRC based on 1,394 SNPs called by mapping JRC resequencing data against the O. sativa ssp. japonica Nipponbare genome. Five accessions, JRC21, 41, 42, 43 and 44 were classified into the indica group (red).

(B) Unrooted Maximum Likelihood tree of JRC and WRC based on 2,004 SNPs. JRC and WRC were divided into japonica (blue), indica (red) and aus (magenta). WRC01 (Nipponbare), WRC02 (Kasalath) and WRC05 (Naba) are shown in green color as representative cultivars for each group. JRC accessions are shown in black.
Figure 2 Screen shot of *Os06g0275000* (*Hd1*) genotypes in JRC accessions displayed using the TASUKE+ system. Each color represents the effect of sequence changes scored by SnpEff. Blue: modifier. Yellow: low. Orange: moderate. Red: high.
Figure 3 GWAS for amylose content using linear mixed model
(A) Histogram of amylose content (mass %) in JRC.
(B) Manhattan plot for amylose content in JRC (top) and WRC (bottom). Arrows indicate waxy locus. (C) Gene structure of waxy and DNA polymorphism in that gene. Red arrowhead indicates the locus of 23-bp insertion. Blue arrowhead indicates the locus of 67-bp deletion in JRC22. Black arrowhead indicates the locus of transposon insertion in JRC50.
(D) Box plot showing amylose content for JRC accessions bearing Haplotype A or Haplotype B at the waxy gene.
Figure 4 GWAS for leaf blade width using linear mixed model
(A) Histogram of leaf blade width in JRC.
(B) Manhattan plot for leaf blade width in JRC (top) and WRC (bottom). Arrow indicates NAL1 locus. (C) Gene structure of NAL1 and DNA polymorphism in that gene. Red arrowhead indicates the locus of A → G substitution. (D) Box-plot showing leaf blade width for JRC accessions bearing Haplotype A or Haplotype B at the NAL1 gene.
Figure 5 GWAS for days to heading using linear mixed model

(A) Histogram of days to heading (days) in JRC.

(B) Manhattan plot for days to heading in JRC (top) and WRC (bottom). Arrow indicates HESO1 locus. (C) Gene structure of HESO1 and DNA polymorphism in that gene. Red arrowhead indicates the locus of G → A substitution. (D) Box-plot showing days to heading for JRC accessions bearing Haplotype A or Haplotype B at the HESO1 gene.
Table 1 Distribution of functional mutations in JRC previously identified in the flowering-time genes. fs: frame shift. *: Stop codon. Newly identified mutations are shown in blue text.

| QTLs | Indel/SNPs | Substitution | Reference | JRC accessions |
|------|------------|--------------|-----------|----------------|
| Hd1  | 4bp Del    | Glu230fs     | Yano et al., 2000 | JRC7 |
|      | 43bp Del   | Pro237fs     | Yano et al., 2000 | JRC20, 49, 50 |
|      | 2bp Del    | Phe279fs     | Yano et al., 2000 | JRC18 |
|      | G->T       | Gly24*       |           | JRC22 |
|      | C->A       | Ser57*       |           | JRC88 |
|      | 1bp del    | Arg82fs      |           | JRC21, 42, 43 |
|      | 8bp Del    | Lys505fs     |           | JRC44 |
|      | T->C       | Tyr704His    | Koo et al., 2013 | JRC41 |
|      | G->A       | Ala331Thr    | Hori et al., 2013 | JRC50 |
|      | A->T       | *146Lys      | Takahashi et al., 2001 | Except for JRC 23, 27, 30, 50 |
|      | C->A       | Glu53*       | Xue et al., 2008 | JRC17 |
|      | G->A       | Glu105Lys    | Ogiso-Tanaka et al., 2013 | JRC21, 42, 43 |
|      | 1bp Del    | Lys108fs     |           | JRC21, 43, 44 |
|      | A->G       | Leu558Ser    | Matsubara et al., 2012 | JRC1-17, 21, 26-29, 40-44, 46-48, 50, 54 |