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# Measurements of Antibacterial Activity of Seed Crude Extracts in Cultivated Rice and Wild *Oryza* Species

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# Abstract

Seeds are continuously exposed to a wide variety of microorganisms in the soil. In addition, seeds contain large amounts of carbon and nitrogen sources that support initial growth after germination. Thus, seeds in the soil can easily promote microbial growth, and seeds are susceptible to decay. Therefore, seed defense against microorganisms is important for plant survival. Seed-microbe interactions are also important issues from the perspective of food production, in seed quality and shelf life. However, seed-microbe interactions remain largely unexplored. In this study, we established a simple and rapid assay system for the antibacterial activity of rice seed crude extracts by colorimetric quantification methods by the reduction of tetrazolium compound. Using this experimental system, the diversity of effects of rice seed extracts on microbial growth was analyzed using *Escherichia coli* as a bacterial model. We used collections of cultivated rice, comprising 50 accessions of Japanese landraces, 52 accessions of world rice core collections, and of 30 wild *Oryza* accessions. Furthermore, we attempted to find genetic factors responsible for the diversity by genome-wide association analysis. Our results demonstrate that this experimental system can easily analyze the effects of seed extracts on bacterial growth. It also suggests that there are various compounds in rice seeds that affect microbial growth. Overall, this experimental system can be used to clarify the chemical entities and genetic control of seed-microbe interactions and will open the door for understanding the diverse seed-microbe interactions through metabolites.

Keywords: Rice, Oryza, Core collection, Wild rice, Seed, Antibacterial activity, GWAS

# Background

Plants are exposed to a wide variety of microorganisms throughout their life cycle and constantly interact with these microorganisms. Among the microorganisms that interact with plants, symbiotic microorganisms such as mycorrhizal fungi absorb nutrients from the soil and supply them to plants, thereby positively affecting plant growth. On the other hand, infection and proliferation by microorganisms can negatively affect plant growth. It is known that plants prevent infection by releasing a group of compounds with antimicrobial activity against

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pathogenic microorganisms (Bednarek and Osbourn 2009). Many of these compound groups are secondary metabolites, and plants have acquired the ability to produce a variety of antimicrobial secondary metabolites in their interactions with microorganisms. Phytoanticipin and phytoalexin are antimicrobial compounds that are synthesized inducibly or not, respectively, and the same compound may act as a phytoanticipin or phytoalexin depending on plant type and organs (VanEtten et al. 1994; Morrissey and Osbourn 1999; Jeandet 2018).

Plant chemical defense using these antimicrobial secondary metabolites has been extensively studied, and to date, numerous antimicrobial secondary metabolites have been found in various plant species. Saponins are well known as phytoanticipins, with  $\alpha$ -tomatin in



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tomatoes and avenacin in oats (Oros and Kállai 2019). For phytoalexins, their bioactivity, synthetic pathways, and induction mechanisms have been analyzed in various plant species (Liu et al. 2010; Schmelz et al. 2011; Ube et al. 2021).

Seed defense against microorganisms is especially important because seeds store sufficient nutrients for seedling establishment, and these seed reserves are also a source of nutrients for microorganisms. Seed defense mechanisms against soil microorganisms can be broadly classified into physical, biochemical, and chemical defenses (Dalling et al. 2020). In physical defense, the seed coat and pericarp serve as a physical barrier between the seed, including the embryo, and its external environment, and suppress microbial invasion (Gergerich and Dolja 2006). Polyphenol oxidase (PPO) is a known defense enzyme involved in biochemical defense, which accumulates in wild oat and wheat glumes (Jerkovic et al. 2010; Fuerst et al. 2011, 2018). PPO catalyzes the conversion of phenolic compounds to quinones, and the quinones may protect seeds from microbes by damaging microbial cell walls (Baltas et al. 2017; Alibi et al. 2021).

Various antimicrobial secondary metabolites identified in studies with rhizosphere and aboveground parts of plants (leaves) have also been identified in seeds (Dalling et al. 2011; Ben-Abu and Itsko 2021; Ishihara 2021). This suggests that chemical defense against microorganisms via antimicrobial secondary metabolites may also operate in seeds.

Momilactones, well-known antimicrobial secondary metabolites of rice, have been studied not only for their functions as phytoalexins but also for their biosynthetic pathways and diversity (Kato et al. 1973; Izawa and Shimamoto 1996; Cartwright et al. 1981; Peters 2006; Schmelz et al. 2014). More than 20 phytoalexins have been reported to accumulate in rice (Kodama et al. 1992; Yamane 2013; Park et al. 2013, 2014; Ishihara et al. 2008; Morimoto et al. 2018; Kariya et al. 2020). The World Rice Core Collection (WRC) (Kojima et al. 2005) and the Rice Core Collection of Japanese Landraces (JRC) (Ebana et al. 2008), which are provided by the National Agriculture and Food Research Organization (NARO, Japan), cover a wide range of genetic diversity of landraces in rice, and they are useful material for the isolation of related quantitative trait loci by GWAS (genome-wide association studies) and for the elucidation of loci associated with phenotypic diversity (Tanaka et al. 2020, 2021). Analysis using the WRC revealed that the amount and type of phytoalexins induced in stress-treated leaves differ between accessions, and novel antimicrobial secondary metabolites have been found in the leaves of several accessions (Kariya et al. 2019, 2020; Murata et al. 2020). In addition, Friedman (2013) reported that different rice secondary metabolites are produced in specific organs and tissues.

The genus Oryza consists of 23 species, of which 2 are cultivated and the remaining 21 are wild species, with 11 genome types (AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ, HHKK) (Lu et al. 2009; Jacquemin et al. 2013; Nonomura et al. 2010; Sato et al. 2021; Kajiya-Kanegae et al. 2021). Remarkable differences have been observed between metabolite diversity in wild and cultivated Oryza species (Atwell et al. 2014). Among the wild Oryza species, momilactones are produced in species with AA genome (O. barthii, O. glumaepatula, O. meridionalis and O. rufipogon) and BB genome (O. punctata), but not in O. brachyantha with FF genome, and phytocasans are produced in species with AA genome but not in species with BB genome or FF genome (Miyamoto et al. 2016). These findings suggest that the composition and the amount of secondary metabolites produced by wild and cultivated Oryza species are different. Therefore, wild Oryza species may accumulate antimicrobial secondary metabolites that are not present in cultivated species, and wild Oryza species are attractive materials to search for novel and useful secondary metabolites.

Disk diffusion method is the most common method for measuring the antimicrobial activity. However, quantification of antimicrobial activity is difficult with this method. To overcome these issues, we performed colorimetric assays using tetrazolium salts, which are widely used to measure cell growth, mainly for mammalian cells but also for bacteria (Eloff 1998; Tsukatani et al. 2009; Haase et al. 2017; Grela et al. 2015; Benov 2021).

To understand the chemical defense of seeds mediated by antimicrobial secondary metabolites, we developed a simple and rapid assay system to evaluate the antibacterial activity of rice seed crude extracts using disk diffusion and colorimetric quantification methods (Fig. 1). Furthermore, we investigated the diversity of antibacterial activity of rice seeds using the rice core collection of landraces WRC, JRC and wild Oryza species, and searched for genetic factors associated with the diversity of antibacterial activity in the landraces using GWAS. Here we demonstrate a colorimetric assay system that can detect antibacterial activity of rice seed crude extracts with higher sensitivity than the disk diffusion method. The results of the measurements of antibacterial activity of rice seeds of landraces and wild Oryza species using this assay system suggest that the antibacterial activity differs between accessions and that several types of antibacterial secondary metabolites are extracted with different solvents. Through comparing the antibacterial activity of husk and brown rice extracts, different types and amounts of antibacterial compounds are suggested to accumulate in these organs. Furthermore, GWAS



using the antibacterial activity of husk and brown rice extracts measured by our assay system detected several genomic regions related to the diversity of antibacterial activity. The assay system established in this study using diverse genetic resources is expected to serve as a stepping stone to explore seed-microbe interactions and antibacterial secondary metabolites produced by seeds and to contribute to the elucidation of their genetic basis.

# **Materials and Methods**

#### **Plant Materials**

The seeds of World Rice Core Collection (WRC) (Kojima et al. 2005) and Rice Core Collection of Japanese landraces (JRC) (Ebana et al. 2008) were obtained from the National Agriculture and Food Research Organization Genebank (NARO gene bank, Japan, http://www.gene. affrc.go.jp/index\_j.php). The seeds of cultivated rice (*Oryza sativa* L. 'T65') and wild *Oryza* species were provided by the National Institute of Genetics (NIG, Japan, https://www.nig.ac.jp/nig/ja/). Plants were grown under natural light condition in a green-house and transplanted to paddy fields at NIG in Mishima, Japan, and the collected seeds were analyzed in this study.

#### Preparation of Seed Crude Extracts

The collected seeds were separated into husks and brown rice using testing rice husker (Fujiwara factory, Tokyo, Japan). The weight of husks and brown rice in 10 grains were measured (Additional file 1: Table S1). Because the weight of husks and brown rice in one accession are constant among grains, we use fix number of grains in all experiments so that weight of tissues used for extraction become constant in replicated experiments. To prepare husk and brown rice crude extracts, rice husks of 20 grains and 10 grains of brown rice were pulverized in 5 mL microtubes, respectively, by a multi-beads shocker (Yasui Kikai Corporation, Japan). Powdered samples in 5 mL microtubes were immersed in 4 mL of four different solvents (80% MeOH, diethyl ether, acetone, and sterilized water, respectively) under two different temperature (room temperature for diethyl ether and acetone extraction, 80 °C for 80% MeOH and sterilized water extraction) for 24 h. For brown rice crude extracts, diethyl ether was not used as solvent. After filter-sterilization using 0.22  $\mu$ m membrane filters (Membrane Solution Limited, USA), solvents were removed by a centrifugal concentrator and each crude extracts was stored at – 20 °C for further analysis.

#### Antibacterial Activity Test by Disk Diffusion Method

Antibacterial activity of the rice husk crude extract was determined against Eschelicia coli DH5a by disk diffusion method according to Fukuta et al. (2007), with some modifications. Muller-Hinton (MH) broth medium was used to grow the bacteria. Single colony of E. coli were inoculated to 4 mL of MH broth and incubated at 37 °C for overnight at 180 rpm in the shaker. Cell density of overnight cultures of E. coli was determined by spectrophotometer (Bio-Rad Laboratories, USA) and suspended in 0.9% sterilized saline to  $2.0 \times 10^5$  cell/mL. 500 µL of the bacteria suspension was spread evenly on each MH agar plate (9 cm diameter). Crude extracts of 20 grains of rice husk (solvents: MeOH, acetone, and sterilized water, respectively) and 80 grains of rice husk (solvent: diethyl ether) were dried and dissolved in 30 µL of 100% dimethyl sulfoxide (DMSO). The crude extracts of 20 grains of rice husk, and only diethyl ether crude extracts were equal to 80 grains. Sterilized paper disks (6 mm diameter, ADVANTEC) were impregnated by 30  $\mu$ L of each extract dissolved in 100% DMSO and laid on the surface of MH agar plates. Ampicillin (10  $\mu$ g/mL) and 100% DMSO were used as positive and negative control, respectively. The plates were incubated for 24 h at 37 °C and inhibition zone was measured.

# Antibacterial Activity Test by Colorimetric Quantification Assay

Antibacterial activity of rice husk and brown rice crude extracts was determined by microtiter plate bioassay method using the tetrazolium compound [3-(4,5-dimeth-ylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)] as a color indicator (CellTiter 96<sup>®</sup> AQueous One Solution Cell Proliferation Assay, Promega, USA) according to the manufacture's instructions. Relative growth rate of *E. coli*, which represents the antibacterial activity of the sample extracts, were measured for each sample extracts. DMSO and antibiotics (ampicillin and kanamycin) are negative and positive controls of antibacterial activity.

### Concentration-Dependent Growth Inhibitory Effect of Seed Crude Extracts on *Escherichia coli*

Rice husk crude extracts were prepared from accessions that showed antibacterial activity by the disk diffusion method and their concentration-dependent growth inhibitory effect on *E. coli* was investigated. To determine concentration-dependent effects, rice husk crude extracts were prepared from 20 grains of rice husk. The extracts were dried and dissolved in 40  $\mu$ L DMSO for 80% MeOH extracts, 20  $\mu$ L DMSO for acetone extracts and 10  $\mu$ L DMSO for sterilized water extracts, respectively, as stock solutions. Brown rice crude extracts were dried and dissolved in 10  $\mu$ L of DMSO as stock solution.

20  $\mu$ L rice crude extracts were transferred to the first well of 96-well plate and serial dilution was done by transferring 10  $\mu$ L mixture via multichannel pipette. Then, 180  $\mu$ L of MH broth was poured into each of the 96 wells of the assay plate. Bacterial cells were added at a density of 4.0 × 10<sup>6</sup> cells/mL (10  $\mu$ L) in 96 wells. Ampicillin and kanamycin were used as a positive control. A growth control with 10  $\mu$ L suspension solvent and blank control without bacteria were also loaded in the assay plate. The assay plates were incubated at 37 °C for 5 h in a humid environment. After incubation, 40  $\mu$ L of MTS solution was added to each well and mixed. The plates were then centrifuged at 600 × *g* for 5 min and 100  $\mu$ L of the supernatant was transferred to a new 96-well plate and the absorbance at 450 nm at 0 h was measured using

a microplate reader. The assay plates were incubated again at 37 °C for 4 h. After the second incubation, the plate proceeded to centrifugation at  $600 \times g$  for 5 min and 100 µL of the supernatant was transferred to a new plate and the absorbance of after 4 h was measured. Relative growth rates of bacteria treated with antibiotics and/ or crude extracts were calculated by taking the increase in absorbance of the growth control wells as 1. Relative growth rate was calculated as the change in absorbance after 4 h in wells treated with antibiotics and/or seed crude extract. The increase in absorbance after 4 h in wells of the growth control was set as 100%.

# Evaluation of Antibacterial Activity of Seeds of Cultivated and Wild Rice Core Collections

Antibacterial activity assays for cultivated and wild *Oryza* species were analyzed in the same way using certain dilutions of each crude extract as described above. Based on the results of the measurements of concentration-dependent antibacterial activity, the dilution factors that showed stronger activity than the value of 50% of maximum inhibitory concentration (IC50) in the rice husk and brown rice crude extracts using three different solvents were used in the subsequent assays.

#### **Construction of Genetic Map**

Sequence data for JRC and WRC derived from the previous study in Tanaka et al. (2020) and Tanaka et al. (2021) were downloaded from DNA Data Bank of Japan Sequence Read Archive. Trimming of raw paired-end reads and subsequent mapping against Os-Nipponbare-Reference-IRGSP-1.0 (Kawahara et al. 2013) were performed using Galaxy/NAAC, a web-based platform for a bioinformatics analysis (https://galaxy.dna.affrc.go.jp/ nias/static/register\_en.html). "Trimmomatic" function (Galaxy Version 0.36.3) and "BWA mapping Illumina" workflow were used for the removal of low quality reads and mapping against the reference sequence, respectively. Obtained bam files were used to create gVCF files using GATK (ver.4.2.2.0) HaplotypeCaller (McKenna et al. 2010; DePristo et al. 2011), and then gVCF files were consolidated with CombineGVCFs. Variants called with GenotypeGVCFs were then filtered using "view" function in bcftools (Li 2011) with the following parameters: -m2 -M2 -g ^het --output-type z --excludeuncalled -e "MAF < 0.05 || N\_MISSING > 17 || QD < 2.0 || QUAL<30.0 || SOR>3.0 || FS>60.0 || MQ<40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0" to obtain 1,213,105 variants with the maximum minor allele frequency of 5% and the minimum call rate of 85% without heterozygous haplotype.

#### GWAS

The Weighted Mixed Linear Model in TASSEL (Bradbury et al. 2007) was used for the GWAS with the option of Re-estimate after each marker. The visualization of manhattan plots and qq plots were performed with R package qqman (Turner 2014). The false discovery rate (FDR) was calculated with Benjamini-Hochberg procedure (Benjamini and Hochberg 1995), and SNPs (Single nucleotide polymorphisms) with FDR of less than 5% were considered as significant association. Genomic linkage disequilibrium (LD) decay was estimated based on the coefficients of determination  $(r^2)$  between all pairs of loci using PopLDdecay (ver. 3.41) (Zhang et al. 2019) in a 2000 kb distance, and it was considered that LD was decayed at 940 kb in our genetic map since r<sup>2</sup> was less than 0.25 at this genetic distance. Therefore, subsequent LD analysis was performed within this distance using R package LDheatmap (Shin et al. 2006), and the continuous markers with r<sup>2</sup> more than 0.5 were considered to belong to the same LD block.

#### Results

## Detection of Antibacterial Activity of Rice Seeds Crude Extracts by Disk Diffusion Methods

In order to elucidate the defense mechanism against microorganisms, we aimed to establish assay systems for the antibacterial activity of rice seeds (Fig. 1). First, a crude extract prepared in 80% MeOH from the husks of cultivated rice T65 was used to determine the antibacterial activity against Escherichia coli (E. coli) (Fig. 2a). We adopted the disk diffusion method where growth inhibition circles were formed around paper disks containing antibacterial substances. Ampicillin, a commonly used antibiotic, was selected as a positive control for antibacterial activity. Growth inhibition circles were formed around discs containing ampicillin, and similarly, growth inhibition circles were observed around discs containing rice husk crude extract. From this result, we concluded that the disc diffusion method can detect antibacterial activity derived from rice husks.

Next, the antibacterial activity of rice husk crude extract was analyzed by the disk diffusion method in 52 cultivars from the World Rice Core Collection (WRC) (Kojima et al. 2005) and 50 cultivars from the Rice Core Collection of Japanese Landraces (JRC) (Ebana et al. 2008). The crude extracts used to detect antibacterial activity were prepared from 20 seeds of rice husks, and 80% MeOH, diethyl ether, acetone, and water were used as extraction solvents. The diethyl ether crude extract was prepared from 80 seed husks.

Of a total of 106 cultivars tested, 43 showed antibacterial activity in extracts prepared from any of the solvents (Fig. 2b–e, Tables 1, 2). The cultivated rice core collection is classified into four subspecies: *indica, aus*, temperate *japonica*, and tropical *japonica* (Tanaka et al. 2020, 2021). Of the 43 cultivars that showed antibacterial activity, 12 (43%), 9 (45%), 8 (21%), and 14 (70%) belonged to *indica*, *aus*, temperate *japonica*, and tropical *japonica*, respectively (Fig. 3a). Extracts from many of the 43 varieties showed antibacterial activity in one of the four solvents (Fig. 3b). On the other hand, extracts from several varieties showed antibacterial activity in two or more solvents. These varieties may contain multiple antibacterial compounds extracted by different solvents (Fig. 3b).

## Quantitative Measurements of Antibacterial Activity of Rice Seed Crude Extracts

Since it is difficult to quantify antibacterial activity by the disk diffusion method, we developed a simple and rapid assay system for the quantitative measurement of antibacterial activity (Fig. 1). In living cells, the tetrazolium salt, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt), is reduced to form formazan. In the MTS assay, the production of formazan by bacterial growth is measured by its specific absorbance and thus effects addition of seed crude extracts on the bacterial growth can be quantified in small-scale culture with high sensitivity. The growth inhibition of bacteria cultured in the presence of the antibiotics ampicillin or kanamycin was examined by the MTS assay and we successfully detected a concentration-dependent decrease in the growth of E. coli (Fig. 4a).

Next, we selected four varieties whose antibacterial activity was detected by the disk diffusion method in extracts in each solvent and confirmed the detection of antibacterial activity by the MTS method. As a result, we were able to confirm the growth inhibition effect in extracts from three solvents other than diethyl ether (Fig. 4b). It may be possible that, due to the extremely low solubility of ether extract in DMSO, antibacterial activity is not detected in the MTS assay from diethyl ether extracts. For the above reasons, extracts from three extraction solvents were used for subsequent assays: 80% MeOH, acetone, and water. The dose dependence on bacterial growth inhibition was then examined using husk extracts with the strongest antibacterial activity in each solvent. The half-maximal inhibitory concentrations (IC50) were between two- and three-fold dilutions for 80% MeOH extracts, and around four-fold dilution for acetone and 16-fold dilutions for water extracts, that are equivalent to the amounts of extracts from 2.5, 5, and 2.5 grains dissolved in 10 ul of DMSO, respectively (Fig. 4c-e). Based on these results, we decided to dilute the extracts used in the subsequent assays to fourfold



World rice core collection		Solvents used to prepare husk crude extracts					
Accession #	Subspecies	80% MeOH	Diethyl ether	Acetone	Water		
WRC 01	Temperate japonica	_	_	_	_		
WRC 02	Aus	_	_	_	_		
WRC 03	Indica	_	_	_	_		
WRC 04	Aus	+	_	_	_		
WRC 05	Indica	_	_	_	_		
WRC 06	Indica	_	+	+	_		
WRC 07	Indica	_	_	-	_		
WRC 09	Indica	_	_	_	_		
WRC 10	Indica	_	_	_	_		
WRC 11	Indica	+	_	_	_		
WRC 12	Indica	_	_	_	_		
WRC 13	Indica	_	_	-	_		
WRC 14	Indica	_	_	+	_		
WRC 15	Indica	_	_	_	_		
WRC 16	Indica	+	_	_	_		
WRC 17	Indica	_	_	-	+		
WRC 18	Indica	+	+	-	_		
WRC 19	Indica	+	_	-	_		
WRC 20	Indica	_	+	_	_		
WRC 21	Indica	_	_	_	_		
WRC 22	Indica	_	_	-	_		
WRC 23	Temperate japonica	_	+	+	_		
WRC 24	Indica	_	_	_	_		
WRC 25	Aus	_	_	-	_		
WRC 26	Aus	_	_	_	_		
WRC 27	Aus	_	_	+	_		
WRC 28	Aus	_	_	_	_		
WRC 29	Aus	+	_	_	_		
WRC 30	Aus	_	_	+	_		
WRC 31	Aus	+	_	_	_		
WRC 32	Aus	_	_	_	_		
WRC 33	Aus	_	_	_	_		
WRC 34	Aus	_	+	_	_		
WRC 35	Aus	-	_	_	_		
WRC 36	Aus	+	+	_	_		
WRC 37	Aus	_	_	_	_		
WRC 38	Aus	_	_	_	+		
WRC 39	Aus	-	-	-	_		
WRC 40	Aus	_	+	_	_		
WRC 41	Aus	_	-	-	_		
WRC 42	Aus	-	_	_	-		
WRC 43	Temperate japonica	_	_	_	_		
WRC 44	Indica	_	_	_	_		
WRC 45	Tropical Japonica	_	_	+	+		
WRC 46	Tropical Japonica	+	_	_	_		
WRC 47	Tropical Japonica	-	_	_	+		
WRC 48	Tropical Japonica	_	_	_	_		

# Table 1 Results of antimicrobial activity tests using husk crude extracts from accessions in World Rice Core Collection

World rice core collection		Solvents used to prepare husk crude extracts					
Accession #	Subspecies	80% MeOH	Diethyl ether	Acetone	Water		
WRC 49	Tropical Japonica	_	+	_	_		
WRC 51	Tropical Japonica	+	_	_	+		
WRC 52	Tropical Japonica	+	_	_	_		
WRC 55	Tropical Japonica	+	_	_	+		
WRC 57	Indica	-	+	+	+		

+ and - represent accessions with or without antimicrobial activity

and eightfold for 80% MeOH and acetone, and for water extracts.

# Quantification of Antibacterial Activity in Extracts from Husk and Brown Rice Using Collections of Cultivated Rice

The MTS assay was used to quantify the antibacterial activity of husk extracts derived from 107 cultivated rice varieties (106 core collection varieties + T65) (Fig. 5a–c). 80% MeOH extracts from most of the 107 varieties inhibited bacterial growth to less than 50%, with extracts from 15 cultivars inhibited to less than 20% (Fig. 5a). Acetone extracts from 7 of the 107 cultivars inhibited growth to less than 50%, with extracts from 3 cultivars inhibited growth to less than 20%. (Fig. 5b). Water extracts from most of the 107 cultivars inhibited growth to less than 50%, with extracts from 36 cultivars inhibited growth to less than 20%. (Fig. 5c). Extracts with antibacterial activities in disk diffusion methods also showed strong antibacterial activities in the MTS assay and the MTS assay detect antibacterial activities in a broader range of cultivars. Thus, the MTS assay seems more sensitive in detecting antibacterial activities.

The antibacterial activity exhibited by rice seeds is not only derived from the husks but brown rice is also known to possess antimicrobial activity (Gianinetti et al. 2018; Pumirat and Luplertlop 2013). Therefore, we attempted to detect the antibacterial activity of brown rice by MTS assay using 107 cultivated rice varieties (106 core collection varieties + T65). 80% MeOH extracts from 28 of the 107 varieties inhibited bacterial growth to less than 50% and extracts from none of cultivars inhibited to less than 20% (Fig. 6a). Acetone extracts from 17 of the 107 cultivars inhibited growth to less than 50%, with extracts from 9 cultivars inhibited growth to less than 20% (Fig. 6b). Water extracts from 44 of the 107 cultivars inhibited growth to less than 50%, with extracts from 7 cultivars inhibited growth to less than 20% (Fig. 6c). Brown rice extracts from several varieties promoted the growth of bacteria (Fig. 6a-c). Since brown rice is rich in carbon sources such as starch and sugar, it is possible that these compounds in the brown rice extract promoted the growth of bacteria.

# Genome-Wide Association Study to Detect Genetic Factors Causing the Diversity of Bacterial Growth by Extracts from Husk and Brown Rice from Collections of Cultivated Rice

Genome-wide association study was conducted to detect genetic factors causing the diversity of bacterial growth by extracts from husks and brown rice from collections of cultivated species of rice using the effect of bacterial growth measured by the MTS assay as phenotype data. SNPs significantly associated with the phenotype at  $a - \log 10(p)$  value greater than 5 were detected in the phenotype data of 80% MeOH rice hull extract, acetone brown rice extract, and 80% MeOH brown rice extract (Fig. 7a-c, Additional file 2: Fig. S1). Among these SNPs, a total of 499 SNPs were selected based on filters of FDR < 0.05 and significant associations below the *p*-values, and these SNPs were located in 42 linkage disequilibrium (LD) blocks (Table 3). Next, we examined the effect of each haplotype on the bacterial growth at five SNPs with the lowest *p*-values in an LD block, we found significant differences in bacterial growth among haplotypes for four of the five SNPs (Fig. 7 d-g). The linkage disequilibrium regions where the four SNPs locate cover the 43 kb, 206 kb, 161 kb, and 28 kb regions on chromosomes 3, 4, 7, and 9, respectively. This suggests that there are single or multiple genetic factors affecting bacterial growth by seed extracts in these regions. Thus, the quantitative measurement of antibacterial activity using the MTS method can be used to approach the genetic basis of the production of seed-derived chemicals that affect bacterial growth using GWAS and possibly other methods such as biparental QTL analysis.

Rice Core Collection of Japanese Landraces		Solvents used to prepare husk crude extracts					
Accession #	Subspecies	80% MeOH	Diethyl ether	Acetone	Water		
JRC 01	Tropical Japonica		_	_			
JRC 03	Tropical Japonica	+	_	_	_		
JRC 04	Tropical Japonica	+	_	_	_		
JRC 05	Tropical Japonica	+	_	_	_		
JBC 06	Tropical Japonica	_	_	_	+		
JRC 07	Tropical Japonica	_	_	_	_		
JBC 08	Tropical Japonica	_	_	_	_		
JRC 10	Tropical Japonica	+	_	_	_		
JRC 11	Tropical Japonica	_	_	_	_		
JRC 12	Tropical Japonica	_	_	_	_		
JRC 13	Tropical Japonica	_	_	_	_		
JRC 14	Tropical Japonica	+	_	_	_		
JRC 17	Temperate iaponica	_	_	_	_		
JRC 18	Temperate japonica	_	_	_	_		
JBC 19	Temperate iaponica	_	_	_	_		
JBC 20	Temperate iaponica	_	_	_	_		
JRC 21	Temperate iaponica	_	_	_	_		
JBC 22	Temperate iaponica	_	_	_	_		
JRC 23	Temperate iaponica	+	_	_	_		
JRC 24	Temperate iaponica	_	_	_	_		
JRC 25	Temperate iaponica	_	_	_	+		
JRC 26	Temperate iaponica	_	_	_	_		
JRC 27	Temperate japonica	_	_	_	+		
JRC 28	Temperate iaponica	_	_	_	_		
JRC 29	Temperate iaponica	_	_	_	_		
JBC 30	Temperate iaponica	_	_	_	_		
JBC 31	Temperate iaponica	_	_	_	_		
JRC 32	Temperate iaponica	_	_	+	_		
JRC 33	Temperate japonica	_	_	_	+		
JRC 34	Temperate japonica	_	_	_	_		
JRC 35	Temperate japonica	_	+	_	_		
JRC 36	Temperate japonica	_	_	_	_		
JRC 37	Temperate japonica	_	_	_	_		
JRC 38	Temperate iaponica	_	_	_	_		
JRC 39	Temperate iaponica	_	_	_	+		
JRC 40	Temperate japonica	_	_	_	_		
JRC 41	Indica	_	_	_	_		
JRC 42	Indica	+	+	_	_		
JRC 43	Indica	_	_	+	_		
JRC 44	Indica	_	_	_	_		
JRC 45	Temperate japonica	_	_	_	_		
JRC 46	Temperate japonica	_	_	_	_		
JRC 47	Temperate japonica	_	_	_	_		
JRC 48	Temperate japonica	_	_	_	_		
JRC 49	Temperate japonica	_	_	_	_		
JRC 50	Temperate japonica	_	_	_	_		
JRC 51	Temperate japonica	_	_	_	_		
JRC 52	Temperate japonica	_	_	_	_		
JRC 53	Temperate japonica	_	_	_	_		
JRC 54	Temperate japonica	_	_	_	_		

Table 2 Results of antimicrobial activity tests using husk crude extracts from accessions in Rice Core Collection of Japanese Landraces

 $+ \mbox{ and } - \mbox{ represent accessions with or without antimicrobial activity}$ 



#### in extracts using one or more solvents in cultivated rice

# Quantification of Antibacterial Activity in Extracts of Husk and Brown Rice from Collections of Wild *Oryza* Species

In order to understand the diversity of seed defense against environmental microorganisms among wild *Oryza* species, we measured antibacterial activity in extracts of husk and brown rice from 30 accessions covering 15 wild *Oryza* species with 8 genome types by MTS assay (Kurata et al. 2010; Nonomura et al. 2010). As reference, we also measured antibacterial activity in two cultivars, Nipponbare (NP) and Kasalath (KS). 80% MeOH extracts of husks from 15 of the 30 accessions inhibited bacterial growth to less than 50% and extracts from two of them inhibited to less than 20% (Fig. 8a). Acetone extracts of husks from 8 of

the 30 accessions inhibited growth to less than 50%, with extracts from one accession inhibited growth to less than 20% (Fig. 8b). Water extracts of husk from all 30 accessions inhibited growth to less than 50%, with extracts from 10 of them inhibited growth to less than 20% (Fig. 8c). 80% MeOH extracts of brown rice from none of the 30 accessions inhibited bacterial growth to less than 50% (Fig. 8d). Acetone extracts of brown rice from most of the 30 accessions inhibited growth to less than 50%, with extracts from 10 accessions inhibited growth to less than 20% (Fig. 8e). Water extracts of brown rice from all 30 accessions inhibited growth to less than 20% (Fig. 8e). Water extracts of brown rice from all 30 accessions inhibited growth to less than 50%, with extracts from 16 of them inhibited growth to less than 20% (Fig. 8f). Surprisingly,



**Fig. 4** Measurements of antibacterial activity by MTS assay. **a** Dose dependent antibacterial activity of two antibiotics measured by MTS assay. **b** Antibacterial activities measured by MTS assay using four representative accessions in each solvent used for the disk diffusion method. **c**–**e** Dose dependent antibacterial activities measured by MTS assay using extracts from accessions which showed strong antibacterial activities in **b**. x axis in **a** indicates concentration of antibiotics. x axis in **b** indicates the name of the extracts. x axis in **c** indicates concentration of specific extracts. y axis indicates relative growth rate of *E. coli*, which represents the antibacterial activity of the sample extracts. DMSO and antibiotics (ampicillin and kanamycin) are negative and positive controls of antibacterial activity. Values indicate means. Error bars indicate standard deviations. N.D. indicates undetectable level of antibacterial activity. IC50 indicates 50% of maximum inhibitory concentration of ampicillin, which is a positive control. Measurements were triplicated except for diethyl ether extracts







positive controls of antibacterial activity. Stock solution of extracts were diluted by the concentration of extracts using either 2.5 seeds (**a**, **c**) or 5 seeds (**b**) for extraction and used for the measurement. All measurements were triplicated. Values indicate means. Error bars indicate standard deviation. Red boxes represent accessions with growth inhibition less than 20% in MTS assay. N.D. indicates undetectable level of antibacterial activity. IC50 indicates 50% of maximum inhibitory concentration of ampicillin, which is a positive control





the growth-promoting effect which was often observed in brown rice acetone extracts from cultivated species was barely observed in wild Oryza, instead, brown rice 80% MeOH extracts from several wild Oryza promoted bacterial growth compared to cultivated species. Another important finding in the analysis using wild Oryza is that there are several accessions whose seed extracts from two or more solvents show strong bacterial growth inhibition. For example, both water and 80% MeOH extracts of husks of W1169 (O. glumaepatula) showed strong bacterial growth inhibition. Similarly, brown rice extracts with two different solvents from W1166 (O. latifolia) and W1921 (O. rufipogon) showed strong growth inhibition. In addition, brown rice extracts from all three solvents in W1401 (O. brachyantha) showed very strong (undetectable levels of bacterial growth) or relatively strong growth inhibition. These results suggest that some of wild Oryza accumulate multiple growth inhibitory substances in grains. Overall, the MTS assay is also applicable to assess the effects on bacterial growth by the seed extracts prepared from wild *Oryza*.

# Discussion

Seeds interact with a variety of microorganisms in the environment, and microbial infection and proliferation can cause seed rot and non-germination (Mizobuchi et al. 2018; Fuerst et al. 2014, 2018). Although the interactions between plants and the microorganisms that infect them have been studied mainly in the aboveground plant body and rhizosphere, much is not known about the interactions between seeds and microorganisms, especially the defense mechanisms of seeds against microorganisms. It is known that defense against microorganisms in the environment is important for seeds to germinate. One of the defense mechanisms of seeds is chemical defense by

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
1	1	Yes	1	18,005,951	5.5737002
2	2		1	27,878,359	5.8942855
2	3		1	27,880,379	5.8942855
2	4		1	27,880,453	5.8942855
2	5		1	27,881,781	5.8942855
2	6		1	27,881,834	5.8942855
2	7		1	27,882,081	5.8942855
2	8		1	27,882,248	5.8942855
2	9		1	27,882,325	5.8942855
2	10		1	27,883,352	5.7644463
2	11		1	27,885,038	6.0783968
2	12		1	27,886,765	6.1436155
2	13		1	27,886,937	6.0783968
2	14		1	27,887,170	6.0783968
2	15		1	27,887,655	6.0783968
2	16		1	27,887,678	6.0783968
2	17		1	27,887,813	6.0783968
2	18		1	27,887,821	6.0783968
2	19		1	27,887,894	6.0783968
2	20		1	27,889,339	6.0783968
2	21		1	27,889,390	6.0783968
2	22		1	27,890,608	6.0092839
2	23		1	27,891,417	5.8942855
2	24		1	27,891,708	6.0783968
2	25		1	27,892,211	5.8573603
2	26		1	27,893,209	5.8942855
2	27		1	27,893,722	5.8573603
2	28		1	27,893,863	5.8942855
2	29		1	27,894,089	5.8942855
2	30		1	27,894,427	5.8573603
2	31		1	27,894,659	5.8942855
2	32		1	27,894,833	5.8942855
2	33		1	27,894,855	5.8942855
2	34		1	27,895,567	5.8942855
2	35		1	27,895,950	5.8942855
2	36		1	27,897,746	5.8942855
2	37		1	27,897,749	5.8942855
2	38		1	27,905,911	5.8942855
2	39		1	27,905,926	5.8942855
2	40		1	27,906,578	5.8942855
2	41		1	27,906,583	5.8705357
2	42		1	27,907,200	5.8942855
2	43		1	27,910,437	5.8942855
2	44		1	27,916,341	5.8942855
2	45		1	27,921,389	5.8942855
2	46		1	27,921,462	5.8942855
2	47		1	27,921,505	5.8942855
2	48		1	27,923,341	5.8942855

**Table 3** List of significantly associated SNPs in GWAS using antimicrobial activities in the 107 accessions of landraces from WRC andJRC

2         49         1         2/925,445         S.8942855           2         50         1         2/924,71         S.8942855           2         52         52         1         2/924,71         S.8942855           2         52         53         1         2/924,834         S.8942855           2         53         1         2/936,344         S.8921489           2         55         1         2/986,455         S.8921499           2         56         1         2/988,474         S.8921499           2         56         1         2/988,474         S.8921459           2         56         1         2/988,474         S.8921459           2         56         1         2/958,375         S.8921459           2         61         Yes         1         2/950,333         S.8942855           2         61         Yes         1         2/951,320         S.914289           2         62         1         2/951,320         S.914289           2         64         1         2/951,320         S.914289           2         66         1         2/951,320         S.994489	LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
2       50       1       27,973,741       5,884,285         2       52       5,944,285       5,894,285         2       53       1       27,973,741       5,894,285         2       55       1       27,973,741       5,892,1459         2       55       1       27,973,64,25       5,892,1459         2       55       1       27,973,643       5,892,1459         2       56       1       27,938,64,25       5,892,1459         2       56       1       27,938,64,35       5,892,1459         2       56       1       27,938,351       5,892,1459         2       58       1       27,939,515       5,892,1459         2       60       1       27,950,516       5,894,2855         2       62       1       27,950,516       5,894,2855         2       63       1       27,951,210       2,894,2855         2       66       1       27,951,210       2,894,2855         2       67       1       27,951,210       2,894,2855         2       70       1       27,951,210       2,894,285         2       73       1       27,951,210<	2	49		1	27,925,445	5.8942855
2     1     2/929,443     5494285       2     53     1     2/929,483     5494285       2     54     1     2/95,624     58021459       2     55     1     2/936,624     58021459       2     56     1     2/936,624     58021459       2     56     1     2/936,624     58021459       2     56     1     2/936,624     58021459       2     57     1     2/938,155     58221459       2     58     1     2/939,155     58221459       2     59     1     2/939,155     58221459       2     61     Yes     1     2/959,158     6194607       2     61     Yes     1     2/951,72     58442855       2     66     1     2/951,72     5844285       2     66     1     2/951,72     5842459       2     66     1     2/951,72     5842459       2     70     1     2/951,84     58921459       2     71     1     2/951,84     58921459       2     76     1     2/951,84     58921459       2     76     1     2/954,443     58921459       2	2	50		1	27,925,448	5.8942855
2       52       1       27993485       58921499         2       53       1       2793423       58921499         2       55       1       27938290       58921499         2       56       1       27938290       58921499         2       57       1       27938290       58921499         2       59       1       279392485       58921499         2       59       1       2795033       58924395         2       60       1       2795033       58942855         2       61       Yes       1       2795150       58942855         2       63       1       27951570       58942855         2       66       1       27951570       58942855         2       66       1       27951570       58942855         2       67       1       27951570       58942855         2       68       1       27951470       58921459         2       70       1       27951470       58921459         2       71       1       2795143       58921459         2       73       1       2795143       58921459 <td>2</td> <td>51</td> <td></td> <td>1</td> <td>27,929,471</td> <td>5.8942855</td>	2	51		1	27,929,471	5.8942855
2       34       1       7.936,34       5.8921499         2       55       1       2.736,624       5.8221499         2       56       1       2.738,624       5.8221499         2       56       1       2.738,624       5.8221499         2       56       1       2.7393,631       5.8921499         2       59       1       2.7496,623       5.8421459         2       61       Yes       1       2.7950,338       6.1942655         2       62       1       2.7951,520       5.8424555         2       63       1       2.7951,520       5.8424555         2       64       1       2.7951,520       5.8424555         2       66       1       2.7951,720       5.8424555         2       67       1       2.7951,710       5.8424555         2       68       1       2.7951,710       5.8424555         2       69       1       2.7951,717       5.8214799         2       71       1       2.7951,810       5.821459         2       72       74       1       2.7951,810       5.821459         2       75 <t< td=""><td>2</td><td>52</td><td></td><td>1</td><td>27,929,485</td><td>5.8942855</td></t<>	2	52		1	27,929,485	5.8942855
2       54       1       27380,425       58921499         2       56       1       27380,424       58921499         2       57       1       27380,401       58921499         2       58       1       27380,401       58921499         2       59       1       27390,33       58921499         2       59       1       27390,33       58921499         2       60       1       27390,33       58921499         2       62       1       27390,33       58924855         2       63       1       27391,516       58924855         2       66       1       27391,70       58924855         2       66       1       27391,71       5892489         2       67       1       27951,70       5892489         2       68       1       27951,71       5892489         2       70       1       27951,30       5892489         2       70       1       27951,30       5892489         2       73       1       27954,33       5892489         2       74       1       27954,33       5892489	2	53		1	27,936,334	5.8921459
25512738,6245802145925612738,3595503135258127393,5355503135258127393,53558021459260127395,15158024855261Yes127351,51658024855263127351,51658024855263127351,51658024855264127351,70758024855266127351,70758024855266127351,71758024855267127351,71758024855268127351,71758024855270127354,31758024855271127354,31758024855273127354,31758024859274127354,31758024859273127354,31758024859274127354,31758024859274127354,31758024859275127354,31758024859276127354,31758024859276127354,31758024859276127354,31758024859276127354,31758024859276127354,31758024859276127354,31758024859<	2	54		1	27,936,425	5.8921459
2       56       1       2798,041       5.8021459         2       57       1       2798,051       5.8021459         2       59       1       2799,052       5.804285         2       60       1       2799,053       5.804285         2       60       1       2799,038       6.1916607         2       62       1       2795,151       5.8942855         2       62       1       2795,151       5.8942855         2       63       1       2795,150       5.8942855         2       65       1       2795,157       5.8942855         2       66       1       2795,137       5.8942855         2       67       1       2795,137       5.8942855         2       67       1       2795,137       5.8942855         2       67       1       2795,137       5.8942855         2       70       1       2795,387,17       5.8942855         2       70       1       2795,387,17       5.8921459         2       77       1       2795,387,17       5.8921459         2       76       1       2795,483,87       5.8921459	2	55		1	27,936,624	5.8921459
2       57       1       2798,399       5.031395         2       58       1       2799,315       5.8921499         2       60       1       2799,033       5.8942855         2       60       1       2799,033       5.8942855         2       61       Yes       1       2795,151       5.8942855         2       63       1       2795,151       5.8942855         2       64       1       2795,152       5.8942855         2       66       1       2795,157       5.8942855         2       66       1       2795,157       5.8942855         2       66       1       2795,157       5.8942855         2       66       1       2795,157       5.8942855         2       67       1       2795,167       5.8942855         2       70       1       2795,371       5.8921459         2       71       1       2795,387       5.8921459         2       73       1       2795,436       5.8921459         2       74       1       2795,436       5.8921459         2       75       1       2795,436       5	2	56		1	27,938,041	5.8921459
2       58       1       27,99,515       5,892,489         2       60       1       27,99,033       5,894,285         2       61       Yes       1       27,99,033       5,894,285         2       62       1       27,99,136       5,894,285         2       63       1       27,95,150       5,894,285         2       63       1       27,95,162       5,894,285         2       65       1       27,95,167       5,894,285         2       66       1       27,95,173       5,894,285         2       66       1       27,95,173       5,894,285         2       67       1       27,95,173       5,894,285         2       69       1       27,95,173       5,894,285         2       70       1       27,95,371       5,892,1459         2       72       70       1       27,95,371       5,892,1459         2       73       1       27,95,4243       5,822,1459         2       75       1       27,95,4343       5,822,1459         2       76       1       27,95,434       5,822,1459         2       78	2	57		1	27,938,359	5.5031395
2       59       1       27,949,632       5,894,285         2       60       1       27,950,033       6,894,2855         2       62       1       27,950,035       6,101,607         2       63       1       27,951,500       5,894,2855         2       63       1       27,951,500       5,894,2855         2       66       1       27,951,700       5,894,2855         2       66       1       27,951,700       5,894,2855         2       66       1       27,951,700       5,894,2855         2       66       1       27,951,700       5,894,2855         2       67       1       27,951,700       5,894,2855         2       68       1       27,951,700       5,894,2855         2       70       1       27,953,871       5,892,1459         2       70       1       27,954,343       5,892,1459         2       72       74       1       27,954,443       5,892,1459         2       76       1       27,955,140       5,892,1459         2       77       1       27,955,140       5,892,1459         2       76	2	58		1	27,939,515	5.8921459
2       60       Yes       1       27,950,388       6,1916607         2       63       1       27,951,512       5,514,3779         2       63       1       27,951,522       5,514,3779         2       64       1       27,951,522       5,894,2855         2       66       1       27,951,622       5,894,2855         2       66       1       27,951,730       5,894,2855         2       66       1       27,951,730       5,894,2855         2       66       1       27,951,730       5,894,2855         2       66       1       27,951,730       5,894,2855         2       67       1       27,951,846       5,894,2855         2       67       1       27,953,394       5,894,285         2       70       1       27,953,394       5,892,1459         2       77       1       27,954,433       5,892,1459         2       78       1       27,954,343       5,892,1459         2       76       1       27,954,343       5,892,1459         2       78       1       27,955,180       5,892,1459         2       78	2	59		1	27,949,632	5.8942855
2       61       Yes       1       27,951,516       5,894/2855         2       63       1       27,951,516       5,894/2855         2       64       1       27,951,520       5,514,379         2       65       1       27,951,579       5,894/2855         2       66       1       27,951,730       5,894/2855         2       66       1       27,951,730       5,894/2855         2       67       1       27,951,346       5,894/2855         2       68       1       27,953,717       5,892/1499         2       70       1       27,953,394       5,892/1499         2       70       1       27,954,343       5,892/1499         2       73       1       27,954,343       5,892/1499         2       74       1       27,954,343       5,892/1499         2       75       1       27,954,343       5,892/1499         2       76       1       27,954,343       5,892/1499         2       77       1       27,954,343       5,892/1499         2       76       1       27,954,343       5,892/1499         2       77	2	60		1	27,950,033	5.8942855
2       62       1       27,951,516       5,8942855         2       63       1       27,951,520       5,184795         2       65       1       27,951,679       5,8942855         2       66       1       27,951,730       5,8942855         2       66       1       27,951,730       5,8942855         2       66       1       27,951,734       5,8942855         2       67       1       27,953,717       5,8921459         2       69       1       27,953,871       5,8921459         2       70       1       27,953,934       5,8921459         2       72       1       27,954,243       5,8921459         2       73       1       27,954,343       5,8921459         2       74       1       27,954,743       5,8921459         2       76       1       27,954,743       5,8921459         2       78       1       27,954,743       5,8921459         2       76       1       27,954,743       5,8921459         2       78       1       27,954,743       5,8921459         2       78       1       27,954,743 </td <td>2</td> <td>61</td> <td>Yes</td> <td>1</td> <td>27,950,388</td> <td>6.1916607</td>	2	61	Yes	1	27,950,388	6.1916607
2       63       1       27,951,520       5,5143779         2       64       1       27,951,622       5,942855         2       66       1       27,951,730       5,8942855         2       66       1       27,951,730       5,8942855         2       66       1       27,951,730       5,8942855         2       67       1       27,953,71       5,8942855         2       69       1       27,953,871       5,8921459         2       70       1       27,953,363       5,8921459         2       72       1       27,954,363       5,8921459         2       73       1       27,954,363       5,8921459         2       74       1       27,954,363       5,8921459         2       76       1       27,954,363       5,8921459         2       78       1       27,955,491       5,8921459         2       78       1       27,956,227       5,8921459         2       78       1       27,956,233       5,8921459         2       80       1       27,956,131       5,8921459         2       81       1       27,956,131 <td>2</td> <td>62</td> <td></td> <td>1</td> <td>27,951,516</td> <td>5.8942855</td>	2	62		1	27,951,516	5.8942855
2       64       1       27951,622       5.8942855         2       65       1       27951,730       5.8942855         2       67       1       27951,870       5.8942855         2       67       1       27951,870       5.8942855         2       68       1       27951,871       5.8921459         2       69       1       27953,871       5.8921459         2       70       1       27954,383       5.8921459         2       72       1       27954,383       5.8921459         2       73       1       27954,383       5.8921459         2       74       1       27954,743       5.8921459         2       76       1       27955,740       5.8921459         2       76       1       27955,741       5.8921459         2       78       1       27955,741       5.8921459         2       78       1       27955,741       5.8921459         2       80       1       27956,781       5.8921459         2       81       1       27956,781       5.8921459         2       82       1       27961,827       5.	2	63		1	27,951,520	5.5143779
2       65       1       27951,79       58942855         2       67       1       27951,710       58942855         2       68       1       27951,717       58921459         2       69       1       27953,871       58921459         2       70       1       27953,871       58921459         2       70       1       27953,8361       58921459         2       72       1       27954,363       58921459         2       72       1       27954,363       58921459         2       73       1       27954,363       58921459         2       75       1       27954,946       58921459         2       76       1       27955,491       58921459         2       76       1       27955,491       58921459         2       76       1       27955,491       58921459         2       78       1       27955,491       58921459         2       76       1       27955,491       58921459         2       78       1       27955,491       58921459         2       80       1       27955,491       58921459	2	64		1	27,951,622	5.8942855
2       66       1       27,951,730       5,8942855         2       67       1       27,951,846       5,8942855         2       69       1       27,953,717       5,8921459         2       70       1       27,953,971       5,8921459         2       70       1       27,953,994       5,8921459         2       72       1       27,954,363       5,8921459         2       73       1       27,954,363       5,8921459         2       74       1       27,954,363       5,8921459         2       75       1       27,954,363       5,8921459         2       76       1       27,955,491       5,8921459         2       76       1       27,955,491       5,8921459         2       78       1       27,955,491       5,8921459         2       78       1       27,956,491       5,8921459         2       80       1       27,956,491       5,8921459         2       81       1       27,956,491       5,8921459         2       82       1       27,966,101       5,8921459         2       83       1       27,966,101<	2	65		1	27,951,679	5.8942855
2       67       1       27,951,846       5,8942855         2       68       1       27,953,917       5,8921459         2       70       1       27,953,987       5,8921459         2       70       1       27,953,984       5,8921459         2       71       1       27,954,243       5,8921459         2       72       1       27,954,343       5,8921459         2       73       1       27,954,436       5,8921459         2       74       1       27,954,436       5,8921459         2       75       1       27,954,436       5,8921459         2       76       1       27,954,436       5,8921459         2       76       1       27,954,743       5,8921459         2       76       1       27,954,743       5,8921459         2       78       1       27,955,801       5,8921459         2       78       1       27,956,293       5,8921459         2       80       1       27,961,815       5,8921459         2       81       1       27,961,815       5,8921459         2       82       1       27,961,815<	2	66		1	27,951,730	5.8942855
2       68       1       27,953,717       5,892,1459         2       69       1       27,953,871       5,892,1459         2       70       1       27,953,871       5,892,1459         2       71       1       27,954,243       5,892,1459         2       72       1       27,954,243       5,892,1459         2       73       1       27,954,343       5,892,1459         2       73       1       27,954,343       5,892,1459         2       74       1       27,954,343       5,892,1459         2       76       1       27,954,743       5,892,1459         2       76       1       27,955,180       5,892,1459         2       76       1       27,955,191       5,892,1459         2       78       1       27,956,293       5,892,1459         2       80       1       27,956,193       5,892,1459         2       80       1       27,961,451       5,892,1459         2       83       1       27,961,451       5,892,1459         2       84       1       27,961,451       5,892,1459         2       85       1	2	67		1	27,951,846	5.8942855
2       69       1       27953,871       58921459         2       70       1       27953,994       58921459         2       71       1       27954,243       58921459         2       73       1       27954,243       58921459         2       73       1       27954,363       58921459         2       73       1       27954,387       58921459         2       74       1       27954,443       58921459         2       74       1       27955,474       58921459         2       76       1       27955,474       58921459         2       77       1       27955,491       58921459         2       78       1       27955,293       58921459         2       78       1       27956,227       58921459         2       80       1       27956,237       58921459         2       81       1       27956,131       58921459         2       82       1       27956,131       58921459         2       81       1       27956,131       58921459         2       82       1       27961,157       58921459	2	68		1	27,953,717	5.8921459
2       70       1       27953,994       58921459         2       71       1       27954,243       58921459         2       72       1       27954,363       58921459         2       73       1       27954,363       58921459         2       73       1       27954,363       58921459         2       74       1       27954,365       58921459         2       75       1       27954,743       58921459         2       76       1       27955,491       58921459         2       76       1       27955,491       58921459         2       76       1       27956,293       58921459         2       79       1       27956,293       58921459         2       80       1       27956,293       58921459         2       80       1       27956,293       58921459         2       81       1       27956,293       58921459         2       82       1       27956,193       58921459         2       83       1       27961,451       58921459         2       84       1       27966,103       58921459	2	69		1	27,953,871	5.8921459
2       71       1       27,954,243       5,8921459         2       72       1       27,954,363       5,8921459         2       73       1       27,954,363       5,8921459         2       74       1       27,954,496       5,8921459         2       76       1       27,954,496       5,8921459         2       76       1       27,955,180       5,8921459         2       76       1       27,955,491       5,8921459         2       76       1       27,955,491       5,8921459         2       77       1       27,955,491       5,8921459         2       78       1       27,955,293       5,8921459         2       79       1       27,956,293       5,8921459         2       80       1       27,956,293       5,8921459         2       81       1       27,956,193       5,8921459         2       82       1       27,961,451       5,8921459         2       83       1       27,961,157       5,8921459         2       84       1       27,965,101       5,8921459         2       86       1       27,965,101<	2	70		1	27.953.994	5.8921459
2       72       1       27954,363       5.8921459         2       73       1       27954,363       5.8921459         2       74       1       27954,363       5.8921459         2       75       1       27954,374       5.8921459         2       75       1       27955,701       5.8921459         2       76       1       27955,701       5.8921459         2       77       1       27955,701       5.8921459         2       78       1       27955,701       5.8921459         2       78       1       27956,227       5.8921459         2       78       1       27956,273       5.8921459         2       80       1       27961,451       5.8921459         2       81       1       27961,827       5.793417         2       83       1       27961,827       5.793417         2       83       1       27961,827       5.8921459         2       84       1       27961,827       5.8921459         2       86       1       27965,827       5.8921459         2       87       1       27966,821       5.89	2	71		1	27.954.243	5.8921459
2       73       1       27,954,87       5,8921459         2       74       1       27,954,87       5,8921459         2       75       1       27,954,496       5,8921459         2       76       1       27,955,810       5,8921459         2       76       1       27,955,801       5,8921459         2       76       1       27,956,227       5,8921459         2       79       1       27,956,227       5,8921459         2       79       1       27,956,227       5,8921459         2       80       1       27,956,227       5,8921459         2       80       1       27,956,273       5,8921459         2       80       1       27,956,181       5,8921459         2       81       1       27,961,827       5,793417         2       82       1       27,961,827       5,8921459         2       83       1       27,961,827       5,8921459         2       84       1       27,961,827       5,8921459         2       87       1       27,965,101       5,8921459         2       87       1       27,965,327 <td>2</td> <td>72</td> <td></td> <td>1</td> <td>27.954.363</td> <td>5.8921459</td>	2	72		1	27.954.363	5.8921459
2       74       1       27,954,496       5,8921459         2       75       1       27,954,493       5,8921459         2       76       1       27,955,180       5,8921459         2       76       1       27,955,180       5,8921459         2       76       1       27,955,491       5,8921459         2       78       1       27,956,227       5,8921459         2       78       1       27,956,293       5,8921459         2       80       1       27,956,293       5,8921459         2       80       1       27,956,781       5,8921459         2       81       1       27,961,827       5,793417         2       82       1       27,961,827       5,793417         2       83       1       27,961,827       5,8921459         2       84       1       27,962,184       5,8921459         2       85       1       27,962,184       5,8921459         2       86       1       27,962,184       5,8921459         2       87       1       27,965,101       5,8921459         2       87       1       27,965,103 <td>2</td> <td>73</td> <td></td> <td>1</td> <td>27.954.387</td> <td>5.8921459</td>	2	73		1	27.954.387	5.8921459
2       75       1       27,95,743       5,821459         2       76       1       27,95,743       5,821459         2       77       1       27,95,7491       5,8921459         2       78       1       27,95,7491       5,8921459         2       78       1       27,95,7491       5,8921459         2       78       1       27,956,293       5,8921459         2       80       1       27,956,781       5,8921459         2       80       1       27,956,781       5,8921459         2       81       1       27,961,451       5,8921459         2       82       1       27,961,451       5,8921459         2       83       1       27,961,451       5,8921459         2       84       1       27,961,451       5,8921459         2       85       1       27,962,134       5,8921459         2       85       1       27,966,21       5,8921459         2       86       1       27,966,021       5,8921459         2       89       1       27,967,087       5,8921459         2       90       1       27,960,991	2	74		1	27.954.496	5.8921459
2       76       1       27,955,180       5.8221459         2       77       1       27,955,180       5.8921459         2       78       1       27,955,491       5.8921459         2       79       1       27,956,227       5.8921459         2       79       1       27,956,293       5.8921459         2       80       1       27,956,781       5.8921459         2       80       1       27,956,781       5.8921459         2       82       1       27,956,781       5.8921459         2       83       1       27,961,979       5.8921459         2       84       1       27,961,979       5.8921459         2       84       1       27,961,979       5.8921459         2       85       1       27,965,101       5.8921459         2       86       1       27,965,101       5.8921459         2       86       1       27,965,101       5.8921459         2       89       1       27,966,621       5.8921459         2       90       1       27,966,51       5.8921459         2       91       1       27,960,971 </td <td>2</td> <td>75</td> <td></td> <td>1</td> <td>27 954 743</td> <td>5 8921459</td>	2	75		1	27 954 743	5 8921459
277127,955,4915.8921459278127,956,2275.8921459279127,956,2935.8921459280127,956,7815.8921459281127,961,4515.8921459282127,961,8275.793417283127,961,8275.793417283127,962,1845.8921459284127,962,1845.8921459285127,964,2045.8921459286127,965,2175.8921459286127,965,3275.8921459287127,965,3275.8921459289127,966,6215.8921459290127,966,6215.8921459290127,966,6215.8921459291127,966,9915.8921459292127,970,0715.8921459293127,970,0715.8921459294127,973,3265.8921459295127,973,3335.8921459296127,980,0375.8921459296127,980,0375.8921459296127,9780,0375.8921459296127,9780,0375.8921459296127,980,0375.89214592961<	2	76		1	27.955.180	5.8921459
278127,956,2275,8921459279127,956,2935,8921459280127,958,7815,8921459281127,961,4515,8921459282127,961,8275,793417283127,961,9795,8921459284127,962,1845,8921459285127,962,1845,8921459286127,965,1015,8921459286127,965,3275,8921459287127,966,6215,8921459289127,966,6215,8921459290127,966,8255,8921459290127,967,0875,8921459291127,967,0875,8921459292127,970,0715,8921459293127,970,0715,8921459294127,973,5335,8921459295127,973,5335,8921459296127,980,0575,8921459296127,980,0575,8921459296127,980,0575,8921459296127,980,0575,8921459296127,980,0575,8921459296127,980,0575,8921459296127,980,0575,89214592961 <t< td=""><td>2</td><td>77</td><td></td><td>1</td><td>27.955.491</td><td>5.8921459</td></t<>	2	77		1	27.955.491	5.8921459
279127,956,2935,8921459280127,958,7815,8921459281127,961,8275,793417282127,961,8275,793417283127,961,8275,793417284127,962,1845,8921459285127,966,21845,8921459286127,966,1015,8921459286127,966,2115,8921459287127,966,2115,8921459288127,966,2115,8921459289127,966,2115,8921459290127,966,8255,8921459290127,967,0875,8921459291127,967,0875,8921459292127,970,0715,8921459293127,973,3265,8921459294127,973,3335,8921459295127,973,3335,8921459296127,980,0575,8921459296127,980,0575,89214592971127,980,0575,8921459	2	78		1	27.956.227	5.8921459
2         80         1         27,958,781         5.8921459           2         81         1         27,958,781         5.8921459           2         82         1         27,958,781         5.8921459           2         82         1         27,951,827         5.793417           2         83         1         27,961,827         5.793417           2         83         1         27,961,827         5.793417           2         83         1         27,961,827         5.793417           2         83         1         27,961,827         5.8921459           2         84         1         27,962,184         5.8921459           2         85         1         27,965,101         5.8921459           2         86         1         27,965,101         5.8921459           2         87         1         27,966,621         5.8921459           2         89         1         27,966,621         5.8921459           2         90         1         27,966,855         5.8921459           2         91         1         27,967,087         5.8921459           2         92         1	2	79		1	27.956.293	5.8921459
2       81       1       27,961,451       5,8921459         2       82       1       27,961,827       5,793417         2       83       1       27,961,979       5,8921459         2       83       1       27,962,184       5,8921459         2       84       1       27,962,184       5,8921459         2       85       1       27,964,204       5,8921459         2       86       1       27,965,101       5,8921459         2       86       1       27,966,5101       5,8921459         2       87       1       27,966,621       5,8921459         2       88       1       27,966,621       5,8921459         2       90       1       27,966,585       5,8921459         2       91       1       27,966,585       5,8921459         2       91       1       27,960,091       5,8921459         2       92       1       27,970,071       5,8921459         2       93       1       27,973,335       5,8921459         2       94       1       27,973,533       5,8921459         2       95       1       27,973,533<	2	80		1	27.958.781	5.8921459
282127,961,8275,793417283127,961,9795,8921459284127,962,1845,8921459285127,964,2045,8921459286127,965,1015,8921459287127,965,3275,8921459288127,966,6215,8921459289127,966,6215,8921459290127,966,8555,8921459291127,968,5855,8921459292127,970,0715,8921459293127,970,0715,8921459294127,973,3265,8921459295127,973,335,8921459296127,980,0575,8921459297127,980,1365,8921459	2	81		1	27.961.451	5.8921459
283127,961,9795.8921459284127,962,1845.8921459285127,962,1845.8921459286127,965,1015.8921459286127,965,3275.8921459287127,966,6215.8921459289127,966,6215.8921459290127,967,0875.8921459290127,968,5855.8921459291127,969,9915.8921459292127,970,0715.8921459293127,973,3265.8921459294127,973,5335.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	82		1	27 961 827	5 793417
284127,962,1845.8921459285127,964,2045.8921459286127,965,1015.8921459287127,965,3275.8921459288127,966,6215.8921459289127,967,0875.8921459290127,966,5855.8921459291127,968,5855.8921459292127,969,9915.8921459293127,970,0715.8921459294127,973,3265.8921459295127,973,335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	83		1	27.961.979	5.8921459
285127,964,2045.8921459286127,965,1015.8921459287127,965,3275.8921459288127,966,6215.8921459289127,967,0875.8921459290127,968,5855.8921459291127,969,9915.8921459292127,969,9915.8921459293127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	84		1	27.962.184	5.8921459
286127,96,1015.8921459287127,965,3275.8921459288127,966,6215.8921459289127,967,0875.8921459290127,968,5855.8921459290127,969,9915.8921459291127,970,0715.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	85		1	27.964.204	5.8921459
287127,965,3275.8921459288127,966,6215.8921459289127,967,0875.8921459290127,968,5855.8921459291127,969,9915.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	86		1	27 965 101	5 8921459
288127,966,6215.8921459289127,967,0875.8921459290127,968,5855.8921459291127,969,9915.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	87		1	27 965 327	5 8921459
289127,963,0215.8921459290127,968,5855.8921459291127,969,9915.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,973,5335.8921459297127,980,1365.8921459	2	88		1	27,966,621	5 8921459
290127,968,5855.8921459291127,969,9915.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	89		1	27,967,087	5 8921459
291127,969,9915.8921459292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	90		1	27 968 585	5 8921459
292127,970,0715.8921459293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	- 2	91		1	27 969 991	5 8921459
293127,972,6735.8921459294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	2	92		1	27,900,001	5 8921459
294127,973,3265.8921459295127,973,5335.8921459296127,980,0575.8921459297127,980,1365.8921459	- 2	93		1	27,979,673	5 8071450
2     95     1     27,973,533     5.8921459       2     96     1     27,980,057     5.8921459       2     97     1     27,980,136     5.8921459	2	94		1	27,972,075	5 8071/50
2     96     1     27,980,057     5.8921459       2     97     1     27,980,136     5.8921459	2	95		1	27,973,520	5 8071450
2 97 1 27.980.136 5.8921459	- 2	96		1	27 980 057	5 8071450
	- 2	97		1	27 980 136	5 8921459

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
2	98		1	27,981,282	5.8921459
2	99		1	27,981,536	5.8921459
3	100	Yes	2	5,087,458	5.0276583
3	101		2	5,102,106	4.7984936
3	102		2	5,102,126	4.7984936
4	103		2	24,844,697	5.0179777
4	104		2	24,880,854	4.9434382
4	105		2	24,882,717	4.9434382
4	106	Yes	2	24,884,774	5.2144851
4	107		2	24,915,038	4.9434382
4	108		2	24,915,059	4.9434382
5	109	Yes	2	31,700,860	4.9846402
5	110		2	31,704,278	4.9846402
5	111		2	31,704,685	4.9846402
5	112		2	31,706,449	4.9846402
5	113		2	31,714,007	4.9846402
5	114		2	31,714,952	4.9846402
5	115		2	31,722,293	4.9846402
5	116		2	31,728,246	4.9846402
5	117		2	31,728,257	4.9846402
5	118		2	31,734,360	4.9846402
5	119		2	31,735,654	4.9846402
5	120		2	31,736,321	4.9846402
5	121		2	31,737,988	4.9846402
5	122		2	31,740,565	4.9846402
5	123		2	31,742,890	4.9846402
5	124		2	31,746,201	4.9846402
5	125		2	31,746,203	4.9846402
5	126		2	31,757,993	4.9846402
5	127		2	31,762,177	4.9846402
5	128		2	31,762,204	4.9846402
5	129		2	31,773,582	4.9846402
6	130		3	23,599,267	5.4185624
6	131		3	23,602,834	5.4185624
6	132		3	23,602,836	5.4185624
6	133		3	23,603,512	5.4185624
6	134		3	23,604,571	4.9748581
6	135		3	23.604.576	4.9748581
6	136		3	23,605,736	5.4185624
6	137	Yes	3	23,619,690	6.2848578
7	138		3	23,703,226	5.5702154
7	139		3	23.703.283	5.5702154
7	140		3	23,705,154	6.9193735
7	141		3	23,705,351	6.9085089
7	142		3	23,705,455	8.5849765
7	143		3	23,705.458	7.3314836
7	144		3	23,705,491	7.3314836
7	145		3	23,705,652	7.1631128
7	146		3	23,705,694	7.9566377

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
7	147		3	23,705,695	7.0222627
7	148		3	23,706,631	8.1004531
7	149		3	23,706,709	8.3545679
7	150		3	23,707,032	5.5702154
7	151		3	23,707,299	5.5702154
7	152		3	23,708,043	8.1004531
7	153		3	23,708,062	8.3545679
7	154		3	23,708,620	5.5702154
7	155		3	23,710,275	8.1004531
7	156		3	23,710,359	8.1004531
7	157		3	23,710,419	5.6737461
7	158		3	23,710,504	6.9193735
7	159		3	23,710,544	8.3545679
7	160		3	23,710,576	5.5702154
7	161		3	23,711,028	6.0668419
7	162		3	23,711,636	8.1004531
7	163		3	23,711,749	8.4396629
7	164		3	23,712,310	8.3545679
7	165		3	23,712,325	8.1004531
7	166		3	23,713,421	8.1004531
7	167		3	23,713,435	5.5702154
7	168		3	23,715,323	7.3879052
7	169	Yes	3	23.715.931	9.3375444
7	170		3	23,716,016	9.1989532
7	171		3	23,716,745	5.8389016
7	172		3	23,716,746	8.3650091
7	173		3	23,716,755	8.1796015
7	174		3	23,720,991	8.1004531
7	175		3	23,721,260	8.3545679
7	176		3	23,722,660	5.5702154
7	177		3	23,722,804	5.5702154
7	178		3	23,723,013	5.5702154
7	179		3	23,724,952	5.5702154
7	180		3	23,725,905	8.1004531
7	181		3	23,725,960	8.3545679
7	182		3	23,726,052	8.3545679
7	183		3	23,726,144	5.5702154
7	184		3	23,726,921	5.5702154
7	185		3	23,727,594	5.4542477
7	186		3	23,727,607	5.5702154
7	187		3	23,729,521	5.5702154
7	188		3	23,731,153	5.5702154
7	189		3	23,731,242	5.5702154
8	190	Yes	3	23,899,609	5.1917485
9	191	Yes	3	24,212,274	4.796233
10	192		3	24,446,000	5.7211321
10	193		3	24,446,501	5.7211321
10	194		3	24,447,171	5.7211321
10	195	Yes	3	24,447,470	6.2612987

10     146     3     24,447,579     6.1835733       11     197     Yes     3     27,810,949     50,112573       12     198     Yes     3     33,335,44     5,321,4088       13     199     Yes     3     34,192,011     6,367,8668       13     201     3     34,200,899     6,367,8668       13     201     3     34,200,899     6,367,8668       13     202     3     34,210,399     6,367,8668       13     204     3     34,210,399     6,367,8668       13     205     3     34,210,890     6,27,4997,3       13     206     3     34,210,890     6,27,4997,3       13     206     3     34,210,890     6,27,4997,3       13     206     3     34,210,890     6,27,4997,3       13     206     3     34,210,890     6,27,4997,3       13     209     3     34,210,890     6,36,78668       13     210     3     34,210,890     6,36,78668       13     210     3     34,216,990     6,36,78668       13     211     3     34,218,990     6,36,78668       15     213     3     34,218,990     6,36,78668	LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
11     197     Yes     3     27810.949     5011.2584       13     199     Yes     3     33,33,541     5,271408       13     200     3     34,200,891     6,367666       13     201     3     34,200,891     6,367666       13     202     3     34,210,292     6,367666       13     203     3     34,210,299     6,367666       13     204     3     34,210,299     6,367666       13     205     3     34,210,299     6,376866       13     205     3     34,210,299     6,376866       13     207     3     34,210,299     6,376866       13     209     3     34,210,274     6,3678666       13     209     3     34,215,424     6,3678666       13     210     3     34,215,424     6,3678666       13     213     3     34,225,540     6,3678666       13     214     3     34,225,540     6,3678666       13     215     3     34,225,540     6,3678666       13     216     3     34,225,540     6,3678666       13     217     3     34,226,349     6,3678666       13     216	10	196		3	24,447,579	6.0825733
12     198     Yes     3     33335.44     521408       13     199     3     34,190,211     63676668       13     200     3     34,202.891     63676668       13     201     3     34,202.891     63676668       13     203     3     34,210.393     63676668       13     204     3     34,210.393     63676668       13     205     3     34,210.544     63748973       13     206     3     34,210.546     63748973       13     207     3     34,210.546     63748973       13     207     3     34,210.546     63748973       13     207     3     34,210.546     6376666       13     207     3     34,210.546     6376666       13     210     3     34,210.546     63676666       13     211     3     34,218,517     63676666       13     213     3     34,218,518     63676666       13     216     3     34,228,517     63676666       13     217     3     34,228,517     63676666       13     218     3     34,233,494     64774665       13     220     3     34,230,5	11	197	Yes	3	27,810,949	5.0142548
19       3       44,09,211       6,36,76668         13       201       3       34,200,819       6,36,76668         13       202       3       34,200,281       6,36,76668         13       202       3       34,200,281       6,36,76668         13       203       3       34,210,389       6,36,76668         13       204       3       34,210,690       6,27,48973         13       205       3       34,210,690       6,27,48973         13       206       3       34,210,690       6,27,48973         13       207       3       34,210,690       6,27,48973         13       209       3       34,215,449       6,099243         13       210       3       34,215,233       6,37,6668         13       211       3       34,215,233       6,37,6668         13       212       3       34,215,241       6,36,76668         13       214       3       34,225,810       6,36,76668         13       214       3       34,225,810       6,36,76668         13       214       3       34,276,817       6,36,76668         13       216       3<	12	198	Yes	3	33,333,544	5.3214088
13       200       3       34202831       63678668         13       201       3       34202851       63678668         13       202       3       34707764       63977866         13       203       3       34710399       63678668         13       205       3       34210674       637973         13       206       3       34210660       62749973         13       208       3       3421072       6397868         13       201       3       3421072       6397868         13       201       3       34210772       6397868         13       210       3       3421654       63678693         13       211       3       34218554       63678668         13       212       3       34229546       63678668         13       213       3       34229546       63678668         13       214       3       34229546       63678668         13       215       3       34229546       63678668         13       216       3       34229546       63678668         13       216       3       34278517       6378668 </td <td>13</td> <td>199</td> <td></td> <td>3</td> <td>34,199,211</td> <td>6.3678668</td>	13	199		3	34,199,211	6.3678668
13       201       3       34202,977,64       6,3676668         13       202       3       34210,099       6,3576668         13       204       3       34210,090       6,2746973         13       205       3       34210,090       6,2746973         13       206       3       34210,690       6,2746973         13       207       3       34210,590       6,3576668         13       209       3       34210,540       6,0902,443         13       200       3       34210,540       6,0902,443         13       210       3       34210,544       6,3576668         13       212       3       34210,544       6,3576668         13       213       34210,544       6,3576668         13       214       3       34275,277       6,3576668         13       215       3       344,75,277       6,3576668         13       216       3       342,75,277       6,3576668         13       217       3       342,75,277       6,3576668         13       216       3       342,75,277       6,3576668         14       247       Yes <td< td=""><td>13</td><td>200</td><td></td><td>3</td><td>34,200,889</td><td>6.3678668</td></td<>	13	200		3	34,200,889	6.3678668
13       202       3       34,20,0499       6,3078068         13       204       3       34,210,389       6,3078068         13       205       3       34,210,630       6,2749973         13       205       3       34,210,650       6,2749973         13       205       3       34,210,650       6,2749973         13       206       3       34,210,650       6,2749973         13       207       3       34,217,855       6,3676689         13       208       3       34,217,855       6,3676669         13       212       3       34,217,855       6,3676669         13       213       34,215,544       6,3676669         13       214       3       34,215,544       6,3676669         13       215       3       34,215,544       6,3676669         13       216       3       34,215,547       6,3676669         13       216       3       34,255,46       6,3676669         13       216       3       34,275,817       6,3676669         13       217       3       34,275,817       6,3676669         14       200       3	13	201		3	34,202,851	6.3678668
13       203       3       34210,099       6,3678668         13       204       3       34210,697       6,2748973         13       206       3       34210,696       6,2748973         13       206       3       34210,696       6,2748973         13       206       3       34210,696       6,2748973         13       206       3       34210,696       6,2748973         13       209       3       34217,435       6,3678668         13       210       3       34217,435       6,3678668         13       211       3       34218,549       6,3678668         13       213       3       3425,810       6,3678668         13       214       3       34225,810       6,3678668         13       216       3       34238,17       6,3678668         13       217       3       34238,19       6,3678668         13       218       3       34333,84       6,447246         13       220       3       34333,84       6,447246         14       240       Yes       4       2486,477       5,143572         15       226	13	202		3	34,207,764	6.292728
13       204       3       34210,389       6,3078688         13       205       3       34210,696       6,2748973         13       207       3       34210,696       6,2748973         13       208       3       34210,696       6,2748973         13       209       3       34215,495       6,3078688         13       210       3       34215,495       6,3678688         13       211       3       34218,523       6,3678688         13       212       3       34229,466       6,3678688         13       213       3       34229,466       6,3678688         13       214       3       34228,101       6,3678688         13       216       3       34278,17       6,3678688         13       216       3       34278,17       6,3678688         13       219       Yes       3       34323,594       6,3678688         13       219       Yes       3       34325,99       6,3678688         13       220       3       34335,44       6,477466         13       221       3       34385,99       6,3678688         14       <	13	203		3	34,210,099	6.3678668
13       205       3       34,210,674       6,2748973         13       206       3       34,210,696       6,2748973         13       208       3       34,210,696       6,2748973         13       208       3       34,210,696       6,2748973         13       209       3       34,217,835       6,3676668         13       210       3       34,217,835       6,3676668         13       212       3       34,215,521       6,3676668         13       213       3       34,275,277       6,3676668         13       214       3       34,275,277       6,3676668         13       216       3       34,275,277       6,3676668         13       217       3       34,275,277       6,3676668         13       216       3       34,275,277       6,3676668         13       217       3       34,325,99       6,3676668         13       219       Yes       3       34,325,91       6,3676668         13       219       Yes       3       34,325,91       6,3676668         13       221       3       34,3436,019       6,3676668	13	204		3	34,210,389	6.3678668
13       206       3       34,210,690       6,274,8973         13       207       3       34,210,72       6,37,7608         13       209       3       34,212,735       6,367,7608         13       210       3       34,212,785       6,367,7608         13       210       3       34,212,785       6,367,7608         13       212       3       34,219,544       6,367,7608         13       213       3       34,219,544       6,367,7608         13       214       3       34,225,846       6,367,7608         13       215       3       34,275,877       6,367,7608         13       216       3       34,275,871       6,367,7608         13       217       3       34,278,017       6,367,7608         13       218       3       34,285,99       6,367,7608         13       220       3       34,385,992       6,367,7608         13       221       3       34,385,992       6,367,7608         14       224       Yes       4       2,856,477       5,134572         15       225       4       4,001,336       4,847246         15 <td>13</td> <td>205</td> <td></td> <td>3</td> <td>34,210,674</td> <td>6.2748973</td>	13	205		3	34,210,674	6.2748973
13       207       3       34,210,696       6,274973         13       208       3       34,210,772       6,367668         13       210       3       34,215,493       6,3676668         13       211       3       34,215,531       6,3676668         13       212       3       34,215,541       6,3676668         13       213       3       34,225,810       6,3676668         13       214       3       34,225,810       6,3676668         13       216       3       34,275,017       6,3676668         13       217       3       34,278,017       6,3676668         13       218       3       34,278,017       6,3676668         13       219       Yes       3       34,325,99       6,3676668         13       220       3       34,385,992       6,3676668         13       221       3       34,385,992       6,3676668         14       224       Yes       4       2,86,477       5,144726         15       225       4       5,007,30       5,945422         15       226       4       5,007,30       5,945422         15 <td>13</td> <td>206</td> <td></td> <td>3</td> <td>34,210,690</td> <td>6.2748973</td>	13	206		3	34,210,690	6.2748973
13       208       3       34,210,722       6,3678668         13       209       3       34,215,449       6,0992,343         13       211       3       34,215,243       6,3678668         13       212       3       34,215,243       6,3678668         13       212       3       34,215,243       6,3678668         13       213       34,225,810       6,3678668         13       214       3       34,225,810       6,3678668         13       215       3       34,275,777       6,3678668         13       216       3       34,278,817       6,3678668         13       217       3       34,283,349       6,3678668         13       218       3       34,325,99       6,3678668         13       220       3       34,385,992       6,3678668         14       224       Yes       4       286,477       5,134352         15       226       4       4,904,935       5,528019         15       227       4       5,005,023       4,96495         15       230       4       5,013,58       5,944222         15       230       4 <td>13</td> <td>207</td> <td></td> <td>3</td> <td>34,210,696</td> <td>6.2748973</td>	13	207		3	34,210,696	6.2748973
13       209       3       34,215,449       6.0992343         13       210       3       34,217,855       6.3676668         13       212       3       34,219,544       6.3676668         13       213       3       34,229,546       6.3676668         13       214       3       34,225,810       6.3676668         13       215       3       34,275,777       6.3676668         13       216       3       34,278,017       6.3676668         13       217       3       34,278,017       6.3676668         13       218       3       34,278,017       6.3676668         13       219       Yes       3       34,382,599       6.3676668         13       210       3       34,385,992       6.3676668         13       220       3       34,382,599       6.3676668         13       221       3       34,385,992       6.3676668         14       224       Yes       4       266,477       5.134572         15       226       4       4,914,935       5.58193         15       236       4       5,050,73       4,9456439         15	13	208		3	34,210,772	6.3678668
13       210       3       34,217,835       6.3678668         13       211       3       34,218,523       6.3678668         13       212       3       34,219,544       6.3678668         13       213       3       34,229,546       6.3678668         13       214       3       34,255,810       6.3678668         13       215       3       34,275,277       6.3678668         13       216       3       34,278,817       6.3678668         13       217       3       34,283,349       6.3678668         13       218       3       34,283,349       6.3678668         13       219       Yes       3       34,382,599       6.3678668         13       220       3       34,382,599       6.3678668         13       221       3       34,382,599       6.3678668         13       222       3       34,382,599       6.3678668         14       224       Yes       4       2,856,477       5.134572         15       225       4       4,868,339       5,474895         15       226       4       5,008,730       5,9454252 <t< td=""><td>13</td><td>209</td><td></td><td>3</td><td>34,215,449</td><td>6.0992343</td></t<>	13	209		3	34,215,449	6.0992343
13       211       3       34,218,523       6,367,8668         13       212       3       34,219,544       6,367,8668         13       213       3       34,225,810       6,367,8668         13       214       3       34,275,810       6,367,8668         13       215       3       34,275,077       6,367,8668         13       216       3       34,278,017       6,367,8668         13       217       3       34,278,017       6,367,8668         13       218       3       34,325,84       6,47,78668         13       219       Yes       3       34,335,84       6,447,246         13       221       3       34,385,992       6,367,8668         13       222       3       34,386,019       6,367,8668         13       223       3       34,386,019       6,367,8668         14       224       Yes       4       4,868,339       5,647,4855         15       225       4       4,914,935       5,5280193         15       226       4       5,013,314       6,025279         15       230       4       5,013,303       5,9454252 <tr< td=""><td>13</td><td>210</td><td></td><td>3</td><td>34,217,835</td><td>6.3678668</td></tr<>	13	210		3	34,217,835	6.3678668
13       212       3       34,219,544       6,3678668         13       213       3       34,229,546       6,3678668         13       214       3       34,225,510       6,3678668         13       215       3       34,275,177       6,3678668         13       216       3       34,275,177       6,3678668         13       217       3       34,275,817       6,3678668         13       219       Yes       3       34,375,844       6,4674668         13       220       3       34,385,992       6,3678668         13       221       3       34,385,092       6,3678668         13       222       3       34,385,092       6,3678668         13       223       3       34,385,092       6,3678668         14       224       Yes       4       2,86,477       5,134572         15       226       4       4,914,935       5,528019         15       227       4       5,005,023       4,969605         15       230       4       5,013,303       5,9454252         15       231       4       5,013,303       5,9454252	13	211		3	34,218,523	6.3678668
13       213       3       34,229,546       6,3678668         13       214       3       34,255,810       6,3678668         13       215       3       34,275,277       6,3678668         13       216       3       34,278,177       6,3678668         13       217       3       34,278,177       6,3678668         13       218       3       34,278,177       6,3678668         13       219       Yes       3       34,37,584       6,3678668         13       220       3       34,385,599       6,3678668         13       221       3       34,385,599       6,3678668         13       222       3       34,385,599       6,3678668         13       223       3       34,385,599       6,3678668         14       224       Yes       4       4,868,339       5,647895         15       225       4       4,914,935       5,5280193         15       226       4       5,010,358       5,9454222         15       230       4       5,013,303       5,9454225         15       230       4       5,013,303       5,9454225	13	212		3	34,219,544	6.3678668
13       214       3       34,255,810       6.3678668         13       215       3       34,275,277       6.3678668         13       216       3       34,278,017       6.3678668         13       217       3       34,278,017       6.3678668         13       218       3       34,278,017       6.3678668         13       219       Yes       3       34,373,584       6.472466         13       220       3       34,385,92       6.3678668         13       221       3       34,385,92       6.3678668         13       222       3       34,385,90       6.3678668         13       223       3       34,385,90       6.3678668         14       224       Yes       4       2,866,477       5.1343572         15       226       4       4,914,935       5.5280193         15       228       4       5,001,035       5,9454252         15       230       4       5,001,035       5,9454252         15       234       4       5,025,679       6,205583         15       236       4       5,025,679       6,205583         15 <td>13</td> <td>213</td> <td></td> <td>3</td> <td>34,229,546</td> <td>6.3678668</td>	13	213		3	34,229,546	6.3678668
13       215       3       34275,277       6.3678668         13       216       3       34278,017       6.3678668         13       217       3       3428,817       6.3678668         13       218       3       3428,817       6.3678668         13       219       Yes       3       34328,599       6.3678668         13       220       3       34,385,599       6.3678668         13       221       3       34,385,992       6.3678668         13       222       3       34,385,019       6.3678668         13       223       3       34,389,050       6.3678668         14       224       Yes       4       2,856,477       5.1343572         15       225       4       4,901,935       5.5280193         15       226       4       5,005,023       4,966,027         15       228       4       5,005,023       4,966,027         15       230       4       5,005,023       4,966,027         15       230       4       5,005,023       4,966,027         15       230       4       5,011,558       5,9454252         15	13	214		3	34,255,810	6.3678668
13       216       3       34,278,017       6,3678668         13       217       3       34,278,017       6,3678668         13       218       3       34,278,017       6,3678668         13       219       Yes       3       34,278,017       6,3678668         13       219       Yes       3       34,335,84       6,4772466         13       220       3       34,385,992       6,3678668         13       221       3       34,386,019       6,3678668         13       222       3       34,389,605       6,3678668         14       224       Yes       4       2,856,477       5,1343572         15       225       4       4,9068,339       5,6474895         15       226       4       5,005,023       4,969027         15       228       4       5,005,023       4,996027         15       230       4       5,013,314       6,025279         15       231       4       5,013,03       5,9454252         15       236       4       5,025,728       6,205983         15       236       4       5,027,7101       6,205983 <t< td=""><td>13</td><td>215</td><td></td><td>3</td><td>34,275,277</td><td>6.3678668</td></t<>	13	215		3	34,275,277	6.3678668
13       217       3       34,278,817       6.3678668         13       218       3       34,278,817       6.3678668         13       219       Yes       3       34,278,817       6.3678668         13       219       Yes       3       34,325,99       6.3678668         13       220       3       34,382,599       6.3678668         13       221       3       34,386,019       6.3678668         13       223       3       34,389,605       6.3678668         14       224       Yes       4       2.856477       5.1383572         15       225       4       4,914,935       5.5280193         15       226       4       4,914,935       5.5280193         15       228       4       5,005,023       4,966027         15       230       4       5,013,314       6,025,728         15       230       4       5,013,033       5,9454252         15       234       4       5,013,033       5,9454252         15       235       4       5,025,78       6,205983         15       236       4       5,025,714       6,2059883 <tr< td=""><td>13</td><td>216</td><td></td><td>3</td><td>34.278.017</td><td>6.3678668</td></tr<>	13	216		3	34.278.017	6.3678668
13       218       3       34,28,349       6,3678668         13       219       Yes       3       34,373,584       6,4472466         13       220       3       34,382,599       6,3678668         13       221       3       34,385,992       6,3678668         13       222       3       34,389,605       6,3678668         13       223       3       34,389,605       6,3678668         14       224       Yes       4       2,856,477       5,1343572         15       225       4       4,949,355       5,5280193         15       226       4       4,949,355       5,5280193         15       227       4       5,005,023       4,996052         15       228       4       5,005,023       4,996051         15       230       4       5,013,356       5,9454252         15       231       4       5,013,033       5,9454252         15       232       4       5,013,031       5,9454252         15       233       4       5,013,031       5,9454252         15       234       4       5,025,728       6,2059583         15 <td>13</td> <td>217</td> <td></td> <td>3</td> <td>34.278.817</td> <td>6.3678668</td>	13	217		3	34.278.817	6.3678668
13       219       Yes       3       34,373,584       64472466         13       220       3       34,332,599       6,3678668         13       221       3       34,385,992       6,3678668         13       222       3       34,386,019       6,3678668         13       223       3       34,386,019       6,3678668         14       224       Yes       4       2,856,477       5,1343572         15       225       4       4,868,339       5,6474895         15       226       4       4,914,935       5,5280193         15       227       4       5,005,023       4,9696027         15       228       4       5,001,336       5,9454252         15       230       4       5,011,336       4,8936051         15       231       4       5,013,033       5,9454252         15       232       4       5,013,033       5,9454252         15       233       4       5,025,728       6,2059683         15       236       4       5,027,101       6,2059583         15       236       4       5,027,101       6,2059583         15 <td>13</td> <td>218</td> <td></td> <td>3</td> <td>34 283 349</td> <td>6 3678668</td>	13	218		3	34 283 349	6 3678668
13       20       3       34,382,599       6,3678668         13       221       3       34,382,599       6,3678668         13       222       3       34,382,599       6,3678668         13       223       3       34,389,605       6,3678668         14       224       Yes       4       2,856,477       5,134357         15       225       4       4,868,339       5,6474895         15       226       4       4,914,935       5,520193         15       227       4       5,005,023       4,9696027         15       228       4       5,001,358       5,9454252         15       229       4       5,011,336       4,896051         15       230       4       5,011,336       4,8936051         15       231       4       5,013,003       5,9454252         15       233       4       5,013,003       5,9454252         15       234       4       5,022,565       6,2031621         15       236       4       5,022,7101       6,2055983         15       237       4       5,022,7101       6,2055983         15       240	13	219	Yes	3	34.373.584	6.4472466
13       21       3       34,385,992       6.3678668         13       222       3       34,385,905       6.3678668         13       223       3       34,389,605       6.3678668         14       224       Yes       4       2,856,477       5.1343572         15       225       4       4,868,339       5.6474895         15       226       4       4,868,339       5.6474895         15       226       4       4,914,935       5.5280193         15       228       4       5,005,023       4,9696027         15       228       4       5,001,0358       5,9454252         15       230       4       5,011,336       4.8986051         15       231       4       5,011,336       4.8986051         15       232       4       5,013,314       6.0225279         15       232       4       5,025,728       6.2031621         15       235       4       5,027,514       6.2059583         15       236       4       5,027,514       6.2059583         15       236       4       5,028,468       6.2059583         15       240 <td>13</td> <td>220</td> <td></td> <td>3</td> <td>34.382.599</td> <td>6.3678668</td>	13	220		3	34.382.599	6.3678668
13       22       3       34,386,019       6,3678668         13       223       3       34,389,605       6,3678668         14       224       Yes       4       2,856,477       5,1343572         15       225       4       4,868,339       5,6474895         15       226       4       4,914,935       5,5280193         15       226       4       5,009,730       5,9454252         15       228       4       5,001,358       5,9454252         15       229       4       5,001,358       5,9454252         15       230       4       5,011,336       4,898,6051         15       231       4       5,011,336       4,898,6051         15       232       4       5,013,314       6,0225279         15       233       4       5,022,565       6,2031621         15       234       4       5,022,565       6,2031621         15       236       4       5,022,565       6,2031621         15       236       4       5,027,7101       6,2059583         15       238       4       5,022,514       6,2059583         15       240 <td>13</td> <td>221</td> <td></td> <td>3</td> <td>34.385.992</td> <td>6.3678668</td>	13	221		3	34.385.992	6.3678668
13       223       3       34,389,605       6.3678668         14       224       Yes       4       2,856,477       5.1343572         15       225       4       4,868,339       5.6474895         15       226       4       4,914,935       5.5280193         15       227       4       5,005,023       4.9666027         15       228       4       5,001,358       5.9454252         15       229       4       5,011,336       4.8936051         15       230       4       5,011,658       5.9454252         15       231       4       5,011,658       5.9454252         15       232       4       5,011,658       5.9454252         15       232       4       5,013,003       5.9454252         15       233       4       5,022,565       6.2031621         15       235       4       5,022,728       6.2059583         15       236       4       5,027,514       6.2059583         15       239       4       5,023,1991       6.2059583         15       240       4       5,033,991       6.2059583         15       240	13	222		3	34 386 019	6 3678668
14224Yes42,85,4775,13435721522544,868,3395,64748951522644,914,9355,52801931522745,005,0234,96960271522845,009,7305,94542521522945,010,3585,94542521523045,011,3664,89360511523145,011,0585,94542521523245,013,0135,94542521523345,013,3146,0225721523445,022,5656,20316211523545,027,71016,2059581523645,027,71016,2059581523845,027,5146,2059581523945,028,4686,2059581524045,029,4036,2059581524145,033,4916,2059581524345,033,4755,4984081524445,033,4755,498408	13	223		3	34,389.605	6.3678668
1522544,868,3395,64748951522644,914,9355,52801931522745,005,0234,96960271522845,009,7305,94542521522945,010,3585,94542521523045,011,3364,89360511523145,011,6585,94542521523245,013,0035,94542521523245,013,0035,94542521523345,013,0035,94542521523445,022,5656,20316211523645,027,796,20595831523745,027,796,20595831523945,027,1016,20595831524045,029,4036,20595831524045,031,9916,20595831524145,033,2956,20595831524245,033,2956,20595831524345,033,4755,49840681524445,033,4755,4984068	14	224	Yes	4	2.856.477	5.1343572
1522644/9/4/9355.52801931522745,005,0234.96960271522845,009,7305.94542521522945,011,3585.94542521523045,011,3364.89360511523145,011,6585.94542521523245,013,0035.94542521523345,013,0035.94542521523345,022,5656.20316211523445,022,7286.20595831523645,027,71016.20595831523745,027,1016.20595831523945,027,1016.20595831524045,029,4086.20595831524145,031,9916.20595831524345,033,4755.49840681524445,033,4755.4984068	15	225		4	4.868.339	5.6474895
15       227       4       5,005,023       4,9696027         15       228       4       5,009,730       5,9454252         15       229       4       5,010,358       5,9454252         15       230       4       5,011,336       4.8936051         15       231       4       5,011,658       5,9454252         15       232       4       5,011,658       5,9454252         15       232       4       5,013,003       5,9454252         15       232       4       5,013,003       5,9454252         15       232       4       5,013,003       5,9454252         15       233       4       5,025,728       6,0225279         15       234       4       5,025,728       6,2031621         15       235       4       5,027,101       6,2059583         15       236       4       5,027,514       6,2059583         15       239       4       5,028,468       6,2059583         15       240       4       5,031,991       6,2059583         15       240       4       5,033,295       6,2059583         15       243       4	15	226		4	4.914.935	5,5280193
15       228       4       5,009,730       5,9454252         15       229       4       5,010,358       5,9454252         15       230       4       5,011,336       4.8936051         15       231       4       5,011,058       5,9454252         15       231       4       5,011,058       5,9454252         15       232       4       5,013,003       5,9454252         15       232       4       5,013,014       6,0225279         15       233       4       5,013,014       6,0225279         15       234       4       5,022,565       6,2031621         15       235       4       5,026,779       6,2059583         15       236       4       5,027,514       6,2059583         15       237       4       5,027,514       6,2059583         15       238       4       5,027,514       6,2059583         15       240       4       5,031,991       6,2059583         15       240       4       5,031,991       6,2059583         15       240       4       5,033,295       6,2059583         15       243       4	15	227		4	5.005.023	4.9696027
1522945,010,3585,94542521523045,011,3364.89360511523145,011,6585,94542521523245,013,0035,94542521523345,013,3146,02252791523445,022,5656,20316211523545,026,7796,20595831523645,027,1016,20595831523845,027,5146,20595831523945,028,4686,20595831524045,029,4036,20595831524145,033,2956,20595831524245,033,2956,20595831524345,033,4755,49840681524445,033,4755,49840681524445,033,4755,4984068	15	228		4	5.009.730	5,9454252
1523045,01,0556,010,2551523145,011,6585,94542521523245,013,0035,94542521523345,013,3146,02252791523445,022,5656,20316211523545,025,7286,20595831523645,027,1016,20595831523745,027,1016,20595831523845,027,5146,20595831523945,028,4686,20595831524045,031,9916,20595831524145,033,4756,20595831524345,033,4755,49840681524345,033,4755,49840681524445,033,4556,2059583	15	229		4	5 010 358	5 9454252
1523145,011,6585,94542521523245,013,0035,94542521523345,013,3146,02252791523445,022,5656,20316211523545,025,7286,20595831523645,027,1016,20595831523745,027,1016,20595831523845,027,1016,20595831523945,028,4686,20595831524045,029,4036,20595831524145,031,9916,20595831524245,033,2956,20595831524345,033,4755,49840681524345,033,4755,49840681524445,033,4755,4984068	15	230		4	5,011,336	4 8936051
1523245,01,0005,04542521523345,013,0035,94542521523345,022,5656,20316211523545,025,7286,20595831523645,027,1016,20595831523845,027,5146,20595831523945,028,4686,20595831524045,029,4036,20595831524145,033,2956,20595831524245,033,4755,49840681524345,033,4755,49840681524445,033,4755,49840681524445,033,4755,4984068	15	230		4	5,011,658	5 9454252
1523345,013,3146,02252791523445,022,5656,20316211523545,025,7286,20595831523645,027,1016,20595831523745,027,5146,20595831523845,027,5146,20595831523945,028,4686,20595831524045,031,9916,20595831524145,033,2956,20595831524245,033,4755,49840681524345,033,4755,49840681524445,033,4755,4984068	15	232		4	5,013,003	5 9454252
1523445,022,5656,20316211523545,025,7286,20595831523645,027,796,20595831523745,027,5146,20595831523845,027,5146,20595831523945,028,4686,20595831524045,031,9916,20595831524145,033,2956,20595831524245,033,4755,49840681524345,033,4755,49840681524445,033,4755,4984068	15	233		4	5013314	6.0225279
1523545,025,7286.20595831523645,026,7796.20595831523745,027,1016.20595831523845,027,5146.20595831523945,028,4686.20595831524045,029,4036.20595831524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524345,033,4755.49840681524445,033,4755.4984068	15	233		4	5,022,565	6 2031621
1523645,026,7796.20595831523745,027,1016.20595831523845,027,5146.20595831523945,028,4686.20595831524045,029,4036.20595831524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524345,033,4755.4984068	15	235		4	5,025,728	6 2059583
1520015,022,1136,025,9581523845,027,5146,20595831523945,028,4686,20595831524045,029,4036,20595831524145,031,9916,20595831524245,033,2956,20595831524345,033,4755,49840681524445,033,4755,4984068	15	236		4	5,026,779	6 2059583
1523845,027,5146.20595831523945,028,4686.20595831524045,029,4036.20595831524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524445,033,4755.4984068	15	230		4	5,027,101	6 2059583
1523945,022,4316.20595831524045,029,4036.20595831524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524445,033,5456.2059583	15	238		4	5 027 514	6 2059583
1524045,029,4036.20595831524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524445,033,5456.2059583	15	230		4	5,028,468	6 2059583
1524145,031,9916.20595831524245,033,2956.20595831524345,033,4755.49840681524445,033,5456.2059583	15	240		4	5 029 403	6 2059583
15     242     4     5,033,295     6.2059583       15     243     4     5,033,475     5.4984068       15     244     4     5,033,545     6.2059583	15	241		4	5 021 001	6 205 255
15     243     4     5,033,475     5,4984068       15     244     4     5,033,545     6 2059583	15	242		4	5 033 295	6 205 255
15 244 4 5.033.545 6.2059.583	15	243		4	5,033,475	5 4984068
	15	244		4	5,033,545	6 2059583

15       245       4       \$,833,557       4,005968         15       246       4       \$,533,524       4,005968         15       247       4       \$,533,526       4,005988         15       248       4       \$,535,576       4,005988         15       250       4       \$,535,576       4,005988         15       250       4       \$,503,526       4,005988         15       251       4       \$,503,526       4,005,933         15       252       Yes       4       \$,503,626       6,205988         15       253       4       \$,503,626       6,205988         15       254       4       \$,503,626       6,205988         15       255       4       \$,502,601       4,020,903         15       258       4       \$,100,200       \$,682,1148         16       260       4       \$,134,402       4,482,624         16       261       4       \$,302,802       4,471,4593         16       261       4       30,92,607       5,763,909         16       261       4       30,92,607       5,762,909         16       263 <t< th=""><th>LD block number</th><th>Associated SNP number</th><th>SNP with highest association in the LD</th><th>CHR</th><th>Coordinate</th><th>— log10(<i>p</i>)</th></t<>	LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
15       246       4       5,033,024       6,020983         15       248       4       5,033,024       5,035,75       6,035983         15       249       4       5,035,75       6,035983       6,035983         15       250       4       5,037,825       6,035983       6,035983         15       251       4       5,037,825       6,035983         15       252       Yes       4       5,038,944       6,019477         15       253       4       5,038,944       6,019479         15       254       4       5,045,020       6,039593         15       255       4       5,103,290       5,687,1248         15       256       4       5,103,290       5,687,1248         15       259       4       5,103,290       5,687,020         16       261       4       3,0240,202       4,871893         16       262       4       3,0240,202       4,871893         16       264       3,0240,202       4,871893         16       266       4       3,094,340       4,871893         16       269       4       3,094,340       4,871893	15	245		4	5,033,552	6.2059583
15247450337350849381524945,0335766,20059331525045,035,766,20059331525145,0377436,020593315252Yes45,0387456,20059331525345,0387456,20059331525445,0387466,20059331525545,0387466,20059331525645,038,046,20059331525745,02005,02001525745,128,6104,540001525943,086,5945,76208916260Yes43,086,5945,7620891626143,092,0024,87149331626243,094,8443,7620891626443,094,8454,87149331626643,094,8454,87149331626743,096,53415,7620891626943,096,53415,76208917271Yes43,096,53415,74259318272Yes62,753,8462826743,096,53415,74259317271Yes62,754,8471826976,065,3415,74259319271Yes76,065,3415,74259329274Yes76,065,3415,74259	15	246		4	5,033,624	6.2059583
15       248       4       5.035,76       6.205983         15       250       4       5.035,763       6.205983         15       251       4       5.037,743       6.035983         15       252       Yes       4       5.037,743       6.035983         15       253       4       5.037,743       6.035983         15       254       4       5.037,743       6.035983         15       254       4       5.037,974       6.03967,83         15       256       4       5.032,00       5.0287,244         15       257       4       5.128,601       4.950905         15       259       4       5.128,601       4.950905         16       260       4       30,388,500       5.752999         16       261       4       30,960,071       5.752999         16       262       4       30,960,071       5.752999         16       264       30,960,071       7.7529899         16       266       4       30,960,31       4.91893         16       266       4       30,960,31       4.91893         16       266       7       30	15	247		4	5,034,748	5.9848918
15       249       4       5035,006       6,2095,933         15       251       4       50,37,42,73       6,2095,933         15       252       4       50,37,42,73       6,2095,933         15       253       4       50,43,742,73       6,2095,933         15       254       4       50,45,702       6,2095,933         15       255       4       50,69,702       6,2095,933         15       257       4       51,28,610       4,89,903         15       259       4       51,28,610       4,89,903         15       250       4       30,88,904       5,76,2009         16       260       Yes       4       30,88,904       5,76,2009         16       261       4       30,92,002,2       4,87,8533         16       262       4       30,92,002,2       4,87,8533         16       264       4       30,94,10,2       4,97,8533         16       265       4       30,94,30       4,87,8533         16       266       4       30,96,50,51       4,87,8533         16       270       4       30,96,749       4,87,8533         17	15	248		4	5,035,576	6.2059583
15     250     4     5.037,243     6.205933       15     251     Yes     4     5.037,243     6.205933       15     252     Yes     4     5.038,344     6.205933       15     253     4     5.045,202     6.205933       15     255     4     5.042,020     6.205933       15     255     4     5.102,801     6.205933       15     256     4     5.102,801     6.205933       15     259     4     5.124,661     4.9640005       16     261     Yes     4     5.03,865,90     5.7520989       16     261     Yes     4     3.03,86,594     5.7520989       16     265     4     3.03,26,071     7.5720939       16     265     4     3.03,26,071     7.5720939       16     266     4     3.03,26,071     7.5720939       16     267     4     3.03,26,311     4.871893       16     269     4     3.03,26,311     4.871893       17     271     Yes     6     2.751,3340     5.481933       16     269     7     2.64,311     5.3333       17     271     Yes     7     2.64,311     5.3	15	249		4	5,035,806	6.2059583
152514507,2056,00598315252Yes4508,5076,205,7821525345,008,7166,205,7821525545,008,9046,205,9831525645,128,6614,549,0051525745,128,6614,549,0051525945,128,6614,549,00516261Yes43,038,0945,762,9091626343,026,0024,871,8531626343,026,0125,762,9091626443,026,0125,762,9091626343,026,0134,871,8531626443,026,0134,871,8531626743,026,0134,871,8531626743,056,0514,871,8531626943,066,3034,871,8531626943,066,3514,871,8531626943,066,3514,871,85317270Yes43,066,3514,871,85318272Yes67,51,9405,449,45319273Yes67,51,9405,449,4532827672,64,015,318,8172974,64,0336,449,4536,449,4532927672,64,015,318,8172976,65,5732,445,453292876,65,57	15	250		4	5,037,743	6.2059583
15     252     Yes     4     5,038,844     6,310/07       15     253     4     5,035,716     6,206,758       15     255     4     5,005,903     6,205,983       15     256     4     5,100,200     6,867,124       15     257     4     5,128,610     49,304905       15     258     4     5,128,610     49,304905       16     260     Yes     4     30,865,660     5,722089       16     260     Yes     4     30,865,600     5,722089       16     261     4     30,920,022     4,718593       16     263     4     30,920,027     4,718593       16     264     4     30,948,64     5,720,909       16     266     4     30,948,64     5,720,909       16     267     4     30,948,64     5,762,909       16     267     4     30,965,514     4,871,8593       16     269     4     30,965,314     5,762,909       16     269     4     30,965,314     5,762,909       16     269     7     4     30,965,314     5,762,909       16     269     7     4     30,965,314     5,762,909 </td <td>15</td> <td>251</td> <td></td> <td>4</td> <td>5,037,825</td> <td>6.2059583</td>	15	251		4	5,037,825	6.2059583
15       253       4       5,039,716       6,296,738         15       254       4       5,006,904       6,205,593         15       256       4       5,102,90       5,687,1248         15       257       4       5,128,611       4,954,905         15       259       4       5,134,602       4,838,424         16       261       4       30,86,609       5,762,009         16       262       4       30,920,022       4,871,853         16       263       4       30,920,022       4,871,853         16       264       4       30,945,182       4,871,853         16       265       4       30,945,182       4,871,853         16       266       4       30,945,182       4,871,853         16       267       4       30,945,182       4,871,853         16       269       4       30,945,182       4,871,853         17       2,71       Yes       4       30,945,182       8,057,353         18       2,72       Yes       6       7,51,940       5,449,759         19       2,73       Yes       7       2,94,611       5,338,09	15	252	Yes	4	5,038,844	6.310407
15       254       4       5.045.02       6.205983         15       255       4       5.002.90       6.205983         15       257       4       5.102.80       6.205983         15       257       4       5.128.61       4.954095         15       259       4       3.036.650       5.762089         16       261       4       3.0986.694       5.762089         16       262       4       3.0986.094       5.762089         16       263       4       3.0945.182       4.871853         16       265       4       3.0945.182       4.871853         16       265       4       3.0945.061       4.871853         16       266       4       3.0945.051       4.871853         16       266       4       3.0945.14       5.762089         16       267       4       3.0945.051       4.871853         16       268       4       3.0945.051       4.871853         16       272       Yes       4       3.0945.151       4.893791         17       271       Yes       6       2.759.840       6.1026657         22       2	15	253		4	5,039,716	6.2961758
15       25       4       5.09,694       6.205983         15       256       4       5.102,610       4.94005         15       258       4       5.128,611       4.94005         15       259       4       5.128,610       4.94005         16       261       4       3.038,6594       5.7629089         16       261       4       3.020,022       4.8718593         16       262       4       3.0348,694       5.7629089         16       264       4       3.024,021       4.8718593         16       264       4       3.094,864       5.7629089         16       267       4       3.096,514       4.8718593         16       267       4       3.096,514       4.8718593         16       267       4       3.096,514       5.7629089         16       267       4       3.096,514       8.807.355         16       267       4       3.096,514       8.807.355         16       270       Yes       6       4.785,418       5.60333         17       271       Yes       7       2.406,135       5.38629         18       27	15	254		4	5,045,202	6.2059583
15       256       4       5,100,200       5,6871248         15       257       4       5,128,610       49540905         15       259       4       5,128,610       4954095         16       260       Yes       4       30386,560       5,722089         16       261       4       30386,560       5,722089         16       262       4       30386,594       5,762089         16       263       4       30390,071       5,762089         16       264       30945,182       4,8718593         16       266       4       30945,182       4,8718593         16       266       4       30966,51       4,8718593         16       267       4       3096,544       5,722089         16       269       4       3096,514       4,8718593         16       269       4       3096,514       4,8718593         17       271       Yes       6       7,519,464       5,0334         18       272       Yes       7       2,64,611       5,0334         19       273       Yes       7       2,64,611       5,3386175         22	15	255		4	5,096,994	6.2059583
15       257       4       5,128,610       4,9540905         15       258       4       5,128,611       4,9540905         16       260       Yes       4       30,865,560       5,7629089         16       261       4       30,986,540       5,7629089         16       262       4       30,926,071       5,7629089         16       263       4       30,945,182       4,8718593         16       264       4       30,945,182       4,8718593         16       266       4       30,965,051       4,8718593         16       267       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       270       4       30,965,344       5,7629089         16       270       4       30,965,344       5,7629089         17       271       Yes       6       7,751,940       5,182893         18       272       Yes       7       2,66,8173       5,136292	15	256		4	5,100,290	5.6871248
15       258       4       5,128,681       4,9940905         15       259       4       5,134,602       4,884224         16       260       Yes       4       3,088,6504       5,7629089         16       262       4       3,092,071       5,7629089         16       263       4       3,092,6071       5,7629089         16       264       4       3,094,6182       4,8118593         16       265       4       3,096,5051       4,8118593         16       267       4       3,096,5051       4,8118593         16       268       4       3,096,514       8,7629089         16       269       4       3,096,514       8,7629089         16       269       4       3,096,749       4,8118593         16       269       4       3,097,363       8,815893         17       271       Yes       4       3,674,325       8,8057353         18       272       Yes       6       25,598,090       6,1028625         21       273       Yes       7       264,021       5,134,661         22       276       Yes       7       6,063,511	15	257		4	5,128,610	4.9540905
15       259       4       5,134,602       4,834,224         16       260       Yes       4       30,86,500       5,762909         16       261       4       30,902,021       4,8118593         16       263       4       30,902,021       4,8118593         16       264       4       30,945,182       4,8118593         16       265       4       30,904,300       4,8118593         16       266       4       30,965,511       4,8118593         16       267       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       270       4       30,965,344       5,7629089         16       270       4       30,967,249       4,8118593         17       271       Yes       6       4,784,418       5,60333         18       272       Yes       6       4,784,418       5,60333         21       275       Yes       7       264,621       5,336617         22       276       7       264,621       5,336617	15	258		4	5,128,681	4.9540905
16       260       Yes       4       30,886,560       5,7629089         16       261       4       30,980,594       5,7629089         16       262       4       30,920,022       4,8718593         16       263       4       30,926,071       5,7629089         16       264       4       30,945,182       4,8718593         16       265       4       30,945,302       4,8718593         16       266       4       30,965,301       4,8718593         16       267       4       30,965,301       4,8718593        16       269       4       30,965,301       4,8718593         16       270       4       30,965,301       4,8718593         16       270       4       30,967,249       4,8718593         17       271       Yes       4       30,973,266       4,8718593         18       272       Yes       6       7,519,840       5,449539         21       271       Yes       7       2,84,003       5,5136893         22       276       Yes       7       2,84,003       5,5136893         23       280       Yes       7       6,	15	259		4	5,134,602	4.8384224
16       261       4       30,886,994       5,7629089         16       262       4       30,920,022       4,8718593         16       263       4       30,926,071       5,7629089         16       264       4       30,945,182       4,8718593         16       265       4       30,948,864       5,7629089         16       266       4       30,966,350       4,8718593         16       266       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       269       4       30,965,344       5,7629089         16       270       4       30,967,249       4,8718593         17       271       Yes       6       7,519,840       5,60333         20       272       Yes       6       7,519,840       5,60333         21       273       Yes       7       2,64621       5,336817         22       276       Yes       7       6,063,5311       6,189212<	16	260	Yes	4	30,886,560	5.7629089
16     262     4     30,920,022     4,871,893       16     263     4     30,926,071     5,762,9089       16     264     4     30,945,182     4,871,8593       16     266     4     30,964,390     4,871,8593       16     266     4     30,965,344     5,762,9089       16     267     4     30,965,344     5,762,9089       16     268     4     30,967,249     4,871,8593       16     269     4     30,967,249     4,871,8593       16     270     4     30,973,636     4,871,8593       16     270     4     30,973,636     4,871,8593       17     271     Yes     4     3,167,43,25     8,805,7355       18     272     Yes     6     7,519,840     5,403,335       20     274     Yes     6     7,519,840     5,108,823       21     275     Yes     7     2,64,621     5,336,817       22     276     7     2,64,621     5,336,817       23     280     Yes     7     6,063,531     6,189,921       23     280     Yes     7     6,065,313     7,2457358       23     281     7     6,065,	16	261		4	30.886.994	5,7629089
16     263     4     30,926,071     5,762,9089       16     264     4     30,945,182     4,871,8593       16     265     4     30,964,390     4,871,8593       16     267     4     30,965,051     4,871,8593       16     267     4     30,965,051     4,871,8593       16     268     4     30,965,051     4,871,8593       16     269     4     30,967,249     4,871,8593       16     269     4     30,967,363     4,871,8593       16     270     4     30,973,636     4,871,8593       17     271     Yes     4     3,674,325     8,805,7355       18     272     Yes     6     7,519,840     5,449,539       20     274     Yes     6     7,519,840     5,449,539       21     275     Yes     7     264,621     5,336,8175       22     276     7     284,003     5,136,829       23     280     Yes     7     6,064,330     7,245,7358       23     281     7     6,064,330     7,245,7358       23     281     7     6,065,571     7,245,7358       23     282     7     6,065,571     7	16	262		4	30,920,022	4.8718593
16     264     4     30,945,182     4,8718593       16     265     4     30,945,864     5,7629089       16     266     4     30,965,051     4,8718593       16     267     4     30,965,051     4,8718593       16     269     4     30,965,344     5,7629089       16     269     4     30,97,249     4,8718593       16     269     4     30,97,365     4,8718593       17     271     Yes     4     30,97,365     4,8718593       18     272     Yes     4     30,97,365     4,8937411       19     273     Yes     6     7,519,840     5,449539       21     275     Yes     6     7,519,840     5,449539       22     276     7     284,621     5,3366175       23     280     Yes     7     29,6531     6,88921       23     281     7     6,065,531     6,188921       23     281     7     6,065,531     7,2457358       23     282     7     6,065,731     7,2457358       23     284     7     6,065,731     7,2457358       23     286     7     6,070,349     7,2457358	16	263		4	30.926.071	5.7629089
16     265     4     30,948,864     5,7629089       16     266     4     30,965,354     4,8718593       16     267     4     30,965,051     4,8718593       16     269     4     30,967,249     4,8718593       16     269     4     30,967,249     4,8718593       16     270     4     30,973,636     4,8718593       17     271     Yes     4     30,767,249     4,893711       19     273     Yes     6     4,785,118     5,60333       20     274     Yes     6     2,598,090     6,028,525       21     275     Yes     6     2,598,090     6,028,525       22     276     7     2,646,21     5,336175       23     277     Yes     6     2,598,090     6,028,525       24     277     Yes     7     2,646,21     5,336175       25     276     7     2,646,21     5,336175       28     277     Yes     7     6,063,331     6,189212       29     277     Yes     7     6,063,331     2,457358       23     280     Yes     7     6,065,031     7,2457358       23     281	16	264		4	30.945.182	4.8718593
16       266       4       30963,390       48718593         16       267       4       30965,051       48718593         16       268       4       30965,344       5,729089         16       269       4       3097,249       48718593         16       270       4       3097,363       48718593         17       271       Yes       4       3097,363       48718593         18       272       Yes       4       3097,435       48937411         19       273       Yes       6       4,785,157       4893741         19       273       Yes       6       25,580,00       6102862         20       274       Yes       7       264,621       5,3368175         21       275       Yes       7       264,621       5,3368175         22       276       7       7       264,621       5,3368175         23       280       Yes       7       264,621       5,3368175         23       280       Yes       7       6,064,370       7,2457358         23       281       7       6,065,371       7,2457358         23       282 <td>16</td> <td>265</td> <td></td> <td>4</td> <td>30.948.864</td> <td>5,7629089</td>	16	265		4	30.948.864	5,7629089
16       267       4       30,950,51       4,871,8593         16       268       4       30,965,244       5,7629089         16       269       4       30,967,249       4,871,8593         16       270       4       30,967,249       4,871,8593         16       270       4       30,967,249       4,871,8593         16       270       4       30,967,249       4,871,8593         16       270       4       31,674,325       8,8057,355         18       272       Yes       4       31,674,325       8,8057,355         19       273       Yes       6       4,785,418       5,60333         20       274       Yes       6       2,598,990       6,102,8625         21       275       Yes       7       24,603       5,513,6825         22       276       7       24,603       5,513,6825         23       280       Yes       7       6,064,370       7,2457,358         23       281       7       6,064,370       7,2457,358         23       282       7       6,065,571       7,2457,358         23       284       7       6,070,345	16	266		4	30,964,390	4.8718593
16       268       4       30,965,344       5,7629089         16       269       4       30,965,249       4,8718593         16       270       4       30,973,36       4,8718593         16       270       4       30,973,36       4,8718593         17       271       Yes       4       31,674,325       8,8057355         18       272       Yes       6       4,785,418       5,0333         20       274       Yes       6       7,519,840       5,4149539         21       275       Yes       6       2,558,090       6,1028625         22       276       7       264,621       5,336175         22       277       Yes       7       284,003       5,5136829         23       280       Yes       7       6,063,531       6,189212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,873       7,2457358         23       282       7       6,065,873       7,2457358         23       284       7       6,065,873       7,2457358         23       287       7	16	267		4	30.965.051	4.8718593
16       269       4       30,967,249       4,8718593         16       270       4       30,967,249       4,8718593         17       271       Yes       4       31,674,325       8,8057355         18       272       Yes       4       34,786,157       4,8937411         19       273       Yes       6       4,785,418       5,60333         20       274       Yes       6       4,785,418       5,60333         21       275       Yes       6       25,598,090       6,1028625         22       276       7       264,621       5,3368175         22       277       Yes       7       264,621       5,3368175         23       280       Yes       7       206,621       5,3368175         24       275       Yes       7       6,063,531       6,188212         23       280       Yes       7       6,064,303       7,2457358         23       280       Yes       7       6,065,731       7,2457358         23       281       7       6,065,731       7,2457358         23       282       7       6,070,346       7,2457358      <	16	268		4	30.965.344	5.7629089
16       270       4       30,973,636       4,8718893         17       271       Yes       4       31,674,325       8,8057355         18       272       Yes       4       34,786,157       4,8937411         19       273       Yes       6       4,785,418       5,60333         20       274       Yes       6       7,519,840       5,449393         21       275       Yes       6       2,598,090       6,1028625         22       276       7       284,003       5,5136829         22       277       Yes       7       294,003       5,5136829         23       279       Yes       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,974       7,2457358         23       283       7       6,065,711       7,2457358         23       284       7       6,070,366       7,2457358         23       285       7       6,070,366       7,2457358         23       287       7       6,070,366       7,2457358         23       289<	16	269		4	30.967.249	4.8718593
17       271       Yes       4       31,674,325       8,805735         18       272       Yes       4       34,786,157       4,8937411         19       273       Yes       6       4,785,418       5,60333         20       274       Yes       6       7,519,840       5,4449539         21       275       Yes       6       2,598,090       6,1028625         22       276       7       264,621       5,3368175         22       277       Yes       7       284,003       5,5136829         23       279       Yes       7       6,063,531       6,189212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,791       7,2457358         23       284       7       6,067,391       7,2457358         23       285       7       6,070,349       7,2457358         23       286       7       6,070,366       7,2457358         23       289       7       6,070,679       7,2457358         23       289 <td>16</td> <td>270</td> <td></td> <td>4</td> <td>30.973.636</td> <td>4.8718593</td>	16	270		4	30.973.636	4.8718593
18       272       Yes       4       34,786,157       4,8937411         19       273       Yes       6       4,785,418       5,60333         20       274       Yes       6       7,519,840       5,4449539         21       275       Yes       6       25,598,090       6,1028625         22       276       7       264,621       5,3368175         22       277       Yes       7       264,621       5,3368175         23       279       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,094       7,2457358         23       284       7       6,067,391       7,2457358         23       285       7       6,070,349       7,2457358         23       286       7       6,070,358       7,2457358         23       287       7       6,070,364       7,2457358         23       287       7       6,070,364       7,2457358         23       289       7       6,070,	17	271	Yes	4	31 674 325	8 8057355
19       273       Yes       6       4785,418       560333         20       274       Yes       6       7,519,840       5,4449539         21       275       Yes       6       25,598,090       6,1028625         22       276       7       264,621       5,3368175         22       277       Yes       7       284,003       5,5136829         22       278       7       291,471       5,432903         23       279       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,73       7,2457358         23       284       7       6,065,71       7,2457358         23       285       7       6,070,349       7,2457358         23       286       7       6,070,366       7,2457358         23       287       7       6,070,366       7,2457358         23       289       7       6,070,366       7,2457358         23       290       7       6,070,679       7,245735	18	272	Yes	4	34,786,157	4.8937411
111	19	272	Yes	6	4 785 418	5 60333
21       275       Yes       6       25,598,090       6,1028625         22       276       7       264,621       5,3368175         22       277       Yes       7       284,003       5,5136829         22       278       7       291,471       5,432903         23       279       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,571       7,2457358         23       283       7       6,066,571       7,2457358         23       284       7       6,067,391       7,2457358         23       285       7       6,070,349       7,2457358         23       286       7       6,070,358       7,2457358         23       287       7       6,070,366       7,2457358         23       289       7       6,070,366       7,2457358         23       289       7       6,070,418       7,2457358         23       290       7       6,070,418       7,2457358	20	274	Yes	6	7.519.840	5.4449539
22       276       7       264,621       5.3368175         22       277       Yes       7       264,621       5.3368175         22       278       7       291,471       5.432903         23       279       7       6,063,531       6.1889212         23       280       Yes       7       6,064,303       7.2457358         23       281       7       6,065,094       7.2457358         23       282       7       6,065,094       7.2457358         23       282       7       6,065,094       7.2457358         23       282       7       6,066,571       7.2457358         23       284       7       6,067,391       7.2457358         23       285       7       6,070,349       7.2457358         23       286       7       6,070,358       7.2457358         23       287       7       6,070,349       7.2457358         23       286       7       6,070,356       7.2457358         23       287       7       6,070,356       7.2457358         23       289       7       6,070,366       7.2457358         23 <t< td=""><td>21</td><td>275</td><td>Yes</td><td>6</td><td>25 598 090</td><td>6 1028625</td></t<>	21	275	Yes	6	25 598 090	6 1028625
22       277       Yes       7       284,003       5,5136829         22       278       7       291,471       5,432903         23       279       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,73       7,2457358         23       283       7       6,065,873       7,2457358         23       284       7       6,066,571       7,2457358         23       285       7       6,067,391       7,2457358         23       286       7       6,070,349       7,2457358         23       287       7       6,070,358       7,2457358         23       289       7       6,070,366       7,2457358         23       289       7       6,070,366       7,2457358         23       290       7       6,070,418       7,2457358         23       291       7       6,084,536       7,2457358         23       291       7       6,084,536       7,2457358         23       <	22	276		7	264.621	5.3368175
22       278       7       291,471       5,432903         23       279       7       6,063,531       6,1889212         23       280       Yes       7       6,064,303       7,2457358         23       281       7       6,065,094       7,2457358         23       282       7       6,065,094       7,2457358         23       283       7       6,065,71       7,2457358         23       284       7       6,066,571       7,2457358         23       285       7       6,066,571       7,2457358         23       286       7       6,067,391       7,2457358         23       286       7       6,070,349       7,2457358         23       286       7       6,070,358       7,2457358         23       287       7       6,070,358       7,2457358         23       289       7       6,070,366       7,2457358         23       289       7       6,070,418       7,2457358         23       290       7       6,070,679       7,2457358         23       291       7       6,084,536       7,2457358         23       291	22	277	Yes	7	284.003	5.5136829
2327976,063,5316,188921223280Yes76,064,3037.24573582328176,065,0947.24573582328276,065,8737.24573582328376,065,8737.24573582328476,066,5717.24573582328576,067,3917.24573582328676,070,3497.24573582328776,070,3587.24573582328976,070,3667.24573582329076,070,3667.24573582329176,070,6797.24573582329276,084,5367.24573582329176,084,5367.24573582329276,084,5507.24573582329376,084,5507.24573582329176,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,084,5507.24573582329376,085,5525.211758	 22	278		7	291 471	5 432903
23280Yes76,064,3037.24573582328176,064,3707.24573582328276,065,0947.24573582328376,065,8737.24573582328476,066,5717.24573582328576,067,3917.24573582328676,070,3497.24573582328776,070,3587.24573582328976,070,3667.24573582328976,070,3667.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329176,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,086,5507.24573582329376,086,5507.24573582329376,086,5507.24573582476,086,5507.245735825	23	279		7	6.063.531	6.1889212
2328176,064,3707.24573582328276,065,0947.24573582328376,065,8737.24573582328476,066,5717.24573582328576,067,3917.24573582328676,070,3497.24573582328776,070,3587.24573582328976,070,3667.24573582329076,070,4187.24573582329176,070,6797.24573582329276,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,085,6525.2117538	23	280	Yes	, 7	6,064,303	7 2457358
121314 <td>23</td> <td>281</td> <td></td> <td>7</td> <td>6.064.370</td> <td>7.2457358</td>	23	281		7	6.064.370	7.2457358
232676,065,8737.24573582328476,066,5717.24573582328576,067,3917.24573582328676,070,3497.24573582328776,070,3587.24573582328976,070,3667.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	287		, 7	6,065,094	7 2457358
2328476,066,5717.24573582328576,067,3917.24573582328676,070,3497.24573582328776,070,3587.24573582328976,070,3667.24573582328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	283		, 7	6,065,873	7 2457358
2326176,060,3117.24573582328576,070,3497.24573582328776,070,3587.24573582328876,070,3667.24573582328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	284		, 7	6,066,571	7 2457358
232676,070,3497.24573582328776,070,3587.24573582328876,070,3667.24573582328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	285		, 7	6,067,391	7 2457358
2326076,070,3157.21573582328776,070,3587.24573582328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	286		, 7	6,070,349	7 2457358
232676,070,3667.24573582328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5367.24573582329276,084,5507.24573582329376,085,6525.2117538	23	287		7	6,070,358	7 2457358
2328976,070,4187.24573582329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329276,084,5507.24573582329376,085,6525.2117538	23	288		, 7	6,070,366	7 2457358
2329076,070,6797.24573582329176,084,5367.24573582329276,084,5507.24573582329376,085,6525.2117538	23	289		, 7	6 070 418	7 2457358
23     291     7     6,084,536     7.2457358       23     292     7     6,084,550     7.2457358       23     292     7     6,084,550     7.2457358       23     293     7     6,085,652     5,2117538	23	290		, 7	6,070,679	7 2457358
23     292     7     6,084,550     7.2457358       23     293     7     6,085,652     5,2117538	23	291		, 7	6 084 536	7 2457358
23     293     7     6085652     5217538	23	292		, 7	6 084 550	7 7457358
	23	293		7	6.085.652	5 2117538

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
23	294		7	6,085,690	7.2457358
23	295		7	6,085,742	5.2117538
23	296		7	6,085,749	5.2117538
23	297		7	6,085,763	7.2457358
23	298		7	6,085,834	5.2117538
23	299		7	6,086,044	5.2117538
23	300		7	6,086,074	7.2457358
23	301		7	6,086,334	5.2117538
23	302		7	6,086,414	7.2457358
23	303		7	6,086,726	5.2117538
23	304		7	6,086,998	7.2457358
23	305		7	6,087,022	7.2457358
23	306		7	6,087,080	7.2457358
23	307		7	6,087,198	7.2457358
23	308		7	6.087.516	7,2457358
23	309		7	6.087.526	6.153898
23	310		7	6.087.573	7.2457358
23	311		7	6.087.600	7.2457358
23	312		7	6.087.643	7 2457358
23	313		7	6,087,826	7 2457358
23	314		, 7	6.087.834	7 2457358
23	315		, 7	6,088,030	7 2457358
23	316		, 7	6,088,069	7 2457358
23	317		7	6.088.359	5 2117538
23	318		7	6,090,769	4 768021
23	319		7	6,093,126	5.8051805
23	320		7	6,093,120	5 2117538
23	320		7	6,093,671	5 2117538
23	327		7	6,093,908	5 2117538
23	373		7	6,094,396	5 8051805
23	324		7	6 094 482	5 805 1805
23	325		7	6,004,901	5 805 1805
23	325		7	6,004,805	5 8051805
23	227		7	6,004,037	5.0051005
23	220		7	6,094,937	5.0051005
23	220		7	6,094,904	5.0051005
23	220		7	6,005,324	5.0051005
25	221		7	0,095,550	5.0051005
25	222		7	0,095,464	5.4227501
25	222		7	0,095,055	2.2220/22
23	333		/	0,090,273	5.8051805
23	334		/	0,090,485	5.8051805
23	335		/	6,096,497	4.914/451
23	330		/	0,090,521	5.8051805
20	220		/	0,090,54/	5.8051805
23	338		/	6,096,809	5.9043426
23	339		/	0,098,286	5.9043426
23	340		/	0,098,386	5.8051805
23	341		/	6,098,581	5.8051805
23	342		/	6,098,852	5.8051805

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
23	343		7	6,099,226	5.8051805
23	344		7	6,099,258	5.8051805
23	345		7	6,099,332	5.9043426
23	346		7	6,099,594	5.9043426
23	347		7	6,099,686	5.9043426
23	348		7	6,099,725	5.9043426
23	349		7	6,100,039	5.9043426
23	350		7	6,100,437	5.9043426
23	351		7	6,100,990	5.8051805
23	352		7	6,101,657	5.8051805
23	353		7	6,102,160	5.7175365
23	354		7	6,102,520	5.8051805
23	355		7	6,102,592	4.8790972
23	356		7	6,102,638	4.8790972
23	357		7	6,102,693	4.8790972
23	358		7	6.102.735	4.8790972
23	359		7	6.102.903	4.8790972
23	360		7	6.103.174	5.8051805
23	361		7	6103463	4 8790972
23	362		7	6 103 806	4 8790972
23	363		7	6104116	4 8790972
23	364		7	6 104 228	5.8051805
23	365		7	6 104 370	5 8305015
23	366		7	6 104 431	1 8790972
23	367		7	6 104 667	4.8700072
23	368		7	6 104 785	4 8790972
23	369		7	6 105 088	1.07 5057 2
23	370		7	6 105 180	5 8051805
23	370		7	6 105 212	5.8051805
23	377		7	6 105 458	4 8790972
23	372		7	6 105 470	5 805 1 805
23	374		7	6 105 480	5 805 1805
23	275		7	6 105 402	5 905 1 905
20	276		7	6 106 124	6 0901262
25	370		/	6 109 272	0.9601505
23	270		7	6 109 255	4.0790972 5 0051005
25	370		/	6 109 654	5.0051005
25	379		/	0,100,034	2.001000
23	380		/	6,109,115	4.8790972
23	381		/	6,109,415	5.8051805
23	382		/	6,109,443	5.8395915
23	383		/	6,109,481	5.8051805
23	384		/	6,109,866	5.8051805
23	385		/	6,109,868	6.9801363
23	386		/	6,109,916	4.8/90972
23	387		/	6,110,331	4.8/90972
23	388		/	6,111,183	4.8790972
23	389		/	6,111,369	4.8/90972
23	390		/	6,111,870	4.8790972
23	391		7	6,112,133	5.8051805

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
23	392		7	6,112,824	5.8051805
23	393		7	6,113,492	4.8790972
23	394		7	6,113,568	4.8790972
23	395		7	6,114,786	4.8790972
23	396		7	6,116,319	4.788826
23	397		7	6,116,607	4.788826
23	398		7	6,116,670	4.788826
23	399		7	6,117,084	4.788826
23	400		7	6,117,920	4.788826
23	401		7	6,117,951	4.788826
23	402		7	6,118,057	4.788826
23	403		7	6,119,445	4.788826
23	404		7	6,119,850	4.788826
23	405		7	6,120,052	4.788826
23	406		7	6,122,777	4.7337213
23	407		7	6,130,228	4.788826
23	408		7	6,130,337	4.788826
23	409		7	6,132,970	4.788826
23	410		7	6,133,017	4.788826
23	411		7	6,133,379	4.788826
23	412		7	6,169,691	4.8181564
23	413		7	6,199,788	5.0076188
23	414		7	6,199,806	5.0076188
24	415		7	6,655,446	4.9209635
24	416	Yes	7	6,670,111	5.4666983
25	417	Yes	7	15,950,927	5.0870132
25	418		7	15,951,213	5.0870132
25	419		7	15,951,648	5.0870132
25	420		7	15,952,726	5.0870132
26	421	Yes	8	244,512	4.7358436
26	422		8	265,949	4.7358436
26	423		8	272,578	4.7358436
26	424		8	272,721	4.7358436
27	425	Yes	8	25,340,714	5.2533736
28	426	Yes	8	25,532,049	5.2987689
29	427	Yes	9	7,166,403	9.2837879
29	428		9	7,168,080	6.1269237
29	429		9	7,168,949	6.1269237
30	430	Yes	9	7,998,198	4.718603
31	431		9	9,668,007	5.6750183
31	432	Yes	9	9,668,585	5.7282745
31	433		9	9,669,315	5.7282745
31	434		9	9,670,051	5.7282745
31	435		9	9,670,151	5.7282745
31	436		9	9,670,373	5.7282745
32	437		9	9,778,486	5.7282745
32	438		9	9,778,556	5.7282745
32	439		9	9,778,722	4.9079815
32	440		9	9,778,764	5.7282745

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
32	441		9	9,778,873	4.9079815
32	442		9	9,778,945	4.9079815
32	443		9	9,779,063	5.7282745
32	444		9	9,779,075	5.7282745
32	445		9	9,779,123	5.7282745
32	446		9	9,779,138	5.7282745
32	447		9	9,779,269	5.7282745
32	448		9	9,779,315	5.7282745
32	449		9	9,779,351	4.9079815
32	450		9	9,779,353	4.9079815
32	451		9	9,779,376	5.7282745
32	452		9	9,779,470	5.7282745
32	453		9	9,779,619	4.9079815
32	454		9	9,779,834	4.9079815
32	455		9	9,779,839	5.7282745
32	456		9	9,779,846	4.9079815
32	457		9	9,779,938	4.9079815
32	458		9	9,779,996	5.7282745
32	459		9	9,780,618	5.7282745
32	460		9	9,780,698	5.7282745
32	461		9	9.782.756	5.7664962
32	462		9	9.783.743	5.7282745
32	463		9	9.784.795	6.0492653
32	464		9	9.784.952	5.7282745
32	465		9	9,785,403	5.9725688
32	466		9	9.785.621	5.9725688
32	467		9	9 785 899	5 9725688
32	468	Yes	9	9.785.988	6.698189
32	469		9	9.786.291	5.9725688
32	470		9	9,788,095	5.7282745
32	471		9	9.788.526	5,7282745
33	472		9	9.925.206	5.6003263
33	473		9	9.925.891	5.527741
33	474		9	9.926.313	5.1139043
33	475		9	9.926.854	5.6642413
33	476		9	9.927.170	5.3699792
33	477		9	9,927,826	5.3652004
33	478	Yes	9	9.928.848	6.1659864
33	479		9	9.929.137	5.6642413
33	480		9	9935223	5 6642413
33	481		9	9.936.891	5.0602412
33	482		9	9939176	5 6642413
33	483		9	9,939,470	5,6642413
33	484		9	9,941,053	5.0602412
33	485		9	9,941.753	5.6642413
33	486		9	9,941,774	5,6642413
33	487		9	9,942,709	5.6642413
34	488	Yes	9	14,962.678	4.7118402
35	489	Yes	9	15,900,636	4.7943897

LD block number	Associated SNP number	SNP with highest association in the LD	CHR	Coordinate	— log10( <i>p</i> )
36	490		9	20,146,598	4.9416879
36	491		9	20,155,748	4.8397716
36	492	Yes	9	20,209,460	5.0262212
37	493		10	2,057,109	5.453482
37	494	Yes	10	2,057,364	5.4953117
38	495	Yes	10	21,819,222	5.3096533
39	496	Yes	11	21,990,372	4.7907263
40	497	Yes	11	23,007,993	5.0269461
41	498	Yes	12	19,752,661	4.7299037
42	499	Yes	12	25,568,747	4.8310019

Table 3 (continued)

antimicrobial compounds. Rice produces antimicrobial compounds such as momilactones are known to accumulate in the husks of cultivated rice (Kato et al. 1973; Minh et al. 2018). This suggests that momilactones may contribute to the chemical defense of rice seeds. On the other hand, much remains unknown about whether compounds other than momilactones contribute to rice seed chemical defense. To address these issues, we established two bioassay systems suitable for simple and rapid determination of seed-derived antibacterial activity in a variety of materials, including landraces and wild *Oryza* species.

The results of seed antibacterial activity measurements using cultivated rice accessions by the disk diffusion and colorimetric quantification methods established in this study showed generally consistent trends, but overall, the colorimetric method with MTS was able to detect antibacterial activity in a wider range of accessions (Fig. 5a–c). The growth inhibition circle of the disk diffusion method is often affected by the extent of the diffusion of compounds into the medium. Therefore, even if the crude extract contains compounds with antimicrobial activity, the activity may not be detected by the disk diffusion method (Valgas et al. 2007; Bubonja-Sonje et al. 2011). Furthermore, in cases where antimicrobial compounds do not inhibit but slow down the growth of bacteria, it is conceivable that the disk diffusion method may not be able to observe growth inhibition circles. These may be the reasons why antibacterial activity could be detected in more accessions than in the MTS assay.

In this study, the cultivated rice core collections, which cover a wide range of genetic diversity, were used to determine the strength of the seed defense mechanisms against microbial infection. These materials include 28 *indica* and 58 *japonica* lines. Seed antibacterial activity of these subspecies was measured in a crude extract of 80% methanol from rice husks, from which momilactones are efficiently extracted (Lee et al. 1999), by the disk diffusion method, and 42.9% and 37.9% of indica and japonica, respectively, showed growth inhibition circles (Tables 1, 2). Since it is known that momilactones tend to accumulate more in *japonica* than in *indica* (Kariya et al. 2020), it is likely that other antibacterial compounds are present in addition to momilactones at least in *indica* seeds. In fact, momilactones have been reported to exhibit antibacterial activity against E. coli at 400 µg/disc in the disk diffusion assay (Fukuta et al. 2007). However, the amount of momilactones contained in rice husk is extremely small, on the order of ng per hull, and is far less than the concentration that shows a growth inhibitory effect against E. coli (Quan et al. 2019a, 2019b; Kakar et al. 2019). This suggests that compounds other than momilactones are involved in the antibacterial activity of the seeds. Furthermore, Minh et al. (2018) focused on rice husk as a material including biochemicals and analyzed the metabolites in rice husks. They found that rice husks of Koshihikari contain momilactones, phenolic acids, phenols, and long-chain fatty acids, and exhibit antimicrobial activity against bacteria. Taken together, it is suggested that several antibacterial compounds other than momilactones may contribute to the antibacterial activity in several cultivated rice varieties used in this study. If momilactones function as antibacterial compounds in seeds, it may be due to the combined action of momilactones and other antibacterial compounds rather than momilactones alone.

Both in cultivated and wild *Oryza* species, antibacterial activities were detected either in husks or brown rice and in both. This suggests that the defense mechanism against microorganisms by seeds may operate both in husks and brown rice and that the defense by the husks and brown



rice may function independently. In fact, components of rice bran derived from the outermost layer of brown rice aleurone have been reported to exhibit antibacterial activity against bacteria (Arpan et al. 2013; Castanho et al. 2019; Ferdes et al. 2009).

The GWAS performed in this study revealed several genomic regions that are strongly associated with the

inhibition of bacterial growth. Among them, several SNPs showed a strong association with the growth inhibition induced by acetone extracts of brown rice. This suggests that the genetic factors responsible for the production of substances inhibiting bacterial growth, which is contained in brown rice, may be located near these SNPs. Because these SNPs were located in a relatively large linkage disequilibrium region, we could not identify the exact genetic factors through this analysis. In the future, genetic factors can be identified through QTL analysis using the progenies of crossed plants between two cultivars with different haplotypes identified from our GWAS analysis. The simple and high throughput seed antibacterial activity assay developed in this study is a possible significance tool to identify genetic factors related to seed antibacterial activity.

In this study, we comprehensively analyzed the antibacterial activity of seeds from a wide range of wild Oryza genetic resources. It is known that some wild Oryza accessions do not produce known phytoalexins, such as momilactones (Miyamoto et al. 2016; Kariya et al. 2020). Thus, the wild Oryza accessions with strong antibacterial activity could be useful materials for understanding seed defense and interactions with microorganisms and for searching for novel compounds related to these interactions. The quantitative assay of seed antibacterial activity through MTS method developed in this study can be used for analyzing materials with a limited number of seeds, such as wild Oryza species because this method can measure antibacterial activity with a limited amount of samples. Therefore, in the future, this method can be applied for GWAS analysis using wild Oryza genetic resources and for measurement of antibacterial activity after transformation experiments of wild Oryza in the process of identifying loci involved in antibacterial substance production (Shimizu-Sato et al. 2020). It is surprising that the growth-promoting effect seen in the acetone extracts is specific to cultivated species. It is intriguing that, during the process of domestication of rice, cultivated rice strains become to accumulate chemicals with a positive effect on bacterial growth.

#### Conclusion

Effectiveness and easiness of the bioassay system we developed in this study was confirmed with Gram-negative bacteria, *E. coli*. There is a possibility that this assay system is applicable to other microorganisms including Gram-positive bacteria and fungi. Furthermore, it can be used to investigate antibacterial activity against seeddecaying microorganisms and vertically transmitted pathogenic microorganisms. The use of this bioassay to search for varieties that exhibit potent seed antibacterial activity is expected to accelerate the breeding of varieties resistant to vertically transmitted seed-borne diseases, and with high seed quality after storage, and advance the identification of novel antibacterial compounds.

#### Abbreviations

MTS: 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; GWAS: Genome-wide association study.

### **Supplementary Information**

The online version contains supplementary material available at https://doi. org/10.1186/s12284-022-00610-3.

Additional file 1: Table S1. Weight of husks and brown rice from 10 grains of WRC, JRC and wild *Oryza* used in this study.

Additional file 2: Figure S1. Genome-wide association study using antibacterial activities of rice seed crude extracts from 107 accessions of landraces from WRC and JRC as phenotype data. (a–c) Manhattan and Quantile–quantile plots of SNPs associated with antibacterial activity in acetone extracts from husk (a), sterilized water extracts from husk (b), and sterilized water extracts from brown rice (c).

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#### **Author Contributions**

YY, MNT, TY, and YS performed the experiments. YY and YS conceived and designed the study. All authors read and approved the final manuscript.

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#### Availability of Data and Materials

All datasets are available from the corresponding author on reasonable request.

#### Declarations

#### Ethics Approval and Consent to Participate Not applicable.

Consent for Publication

Not applicable.

#### **Competing Interests**

The authors declare that they have no competing interests.

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