1 **Identifying genomic regions and candidate genes selected during the breeding of rice in Vietnam**

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14 **Key Message**

15 We localised regions in the rice genome selected during breeding by comparing allele frequency patterns among
16 Vietnamese rice subpopulations. We characterised candidate genes in the Indica-5 subpopulation with breeding
17 potential.

18

19 **Abstract**

20 *Background and aims*

21 Vietnam harnesses a rich diversity of rice landraces adapted to a broad range of conditions, which constitute a
22 largely untapped source of genetic diversity for the continuous improvement of rice cultivars. We previously
23 identified a strong population structure in Vietnamese rice, which is captured in five Indica and four Japonica
24 subpopulations, including an outlying *Indica-5* group. Here, we leveraged on that strong differentiation, and the
25 672 rice genomes generated, to identify genes within genomic regions putatively selected during domestication
26 and breeding of rice in Vietnam.

27 *Methodology*

28 We identified significant distorted patterns in allele frequency (XP-CLR method) and population differentiation
29 scores (F_{ST}), resulting from differential selective pressures between native subpopulations, and compared them
30 with QTLs previously identified by GWAS in the same panel. We particularly focused on the outlying *Indica-5*
31 subpopulation because of its likely novelty and differential evolution.

32 *Results*

33 We identified selection signatures in each of the Vietnamese subpopulations and carried out a comprehensive
34 annotation of the 52 regions selected in *Indica-5*, which represented 8.1% of the rice genome. We annotated the
35 4,576 genes in these regions, verified the overlap with QTLs identified in the same diversity panel and the
36 comparison with a F_{ST} analysis between subpopulations, to select sixty-five candidate genes as promising
37 breeding targets, several of which harboured alleles with non-synonymous substitutions.

38 *Conclusions*

39 Our results highlight genomic differences between traditional Vietnamese landraces, which are likely the
40 product of adaption to multiple environmental conditions and regional culinary preferences in a very diverse
41 country. We also verified the applicability of this genome scanning approach to identify potential regions
42 harbouring novel loci and alleles to breed a new generation of sustainable and resilient rice.

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44 **KEYWORDS:** Rice, breeding, selection, landraces
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Introduction

Vietnam harnesses a rich novel rice diversity due to the presence of native and traditional rice varieties adapted to its broad latitudinal range, diversity of ecosystems and regional food preferences (Fukuoka et al. 2003). This diversity constitutes a largely untapped and highly valuable genetic resource for local and international breeding programs (Khanh et al. 2021). Vietnamese rice shows a strong population structure, which is captured within five Indica and four Japonica subpopulations that we have recently described (Higgins et al. 2021). These subpopulations were characterised in relation to the fifteen subpopulations of Asian rice described by the rice 3,000 rice genomes project (3K RGP) (Zhou et al. 2020). Among these nine populations described in Vietnam, the *Indica*-5 (I5) subpopulation is an outlier and is expanded in Vietnam and, therefore, a potential source of novel variation compared to the wider Asian diversity.

Genetic variation and differentiation are influenced by natural processes, such as adaption and random drift, as well as conscious breeding selection and unconscious selection, due to the agricultural practices of local farmers. Selection causes detectable changes in allele frequencies at the selected sites and their flanking regions. By modelling differences in allele frequency in close loci between neutrality and selection scenarios, the cross-population composite likelihood ratio test (XP-CLR) can detect selective sweeps (Chen, Patterson, and Reich 2010). Any distorted pattern in allele frequency in contiguous SNP sites would have occurred too quickly (speed of change is assessed over expanding windows based on the length of the affected region) to be explained by random drift, so allowing the identification of selected regions. XP-CLR has been used to identify regions of selection associated with domestication and improvement in a wide range of crops, such as apple (Duan et al. 2017), soybean (Zhou et al. 2015), cucumber (Qi et al. 2013) and wheat (Joukhadar et al. 2019); and has been increasingly used in rice. Lyu et al.(2014) identified a list of differentiated genes that may account for the phenotypic and physiological differences between upland and irrigated rice. Xie et al (2015) compared Indica semi-dwarf modern bred varieties (IndII) with taller Chinese landraces (IndI) to identify signatures of rice improvement and detected 200 regions spanning 7.8% of the genome. Meyer et al. (2016) identified genomic regions associated with adaptive differentiation between *O. glaberrima* populations in Africa. He et al. (2017) tested for positive selection between weedy and landrace rice using five different approaches. Cui et al. (2019) identified potential selective sweeps in both Indica and Japonica genomes showing that there were multiple loci responding to selection and that loci associated with agronomic traits were particularly targeted by selection. Lin et al. (2020) used XP-CLR to demonstrate how introgressed regions were selected through hybrid rice breeding.

76 While these studies were trying to answer difference questions, all used XP-CLR to detect selected regions. In
77 addition, many of the studies used other metrics, such as the fixation index (F_{ST}), to verify selected regions.
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79 Here, we identified regions in the rice genome which have been selected by human breeding by leveraging the
80 strong population structure among Vietnamese-native rice varieties and landraces, which has resulted from its
81 diverse geography and agronomic practices. Unravelling the genomic differences and identifying regions
82 selected between these nine subpopulations is the first step towards understanding their breeding potential. To
83 assess the potential of our approach, we later focused on the outlying *indica-5* (I5) subpopulation to identify
84 candidate loci for breeding targets, since this subpopulation constitutes a gene-pool not used in rice
85 improvement. To assess the putative role of these selected regions and whether these selected regions may
86 contain loci that potentially could control agronomic traits, we looked for overlaps with previously mapped
87 QTLs in the same diversity panel, and regions enriched in gene ontology (GO) terms. QTLs have been
88 described for a range of agronomic traits using the complete set of 672 native rice accessions (Higgins et al.
89 2021), while a subset of 182 of these traditional Vietnamese accessions (Phung 2014) were used for genome-
90 wide phenotype-genotype association studies (GWAS) relating to root development (Phung et al. 2016), panicle
91 architecture (Ta et al. 2018), drought tolerance (Hoang, Van Dinh, et al. 2019), leaf development (Hoang,
92 Gantet, et al. 2019), Jasmonate regulation (To et al. 2019) and phosphate starvation and efficiency (Mai et al.
93 2020; To et al. 2020). Finally, we studied alleles with non-synonymous substitutions in candidate genes in
94 selected regions of the outlying and highly selected I5 subpopulation.

95

96 **Materials and Methods**

97 **Sequencing and SNP calling and annotation**

98 A set of 616 Vietnamese samples were sequenced by us as described previously (Higgins et al. 2021). We added
99 56 samples from the “3000 Rice Genomes Project” (3K RGP), which originated from Vietnam, to give a total of
100 672 samples. These samples were genotyped as described previously (Higgins et al. 2021) to obtain a set of
101 imputed SNPs which were filtered to remove SNPs with excess heterozygosity, followed by filtering for a
102 minimal allele frequency of 5%. We utilized a set of 2,027,294 SNPs for the 426 Indica samples and 1,125,716
103 SNPs for the 211 Japonica samples. Detailed information for each sample is available in Higgins et al. (2021). A
104 summary of the number and source of each subpopulation is available in Table 1 (47 Indica samples and 9
105 Japonica samples native to Vietnam were obtained from the 3K RGP project). The putative effects of the bi-

allelic SNPs (low, medium and high effects) on the genome were determined using SnpEff (Cingolani et al. 2012) as detailed in Higgins et al. (2021) and the pre-built release 7.0 annotation from the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>).

Identification of selective sweeps using XP-CLR

Selective sweeps across the genome were identified using XP-CLR (Chen, Patterson, and Reich 2010), a method based on modelling the likelihood of multilocus allele frequency differentiation between two populations. An updated version of the original code was used (<https://github.com/hardingnj/xpclr>). We used 100 kbps sliding windows with a step size of 10 kbps and the default option of a maximum of 200 SNPs in any window. XP-CLR was run comparing the five Indica subpopulations to each other and the four Japonica subpopulations to each other. Selected regions were extracted using the XP-CLR score for each 100 kbps window as follows: 200 kbps centromeric regions were removed. The mean and 99th percentile of the XP-CLR scores were calculated for each comparison between one subpopulation against the remaining ones (e.g. I5 vs I1, I2, I3, I4). The mean 99th percentile was used to define the cut-off level for selection in that subpopulation. 100 kbps regions with an XP-CLR score higher than the cut-off were extracted and contiguous regions were merged using BEDTools v2.26.0 (Quinlan and Hall 2010) specifying a maximum distance between regions of 100 kbps. Regions shorter than 80 kbps were removed to give a final set of putatively selected regions for each comparison. Putative regions observed selected in at least two comparisons for Japonica subpopulations, or three comparisons for Indica subpopulations, were merged to obtain a final set of selected regions for each subpopulation. BEDTools map was used for finding any overlap of selected regions with QTLs. QTL regions using the same, or a subset of, the samples were previously identified by reviewing the literature. Genes lying within the selected regions were extracted and checked for enrichment in Protein Domain and Pathway using a maximum Bonferroni FDR value of 0.05 in PhytoMine (<https://phytozome.jgi.doe.gov/>), a service implemented within Phytozome (Goodstein et al. 2012).

Calculating F_{ST}

We calculated F_{ST} per SNP between the 43 samples in the I5 subpopulation and the 190 samples in the I2, I3 and I4 subpopulations with VCFtools using the "weir-fst-pop" option, which calculates F_{ST} according to the method of Weir and Cockerham (Weir and Cockerham 1984). Sites which are homozygous between these

populations were removed, and negative values were changed to zero. The mean F_{ST} was calculated per gene and per specified region.

Enrichment analysis of GO terms in selected regions

The enrichment analysis was done with the library topGO (Alexa A 2010) in R, using as inputs the lists of genes in each selected region, and the functional annotation of the rice genome (Rice MSU7.0) from agriGO (<http://bioinfo.cau.edu.cn/agriGO>). The method in topGO compared the genes observed in each selected region annotated with a given GO term with the expected number of genes annotated with that term in the whole transcriptome. The statistical test was a F-Fisher test ($FDR \leq 0.05$) with the “weight01” algorithm in topGO. The “weight01” algorithm resolves the relations between related GO ontology terms at different levels. The number of genes and selected regions that were enriched terms for the different subpopulations were plotted using ggplot2 (Wickham 2016).

Results

Identification of selective sweeps among Vietnamese subpopulations

To identify genomic regions that have been selected during the breeding of rice in Vietnam, we searched for genomic regions with distorted patterns of allele frequency that cannot be explained by random drift using XP-CLR (Chen, Patterson, and Reich 2010). We used our previously described dataset of 672 genomes from Vietnamese-native landraces and varieties (Table 1), which have been divided into nine subpopulations (Higgins 2021). We compared all the five Indica subpopulations to each other and all the four Japonica subpopulations to each other. Firstly, we obtained the mean XP-CLR score over the whole genome, as summarised in Table 2, with the reciprocal differences in the comparisons between a pair of subpopulations in Supplementary Table S1. Among the Japonica subpopulations, the J4 subpopulation had consistently the highest selection scores especially against the J1 subpopulation. Among the Indica subpopulations, the I1 subpopulation had consistently the lowest selection scores. The I5 subpopulation had the highest selection scores except in comparison with the I3 subpopulation. We calculated the 99th percentile for each comparison between a pair of subpopulations and used the mean value for each subpopulation as a cut-off to identify selected regions (detailed in Supplementary Table S2 and summarised in Table 3). We merged selected regions within 100kb of each other, so the final set of selected regions for each comparison were of variable length. Selected regions covered a higher proportion of the genome where the XP-CLR score was higher. The regions selected in the comparisons between a pair of subpopulations were plotted along each chromosome for the Indica subpopulations (Supplementary Fig. S1) and the Japonica subpopulations (Supplementary Fig. S2). In order to define a final set of selected regions in a given subpopulation, we retained and merged regions selected in at least three comparisons between that subpopulation and any other subpopulation in the case of the Indica ones, or in at least two comparisons in the case of the Japonica subpopulations. This procedure is described in detail for the I5 subpopulation in a subsequent section. The final set of selected regions in each subpopulation were plotted along each of the rice chromosomes in Fig. 1A and 1B for the Indica and Japonica subtypes, respectively. The selected regions ranged from 98,583 to 2,787,579 bases for the Japonica subpopulations, and from 106,844 to 2,309,615 bases for the Indica subpopulations. We observed slightly different patterns in length variation per subtype and subpopulation (Supplementary Fig. S3). Overall, the Japonica subpopulations had fewer selected regions, which represented from 3.7 to 4.9% of the genome, while Indica subpopulations ranged from 5.3 to 8.1% of the genome. Gene lists for the selected regions are available in Supplementary Table S3. The Japonica subtypes had a higher proportion

of long selected regions. These regions were confined to specific areas of the genome and absent from large chromosome regions. All four Japonica subpopulations were selected on the long arm of chromosome 2 and in both flanks of the centromeric region of chromosome 4. The selected regions in the Indica subpopulations were spread throughout the genome and very variable in length. We particularly observed a high proportion of shorter than average selected regions and a lower proportion of longer than average selected regions in the I1 subpopulation. The I5 subpopulation stands out as having the highest proportion of the genome under selection, overlapping with the other landrace subpopulations (I2, I3 and I4) on the short arm of chromosome 1 and the long arm of chromosome 9. However, selected regions in I5 were absent on the long arm of chromosome 4, where all other landrace subpopulations overlapped with the elite I1 subpopulation.

Putative roles of the regions under selection

We looked for the overlap of the selected regions with sets of QTLs previously reported in the literature (Supplementary Tables S4 and S5); 21 QTLs for basic plant and seed architecture traits identified using the same complete set of Vietnamese rice samples (Higgins et al. 2021); and 88 QTLs associated with root development traits (Phung et al. 2016), 29 QTLs for panicle morphological traits (Ta et al. 2018), 17 QTLs for tolerance to water deficit (Hoang, Van Dinh, et al. 2019), 13 QTLs for leaf mass traits (Hoang, Gantet, et al. 2019), 25 QTLs for growth mediated by jasmonate (To et al. 2019), 21 QTLs for phosphate starvation (Mai et al. 2020) and 18 QTLs for phosphate efficiency (To et al. 2020) reported for a subset of 180 samples of the whole dataset. The selected regions in the Japonica subpopulations had overlaps with all the QTLs sets, except QTLs associated with growth regulation by jasmonate (Supplementary Table S5). The region on chromosome 2 that was selected in all Japonica subpopulations overlapped with a QTL for grain length (2_GL) and two related QTLs for panicle morphology, secondary branch number (SBN) and spikelet number (SpN). These QTLs collocate with osa-MIR437 (Ta et al. 2018), a monocot preferential miRNA that targets LOC_Os02g18080 (<https://rapdb.dna.affrc.go.jp>). J2 and J4 lowland varieties were both selected on the long arm of chromosome 5 and at the start of chromosome 9. The region on chromosome 5 overlaps with a QTL for drought sensitivity observed after 4 weeks of drought stress (q4_Score4). The selected region on chromosome 9 overlaps with a QTL for rachis length (RL), which is associated with the size of the panicle, a key component of yield. The region towards the end of chromosome 11, which was selected in J1, J2 and J3, overlaps with qRTW11.19 as well as several QTLs associated with root traits: Rq13_J_TIL, Rq29_J_DEPTH, Rq30_J_DEPTH, Rq46_F_NCR, Rq63_J_THK. The selected regions in the Indica subtypes overlapped with all the QTL sets

(Supplementary Table S4). Most overlaps that occurred in more than one subpopulation were also observed in the I5 subpopulation, so are discussed in the next section. In addition, the region on the long arm of chromosome 11, which is selected in both I3 and I4, overlaps with QTLs for drought sensitivity (Tq17 Score4), rachis length (QTL25 RL) and response to jasmonate (qSHL5).

The total number of genes within the selected regions are shown in Table 2. For the Japonica subtypes, the number of genes ranged from 2,007 genes within the selected regions of the J3 subpopulation to 2,643 genes within the selected regions of the J4 subpopulation. For the Indica subtypes, the number of genes ranged from 2,993 to 3,465 in the I1 to I4 subpopulations, whilst the I5 subpopulation had 4,576 genes within 52 selected regions (gene listed in Supplementary Table S3). The overlap between genes selected in each subpopulation showed that around half of the genes selected in a subpopulation were unique to that subpopulation (Supplementary Fig. S4). No genes were selected in all subpopulations, but 230 genes were selected in all four Japonica subpopulations, and 44 genes were selected in all the Indica landrace subpopulations I2 to I5.

An enrichment analysis of the GO terms enriched in each selected region was obtained by comparing the annotations in each selected region with the whole genome annotation, as background (Supplementary Table S6). The number of genes associated with enriched terms in different regions from the same subpopulation were added up and plotted (Fig. 2). A large proportion of genes in selected regions were associated with the same biological functions in the different Indica subpopulations, e.g. Lipid and protein metabolic process, or “Biosynthetic process”. However, we also evidenced specific selections in particular subpopulations, such as “Photosynthesis” genes in I5 and J1; biotic response genes in I2, I5 and J1; abiotic response genes in I1 and I5; and “flower development” genes in I2. Selected regions were more clearly associated to specific GO terms in the Indica subpopulations than in the Japonica ones. The enrichment of GO terms was not correlated with the total number of genes or genome length in each subpopulation (Supplementary Table S2).

Selected regions in the outlying Indica-5 (I5) subpopulation

Overall, the I5 subpopulation had the highest XP-CLR selection scores, this is reflected in I5 having the greatest number of selected regions covering the highest proportion of the genome. I5 is an outlier subpopulation, which contains a gene-pool not present in the modern bred improved varieties that comprise subpopulation I1 (Higgins et al 2021). The XP-CLR score of the I5 subpopulation compared to the other four Indica subpopulations in 100

kbps windows is shown in Fig. 3. A cut-off XP-CLR score of 440 was used to defined selected regions in I5; I5 vs I1 produced 207 regions with a mean length of 267 kbp (14.8% of the genome); I5 vs I2 produced 120 regions with a mean length of 204 kbp (6.57% of the genome); I5 vs I3 produced 14 regions with a mean length of 162 kbp (0.61% of the genome); I5 vs I4 produced 122 regions with a mean length of 122 kbp (6.02% of the genome). Regions selected in three or more subpopulations were merged to give 52 selected regions in I5, the regions are listed in Supplementary Table S7 and the functional annotation of each region detailed in Supplementary Table S8. These regions had a mean length of 584 kbp, covered 30 Mbp, which represents 8.13% of the rice genome, and contained 4,576 genes (Supplementary Table S9). To gain further information on the uniqueness of these 52 regions selected in I5, we calculated the F_{ST} per SNP between the 43 samples in the I5 subpopulation and the 190 samples in the landrace subpopulations, I2, I3 and I4. The variation of F_{ST} and diversity along each chromosome are shown in Fig. 3e and 3f. Both F_{ST} and diversity varied widely along the genome and did not show the clear peaks seen in the XP-CLR score, but peaks can be seen in F_{ST} pattern coinciding with XP-CLR peaks. This is clearest on chromosome 12 where F_{ST} and XP-CLR score showed a similar pattern and the diversity scores showing the opposite pattern. Our aim was to localise regions in the genome with both high F_{ST} between the I5 subpopulation compared to the other Vietnamese subpopulations and low diversity in the I5 subpopulation. High F_{ST} but low diversity would be expected in recently selected regions, as can be seen on chromosome 10. Chromosome 3 also showed this pattern and contained a large number of selected regions. The mean F_{ST} per gene for the 4,576 genes selected in I5 is listed in Supplementary Table S10, and the mean F_{ST} per selected region is shown in Supplementary Table S7. The 1,983,066 heterozygous SNPs in subpopulations I2, I3, I4 and I5 had a mean F_{ST} of 0.185, and this mean value increased to 0.305 for the subset of 177,874 SNPs within the I5 selected regions.

The overlap of the 52 selected regions in the I5 subpopulation with the eight sets of QTLs is shown in Fig. 4. Fourteen regions showed significant overlaps, these were shaded in Fig. 4 and listed in Table 4, detailing the individual QTLs in Supplementary Table S11. Region 'a' was selected in I2, I4, I5, J1, J3 and J4, and coincided with a QTL for spikelet number (SpN) and two QTLs for root traits (q73, q84) where the following candidate genes LOC_Os01g10900, LOC_Os01g11010 and LOC_Os01g11860 were located. Region 'b' was selected in I1 and I5, and coincided with a QTL for Leaf weight (FW_TW) and a QTL for relative phosphate uptake (qRPUe1.5) where the candidate gene LOC_Os01g66070 was located. Region 'c' was selected in I3 and I5 and overlapped a QTL for response of root length to jasmonate (qRTL1), an overlap of 37 candidate genes,

including the transcription factor *OsBLR1* (LOC_Os02g47660), which regulates leaf angle in rice via brassinosteroid signalling (Wang et al. 2020). Region ‘d’ partially overlapped a QTL for panicle length (9_PL) and a region selected during recent domestication by farmers in China (Cui et al. 2019). Region ‘e’ fully overlapped a QTL for grain length (12_GL). Region ‘f’ was selected in I3, I4 and I5 and coincided with a QTL for leaf length (Rq2), which is only found in the Japonica subtype. Both regions ‘e’ and ‘f’ overlapped with two large regions selected during recent domestication by farmers in China. Gene *SSIIa* (LOC_Os06g12450) and *SDL/RNRS1* (LOC_Os06g14620) fall within this region. *SSIIa* is required for the edible quality of rice and plays an important role in grain starch synthesis (Zhang et al. 2011). *SDL/RNRS1* (LOC_Os06g14620) encodes the small subunit of ribonucleotide reductase, which is required for chlorophyll synthesis and plant growth development (Qin et al. 2017). Region ‘g’ was selected in I1 and I5, and coincided with a QTL for panicle length (14_PL) and a QTL for maximum root length (Rq35). Region ‘h’ was selected in J4, I1 and I5, and coincided with a QTL for relative water content (Tq7) observed after 3 weeks of drought stress. Region ‘i’ was selected in I3 and I5, and coincided with a QTL for root depth (Rq25). Region ‘j’ was selected in I3, I4 and I5, and overlapped with a QTL for response of shoot length to jasmonate (qSHL4) and 4 candidate genes, including a G2-like transcription factor (LOC_Os08g06370). Region ‘k’ was selected in I3, I4 and I5, and coincided with two QTLs for panicle traits, primary branch number (PBN) and primary branch average length (PBL) that include the gene Auxin Response factor, *OsPILS2* (LOC_Os08g09190). Region ‘l’ coincided with a QTL for the number of crown roots in response to Pi deficiency (qNCR8.13), which contained two candidate genes, *OsPP2C66* (LOC_Os08g39100) encoding PHOSPHATASE 2C, and transcription factor *OsWKKY30* (LOC_Os08g38990). Region ‘m’ was selected in I1, I4 and I5, and coincided with four QTLs related to response of plants to drought (Tq12), which contained *OsbZIP80* (LOC_Os11g05640), a transcription factor involved in dehydration stress response (Nijhawan et al. 2008). Region ‘n’ was selected in J1 and I5, and coincided with a QTL for number of crown roots (Rq43).

Candidate genes and non-synonymous alleles in selected regions of I5

We carried out three further analysis on the I5 subpopulation selected regions; a functional enrichment of the genes found within the 52 regions (Supplementary Table S8), a detailed annotation of the 4,576 genes in the 52 regions selected in the I5 subpopulation (Supplementary Table S10), and an analysis of the location of twenty-one genes with a putative role in salt tolerance in rice (Ganie et al. 2019) within the regions selected in the I5 subpopulation (Supplementary Table S12). Eventually, sixty-five candidate genes among the genes identified in

the previous three analysis were short-listed using the following criteria (Table 5, details in Supplementary Table S13); F_{ST} over 0.5 in the whole selected region or in the functionally enriched genes within regions, presence of “*High impact*” SNPs, and presence of candidate genes from overlapping QTL. Ten of the 65 genes contained SNPs classified using SnpEff as having a “*High impact*”, i.e. predicted to cause deleterious gene effects (five stop gains, two start loses and three frame swifts). The alleles of eight of the genes containing “*High impact*” SNPs were different in the I5 subpopulation compared to the other Indica subpopulations (Fig. 5 and Supplementary Table S14). Among these eight genes, five of them showed the same allele than the Japonica subpopulations. However, two genes (LOC_Os10g35604 and LOC_Os11g10070/OsSEU2) had alleles unique to the I5 subpopulation.

Discussion

Vietnam has one of the richest rice germplasm resources with over 4000 years of rice-cultivating experience. Local farmers have bred varieties to suit their ecosystem and regional culinary preferences. These conscious and unconscious selection processes have resulted in detectable changes in allele frequencies at selected sites and their flanking regions. We used a well-tested method, named XP-CLR, to identify distorted allele frequency patterns in contiguous SNP sites that cannot be explained by random drift. To identify regions under selection, we leveraged the strong population structure recently described in Vietnam (Higgins et al. 2021), which comprised five Indica and four Japonica subpopulations of native rice accessions adapted to variable geography and latitude range. We observed a stronger signature of selection in the Indica subtypes than in the Japonica subtypes, which may reflect the higher diversity within the Indica subtypes in Vietnam. Taking into consideration the size and diversity in each subpopulation (Higgins et al. 2021), the whole-genome XP-CLR score was lower in the larger subpopulations (I1 and J1) and the subpopulations with the lower diversity. However, this trend was not true in the subpopulation indica-5 (I5), which showed a higher selection score than the other subpopulations with comparable size and diversity. Within the Indica subtypes, the subpopulation I5 showed the highest XP-CLR score against the subpopulation I1, which supports a strong signature for selection in I5 compared to the modern bred varieties in I1. On the contrary, the lowest XP-CLR score was obtained when I5 was compared to the I3 subpopulation, which is adapted to upland ecosystems (Phung et al. 2014). This suggest I5 shares selection pressures and resilient traits with upland varieties. Intermediate XP-CLR scores were obtained for the comparison of I5 with the two lowland subpopulations I2 (Mekong Delta) and I4 (Red River Delta).

Diversity is reduced when regions are under selection, but the observed diversity depends on many factors, including how long ago the selection occurred and the type of alleles selected alongside. This is referred to as the hitchhiking effect (Pavlidis and Alachiotis 2017). The fixation index (F_{ST}) is a measure of population differentiation due to genetic structure. Both measurements vary highly along the genome but can provide additional information about the selected regions identified using XP-CLR. In this study, we calculated F_{ST} by comparing the I5 accessions to accessions in subpopulations I2, I3 and I4. We did not include the accessions in the elite I1 subpopulation, as we are specifically interested in genes that have been selected during the breeding of landraces within Vietnam. We used F_{ST} as an additional measure for identifying regions and genes under strong selection in the I5 subpopulation, and in support of the selection measurements obtained using XP-CLR.

Assigning functional roles to both regions and genes within the regions was the following natural step to identify breeding targets. We used two approaches, overlap with QTLs and functional enrichment. Seven QTL studies have been carried out on this dataset, finding associations for a range of traits relating to yield, this enables us to propose functional associations for around a third of the selected regions. A functional enrichment analysis evidenced selected regions were more clearly associated to specific GO terms in the Indica subpopulations than in the Japonica ones. The enrichment of GO terms was not correlated with the total number of genes or genome length in each subpopulation.

Looking in more detail at the 52 regions selected in the I5 subpopulation using a range of criteria, we identified 65 candidate genes within 20 of the selected regions. Six of these regions had a mean F_{ST} over 0.5 and we highlighted the following candidate genes within these regions. In region I5_35, we identified the transcription factor *WOX11* involved in crown root development (Zhang et al. 2018) and *OsCam1*, *OsbZIP63*, and *OsSDR*, which have putative roles in defence (Kim et al. 2009). Further genes of interest were (i) *OsAAP6*, a regulator of grain protein content (Peng et al. 2014), in region I5_5, (ii) *OsBSK3* (Zhang et al. 2016) and *WSL5* (Liu et al. 2018), which play roles in growth, in region I5_29, (iii) *OsABP*, which is upregulated in response to multiple abiotic stress treatments (Macovei et al. 2012), falls within region I5_33; and (iv) *OsSFR6*, a cold-responsive gene (de Freitas et al. 2019), in region I5_47. In addition, eight of the ten genes containing “high impact” mutations showed a different allelic content in the I5 subpopulation compared to the other Indica subpopulations, and in six cases these alleles were similar to the Japonica ones. Two genes containing “high

impact” mutations were *OsFBX398*, an F-box gene with a potential role in both abiotic and biotic stresses (Jain et al. 2007; Vemanna et al. 2019), in region I5_49; and *bip130* (Zhou et al. 2019) in region I5_30, which regulates abscisic acid-induced antioxidant defence and fall within our QTL for panicle length (9_PL). In order to pinpoint candidate genes for a range of agronomic traits, we looked for overlap of selected regions with relevant QTLs. 14 of the 52 regions selected in the I5 subpopulation had overlaps with a wide range of QTLs, two of most relevant genes in these regions were *SSIIa*, which is responsible for the eating quality of rice (Zhang et al. 2011), and *OsZIP80*, which is a transcription factor involved in dehydration stress response (Nijhawan et al. 2008).

Finally, we looked for overlaps with selected genes identified in three published studies using XP-CLR in rice (Lyu et al. 2014; Xie et al. 2015; Cui et al. 2019). Lyu et al. (2016) identified 56 Indica-specific genes in selected regions, which may account for the phenotypic and physiological differences between upland and irrigated rice. Thirty-one of these genes were on chromosome 3 and lied within regions also selected in the I4 and I5 subpopulations (I5_23, I5_24). The gene with the highest F_{ST} (0.67) is *ptr8* (LOC_Os03g51050), which encodes a peptide transporter (Ouyang et al. 2010). Xie et al. (2015) identified 2,125 and 2,098 coding genes in regions selected in the Chinese landraces (IndI) and modern-bred (IndII) subpopulations, respectively. We evidenced an overlap of 131 genes in selected regions in the I5 subpopulation with the genes selected in the IndI subpopulation and an overlap of 235 genes with the genes selected in the IndII subpopulation. This includes 7 genes in I5_22 and two genes in I5_23, both regions on chromosome 3, which were selected in all three subpopulations. Cui et al (2018) identified 186 potential selective-sweep regions in the Indica subtypes, of which 33 overlap with 9 of the 52 regions identified in the I5 subpopulation. These 9 regions contained 153 genes (Table 2). Cui et al. were specifically addressing the role of indigenous farmers in shaping the population structure of rice landraces in China, there is the possibility that similar regions may also have been selected in Vietnam. Substantial overlaps were found in three regions. On chromosome 2, 3 regions overlapped with I5_14. On chromosome 6, 11 regions overlapped with I5_31 and I5_32, including gene *SIIa* (LOC_Os06g12450), which is an important agronomic gene which is responsible for the eating quality of rice and plays an important role in grain synthesis. On chromosome 9, 13 regions overlapped with I5_4, including gene LOC_Os09g28280, which is a putative gibberellin receptor GID1L2 detailed in Table 2.

XP-CLR has proved a valuable method for identifying regions selected in the Vietnamese rice subpopulations and provided an insight into how natural selection and agricultural practices of farmers in Vietnam have shaped the population structure. Annotation of these regions with both overlaps with QTLs for a range of agronomic traits and functional enrichment allowed us to prioritise candidate regions as targets for breeding programs. Our results give further support for the Indica I5 subpopulation being an important source of novel alleles for both national and international breeding programmes. Using a range of criteria, F_{ST} and diversity in these regions, we identified 65 genes which could be further investigated for their breeding potential.

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Availability of data All sequence data used in this manuscript have been deposited as study PRJEB36631 in the European Nucleotide Archive.

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Abbai, Ragavendran, Vikas Kumar Singh, Vishnu Varthini Nachimuthu, Pallavi Sinha, Ramchander Selvaraj, Abhilash Kumar Vipparla, Arun Kumar Singh, Uma Maheshwar Singh, Rajeev K. Varshney, and Arvind Kumar. 2019. 'Haplotype analysis of key genes governing grain yield and quality traits across 3K RG panel reveals scope for the development of tailor-made rice with enhanced genetic gains', *Plant Biotechnology Journal*, 17: 1612-22.
- Alexa A, Rahnenfuhrer J 2010. 'topGO: enrichment analysis for gene ontology. R package version 2.42.0. '.
- Chen, H., N. Patterson, and D. Reich. 2010. 'Population differentiation as a test for selective sweeps', *Genome Res*, 20: 393-402.
- Chen, R., Y. Cheng, S. Han, B. Van Handel, L. Dong, X. Li, and X. Xie. 2017. 'Whole genome sequencing and comparative transcriptome analysis of a novel seawater adapted, salt-resistant rice cultivar - sea rice 86', *BMC Genomics*, 18: 655.
- Cingolani, P., A. Platts, L. Wang le, M. Coon, T. Nguyen, L. Wang, S. J. Land, X. Lu, and D. M. Ruden. 2012. 'A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3', *Fly (Austin)*, 6: 80-92.
- Cui, Di, Hongfeng Lu, Cuifeng Tang, Jinmei Li, Xinxiang A, Tengqiong Yu, Xiaoding Ma, Enlai Zhang, Yanjie Wang, Guilan Cao, Furong Xu, Yongli Qiao, Luyuan Dai, Ruiqiang Li, Shilin Tian, Hee-Jong Koh, and Longzhi Han. 2019. 'Genomic analyses reveal selection footprints in rice landraces grown under on-farm conservation conditions during a short-term period of domestication', *Evolutionary Applications*, 13: 290-302.
- de Freitas, G. M., J. Thomas, R. Liyanage, J. O. Lay, S. Basu, V. Ramegowda, M. N. do Amaral, L. C. Benitez, E. J. Bolacel Braga, and A. Pereira. 2019. 'Cold tolerance response mechanisms revealed through comparative analysis of gene and protein expression in multiple rice genotypes', *PLoS One*, 14: e0218019.
- Delteil, A., M. Blein, O. Faivre-Rampant, A. Guellim, J. Estevan, J. Hirsch, R. Bevitore, C. Michel, and J. B. Morel. 2012. 'Building a mutant resource for the study of disease resistance in rice reveals the pivotal role of several genes involved in defence', *Mol Plant Pathol*, 13: 72-82.
- Du, H., L. Liu, L. You, M. Yang, Y. He, X. Li, and L. Xiong. 2011. 'Characterization of an inositol 1,3,4-trisphosphate 5/6-kinase gene that is essential for drought and salt stress responses in rice', *Plant Mol Biol*, 77: 547-63.
- Duan, N., Y. Bai, H. Sun, N. Wang, Y. Ma, M. Li, X. Wang, C. Jiao, N. Legall, L. Mao, S. Wan, K. Wang, T. He, S. Feng, Z. Zhang, Z. Mao, X. Shen, X. Chen, Y. Jiang, S. Wu, C. Yin, S. Ge, L. Yang, S. Jiang, H. Xu, J. Liu, D. Wang, C. Qu, Y. Wang, W. Zuo, L. Xiang, C. Liu, D. Zhang, Y. Gao, Y. Xu, K. Xu, T. Chao, G. Fazio, H. Shu, G. Y. Zhong, L. Cheng, Z. Fei, and X. Chen. 2017. 'Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement', *Nat Commun*, 8: 249.
- Fukuoka, S., N. V. Alpatyeva, K. Ebana, N. T. Luu, and T. Nagamine. 2003. 'Analysis of Vietnamese rice germplasm provides an insight into Japonica rice differentiation', *Plant Breeding*, 122: 497-502.
- Ganie, S. A., K. A. Molla, R. J. Henry, K. V. Bhat, and T. K. Mondal. 2019. 'Advances in understanding salt tolerance in rice', *Theor Appl Genet*, 132: 851-70.

- Goodstein, D. M., S. Shu, R. Howson, R. Neupane, R. D. Hayes, J. Fazo, T. Mitros, W. Dirks, U. Hellsten, N. Putnam, and D. S. Rokhsar. 2012. 'Phytozome: a comparative platform for green plant genomics', *Nucleic Acids Res*, 40: D1178-86.
- Higgins, J., B. Santos, T. D. Khanh, K. H. Trung, T. D. Duong, N. T. P. Doai, N. T. Khoa, D. T. T. Ha, N. T. Diep, K. T. Dung, C. N. Phi, T. T. Thuy, N. T. Tuan, H. D. Tran, N. T. Trung, H. T. Giang, T. K. Nhung, C. D. Tran, S. V. Lang, T. Nghia, N. Van Giang, T. D. Xuan, A. Hall, S. Dyer, L. H. Ham, M. Caccamo, and J. J. De Vega. 2021. 'Resequencing of 672 Native Rice Accessions to Explore Genetic Diversity and Trait Associations in Vietnam', *Rice (N Y)*, 14: 52.
- Hoang, G. T., P. Gantet, K. H. Nguyen, N. T. P. Phung, L. T. Ha, T. T. Nguyen, M. Lebrun, B. Courtois, and X. H. Pham. 2019. 'Genome-wide association mapping of leaf mass traits in a Vietnamese rice landrace panel', *PLoS One*, 14: e0219274.
- Hoang, G. T., L. Van Dinh, T. T. Nguyen, N. K. Ta, F. Gathignol, C. D. Mai, S. Jouannic, K. D. Tran, T. H. Khuat, V. N. Do, M. Lebrun, B. Courtois, and P. Gantet. 2019. 'Genome-wide Association Study of a Panel of Vietnamese Rice Landraces Reveals New QTLs for Tolerance to Water Deficit During the Vegetative Phase', *Rice (N Y)*, 12: 4.
<https://github.com/hardingnj/xpclr>.
- Jain, M., A. Nijhawan, R. Arora, P. Agarwal, S. Ray, P. Sharma, S. Kapoor, A. K. Tyagi, and J. P. Khurana. 2007. 'F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress', *Plant Physiol*, 143: 1467-83.
- Joukhadar, R., H. D. Daetwyler, A. R. Gendall, and M. J. Hayden. 2019. 'Artificial selection causes significant linkage disequilibrium among multiple unlinked genes in Australian wheat', *Evol Appl*, 12: 1610-25.
- Khanh, Tran Dang, Vu Xuan Duong, Phi Cong Nguyen, Tran Dang Xuan, Nguyen Thanh Trung, Khuat Huu Trung, Dong Huy Gioi, Nguyen Huy Hoang, Hoang-Dung Tran, Do Minh Trung, and Bui Thi Thu Huong. 2021. 'Rice Breeding in Vietnam: Retrospects, Challenges and Prospects', *Agriculture*, 11.
- Kim, E. H., Y. S. Kim, S. H. Park, Y. J. Koo, Y. D. Choi, Y. Y. Chung, I. J. Lee, and J. K. Kim. 2009. 'Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice', *Plant Physiol*, 149: 1751-60.
- Kim, S. K., H. Y. Park, Y. H. Jang, K. C. Lee, Y. S. Chung, J. H. Lee, and J. K. Kim. 2016. 'OsNF-YC2 and OsNF-YC4 proteins inhibit flowering under long-day conditions in rice', *Planta*, 243: 563-76.
- Lira-Ruan, V., M. Ruiz-Kubli, and R. Arredondo-Peter. 2011. 'Expression of non-symbiotic hemoglobin 1 and 2 genes in rice (*Oryza sativa*) embryonic organs', *Commun Integr Biol*, 4: 457-8.
- Liu, X., J. Lan, Y. Huang, P. Cao, C. Zhou, Y. Ren, N. He, S. Liu, Y. Tian, T. Nguyen, L. Jiang, and J. Wan. 2018. 'WSL5, a pentatricopeptide repeat protein, is essential for chloroplast biogenesis in rice under cold stress', *J Exp Bot*, 69: 3949-61.
- Lombardo, F., M. Kuroki, S. G. Yao, H. Shimizu, T. Ikegaya, M. Kimizu, S. Ohmori, T. Akiyama, T. Hayashi, T. Yamaguchi, S. Koike, O. Yatou, and H. Yoshida. 2017. 'The superwoman1-cleistogamy2 mutant is a novel resource for gene containment in rice', *Plant Biotechnol J*, 15: 97-106.
- Lyu, J., B. Li, W. He, S. Zhang, Z. Gou, J. Zhang, L. Meng, X. Li, D. Tao, W. Huang, F. Hu, and W. Wang. 2014. 'A genomic perspective on the important genetic mechanisms of upland adaptation of rice', *BMC Plant Biol*, 14: 160.

Macovei, A., N. Vaid, S. Tula, and N. Tuteja. 2012. 'A new DEAD-box helicase ATP-binding protein (OsABP) from rice is responsive to abiotic stress', *Plant Signal Behav*, 7: 1138-43.

Mai, N. T. P., C. D. Mai, H. V. Nguyen, K. Q. Le, L. V. Duong, T. A. Tran, and H. T. M. To. 2020. 'Discovery of new genetic determinants of morphological plasticity in rice roots and shoots under phosphate starvation using GWAS', *J Plant Physiol*, 257: 153340.

Mukherjee, S., S. Sengupta, A. Mukherjee, P. Basak, and A. L. Majumder. 2019. 'Abiotic stress regulates expression of galactinol synthase genes post-transcriptionally through intron retention in rice', *Planta*, 249: 891-912.

Nijhawan, A., M. Jain, A. K. Tyagi, and J. P. Khurana. 2008. 'Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice', *Plant Physiol*, 146: 333-50.

Ouyang, Jie, Zhaoyan Cai, Kuaifei Xia, Yaqing Wang, Jun Duan, and Mingyong Zhang. 2010. 'Identification and analysis of eight peptide transporter homologs in rice', *Plant Science*, 179: 374-82.

Park, S. H., P. J. Chung, P. Juntawong, J. Bailey-Serres, Y. S. Kim, H. Jung, S. W. Bang, Y. K. Kim, Y. Do Choi, and J. K. Kim. 2012. 'Posttranscriptional control of photosynthetic mRNA decay under stress conditions requires 3' and 5' untranslated regions and correlates with differential polysome association in rice', *Plant Physiol*, 159: 1111-24.

Pavlidis, P., and N. Alachiotis. 2017. 'A survey of methods and tools to detect recent and strong positive selection', *J Biol Res (Thessalon)*, 24: 7.

Peng, B., H. Kong, Y. Li, L. Wang, M. Zhong, L. Sun, G. Gao, Q. Zhang, L. Luo, G. Wang, W. Xie, J. Chen, W. Yao, Y. Peng, L. Lei, X. Lian, J. Xiao, C. Xu, X. Li, and Y. He. 2014. 'OsAAP6 functions as an important regulator of grain protein content and nutritional quality in rice', *Nat Commun*, 5: 4847.

Peng, X., H. Wang, J. C. Jang, T. Xiao, H. He, D. Jiang, and X. Tang. 2016. 'OsWRKY80-OsWRKY4 Module as a Positive Regulatory Circuit in Rice Resistance Against *Rhizoctonia solani*', *Rice (N Y)*, 9: 63.

Phung, N. T., C. D. Mai, G. T. Hoang, H. T. Truong, J. Lavarenne, M. Gonin, K. L. Nguyen, T. T. Ha, V. N. Do, P. Gantet, and B. Courtois. 2016. 'Genome-wide association mapping for root traits in a panel of rice accessions from Vietnam', *BMC Plant Biol*, 16: 64.

Phung, N. T., C. D. Mai, P. Mournet, J. Frouin, G. Droc, N. K. Ta, S. Jouannic, L. T. Le, V. N. Do, P. Gantet, and B. Courtois. 2014. 'Characterization of a panel of Vietnamese rice varieties using DArT and SNP markers for association mapping purposes', *BMC Plant Biol*, 14: 371.

Qi, J., X. Liu, D. Shen, H. Miao, B. Xie, X. Li, P. Zeng, S. Wang, Y. Shang, X. Gu, Y. Du, Y. Li, T. Lin, J. Yuan, X. Yang, J. Chen, H. Chen, X. Xiong, K. Huang, Z. Fei, L. Mao, L. Tian, T. Stadler, S. S. Renner, S. Kamoun, W. J. Lucas, Z. Zhang, and S. Huang. 2013. 'A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity', *Nat Genet*, 45: 1510-5.

Qin, R., D. Zeng, R. Liang, C. Yang, D. Akhter, M. Alamin, X. Jin, and C. Shi. 2017. 'Rice gene SDL/RNRS1, encoding the small subunit of ribonucleotide reductase, is required for chlorophyll synthesis and plant growth development', *Gene*, 627: 351-62.

Quinlan, A. R., and I. M. Hall. 2010. 'BEDTools: a flexible suite of utilities for comparing genomic features', *Bioinformatics*, 26: 841-2.

549 Saeng-ngam, Sukhumaporn, Warintra Takpirom, Teerapong Buaboocha, and Supachitra
550 Chadchawan. 2012. 'The role of the OsCam1-1 salt stress sensor in ABA
551 accumulation and salt tolerance in rice', *Journal of Plant Biology*, 55: 198-208.
552 Sakamoto, T., H. Kitano, and S. Fujioka. 2017. 'Rice ERECT LEAF 1 acts in an alternative
553 brassinosteroid signaling pathway independent of the receptor kinase OsBRI1', *Plant*
554 *Signal Behav*, 12: e1396404.
555 Sumiyoshi, M., A. Nakamura, H. Nakamura, M. Hakata, H. Ichikawa, H. Hirochika, T. Ishii, S.
556 Satoh, and H. Iwai. 2013. 'Increase in cellulose accumulation and improvement of
557 saccharification by overexpression of arabinofuranosidase in rice', *PLoS One*, 8:
558 e78269.
559 Ta, K. N., N. G. Khong, T. L. Ha, D. T. Nguyen, D. C. Mai, T. G. Hoang, T. P. N. Phung, I.
560 Bourrie, B. Courtois, T. T. H. Tran, B. Y. Dinh, T. N. La, N. V. Do, M. Lebrun, P. Gantet,
561 and S. Jouannic. 2018. 'A genome-wide association study using a Vietnamese
562 landrace panel of rice (*Oryza sativa*) reveals new QTLs controlling panicle
563 morphological traits', *BMC Plant Biol*, 18: 282.
564 Tanaka, W., T. Toriba, and H. Y. Hirano. 2017. 'Three TOB1-related YABBY genes are required
565 to maintain proper function of the spikelet and branch meristems in rice', *New*
566 *Phytol*, 215: 825-39.
567 To, H. T. M., K. Q. Le, H. Van Nguyen, L. V. Duong, H. T. Kieu, Q. A. T. Chu, T. P. Tran, and N.
568 T. P. Mai. 2020. 'A genome-wide association study reveals the quantitative trait locus
569 and candidate genes that regulate phosphate efficiency in a Vietnamese rice
570 collection', *Physiol Mol Biol Plants*, 26: 2267-81.
571 To, H. T. M., H. T. Nguyen, N. T. M. Dang, N. H. Nguyen, T. X. Bui, J. Lavarenne, N. T. P.
572 Phung, P. Gantet, M. Lebrun, S. Bellafiore, and A. Champion. 2019. 'Unraveling the
573 Genetic Elements Involved in Shoot and Root Growth Regulation by Jasmonate in
574 Rice Using a Genome-Wide Association Study', *Rice (N Y)*, 12: 69.
575 Tu, Bin, Li Hu, Weilan Chen, Tao Li, Binhua Hu, Ling Zheng, Zheng Lv, Shuju You, Yuping
576 Wang, Bingtian Ma, Xuwei Chen, Peng Qin, and Shigui Li. 2015. 'Disruption of
577 OsEXO70A1 Causes Irregular Vascular Bundles and Perturbs Mineral Nutrient
578 Assimilation in Rice', *Scientific Reports*, 5: 18609.
579 Vemanna, R. S., R. Bakade, P. Bharti, M. K. P. Kumar, S. M. Sreeman, M. Senthil-Kumar, and
580 U. Makarla. 2019. 'Cross-Talk Signaling in Rice During Combined Drought and
581 Bacterial Blight Stress', *Front Plant Sci*, 10: 193.
582 Wang, K., M. Q. Li, Y. P. Chang, B. Zhang, Q. Z. Zhao, and W. L. Zhao. 2020. 'The basic helix-
583 loop-helix transcription factor OsBLR1 regulates leaf angle in rice via brassinosteroid
584 signalling', *Plant Mol Biol*, 102: 589-602.
585 Weir, B. S., and C. Clark Cockerham. 1984. 'Estimating F-Statistics for the Analysis of
586 Population Structure', *Evolution*, 38.
587 Wickham, H. 2016. 'ggplot2: elegant graphics for data analysis: Springer'.
588 Xie, W., G. Wang, M. Yuan, W. Yao, K. Lyu, H. Zhao, M. Yang, P. Li, X. Zhang, J. Yuan, Q.
589 Wang, F. Liu, H. Dong, L. Zhang, X. Li, X. Meng, W. Zhang, L. Xiong, Y. He, S. Wang, S.
590 Yu, C. Xu, J. Luo, X. Li, J. Xiao, X. Lian, and Q. Zhang. 2015. 'Breeding signatures of rice
591 improvement revealed by a genomic variation map from a large germplasm
592 collection', *Proc Natl Acad Sci U S A*, 112: E5411-9.
593 Yang, Sheng-hui, Xiang-li Niu, Di Luo, Chang-dong Chen, Xu Yu, Wei Tang, Bao-rong Lu, and
594 Yong-sheng Liu. 2012. 'Functional Characterization of an Aldehyde Dehydrogenase
595 Homologue in Rice', *Journal of Integrative Agriculture*, 11: 1434-44.

- Yi, J., S. R. Kim, D. Y. Lee, S. Moon, Y. S. Lee, K. H. Jung, I. Hwang, and G. An. 2012. 'The rice gene DEFECTIVE TAPETUM AND MEIOCYTES 1 (DTM1) is required for early tapetum development and meiosis', *Plant J*, 70: 256-70.
- Ying, Y., W. Yue, S. Wang, S. Li, M. Wang, Y. Zhao, C. Wang, C. Mao, J. Whelan, and H. Shou. 2017. 'Two h-Type Thioredoxins Interact with the E2 Ubiquitin Conjugase PHO2 to Fine-Tune Phosphate Homeostasis in Rice', *Plant Physiol*, 173: 812-24.
- Yu, M., C. P. Yau, and W. K. Yip. 2017. 'Differentially localized rice ethylene receptors OsERS1 and OsETR2 and their potential role during submergence', *Plant Signal Behav*, 12: e1356532.
- Yuenyong, W., A. Chinpongpanich, L. Comai, S. Chadchawan, and T. Buaboocha. 2018. 'Downstream components of the calmodulin signaling pathway in the rice salt stress response revealed by transcriptome profiling and target identification', *BMC Plant Biol*, 18: 335.
- Zang, D., H. Li, H. Xu, W. Zhang, Y. Zhang, X. Shi, and Y. Wang. 2016. 'An Arabidopsis Zinc Finger Protein Increases Abiotic Stress Tolerance by Regulating Sodium and Potassium Homeostasis, Reactive Oxygen Species Scavenging and Osmotic Potential', *Front Plant Sci*, 7: 1272.
- Zhang, B., X. Wang, Z. Zhao, R. Wang, X. Huang, Y. Zhu, L. Yuan, Y. Wang, X. Xu, A. L. Burlingame, Y. Gao, Y. Sun, and W. Tang. 2016. 'OsBRI1 Activates BR Signaling by Preventing Binding between the TPR and Kinase Domains of OsBSK3 via Phosphorylation', *Plant Physiol*, 170: 1149-61.
- Zhang, G., Z. Cheng, X. Zhang, X. Guo, N. Su, L. Jiang, L. Mao, and J. Wan. 2011. 'Double repression of soluble starch synthase genes SSIIa and SSIIIa in rice (*Oryza sativa* L.) uncovers interactive effects on the physicochemical properties of starch', *Genome*, 54: 448-59.
- Zhang, T., R. Li, J. Xing, L. Yan, R. Wang, and Y. Zhao. 2018. 'The YUCCA-Auxin-WOX11 Module Controls Crown Root Development in Rice', *Front Plant Sci*, 9: 523.
- Zhou, X., L. Ni, Y. Liu, and M. Jiang. 2019. 'Phosphorylation of bip130 by OsMPK1 regulates abscisic acid-induced antioxidant defense in rice', *Biochem Biophys Res Commun*, 514: 750-55.
- Zhou, Y., D. Chebotarov, D. Kudrna, V. Llaca, S. Lee, S. Rajasekar, N. Mohammed, N. Al-Bader, C. Sobel-Sorenson, P. Parakkal, L. J. Arbelaez, N. Franco, N. Alexandrov, N. R. S. Hamilton, H. Leung, R. Mauleon, M. Lorieux, A. Zuccolo, K. McNally, J. Zhang, and R. A. Wing. 2020. 'A platinum standard pan-genome resource that represents the population structure of Asian rice', *Sci Data*, 7: 113.
- Zhou, Z., Y. Jiang, Z. Wang, Z. Gou, J. Lyu, W. Li, Y. Yu, L. Shu, Y. Zhao, Y. Ma, C. Fang, Y. Shen, T. Liu, C. Li, Q. Li, M. Wu, M. Wang, Y. Wu, Y. Dong, W. Wan, X. Wang, Z. Ding, Y. Gao, H. Xiang, B. Zhu, S. H. Lee, W. Wang, and Z. Tian. 2015. 'Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean', *Nat Biotechnol*, 33: 408-14.

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643 **Table 1 Summary of the 426 Indica and 211 Japonica samples.** The complete dataset of 672 samples
644 includes 35 admixed samples, full sample detail in Higgins et al.(2021)
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	Subpopulation	Higgins et al(2021)	3K-RGP(Zhou et al. 2020)	Total
Indica	I1	135	10	145
	I2	77	14	91
	I3	36	1	37
	I4	52	10	62
	I5	38	5	43
	Im	41	7	48

Japonica	J1	113	2	115
	J2	47	3	50
	J3	16	1	17
	J4	20	1	21
	Jm	6	2	8

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648 **Table 2 Whole-genome XP-CLR selection scores**

649 Mean XP-CLR score across the whole genome for each comparison between the four Japonica subpopulations

650 and the five Indica subpopulations. Reciprocal comparisons shown in Supplementary Table S1.

	SCORE	J1	J2	J3	J4
Selected	J1		17.8	7.6	6.1
	J2	19.5		21.6	6.6
	J3	24.4	17.9		5.9
	J4	46.1	17.5	17.9	

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	SCORE	I1	I2	I3	I4	I5
Selected	I1		8.5	4.0	8.2	9.8
	I2	28.8		7.0	15.7	17.3
	I3	40.2	24.5		23.2	23.7
	I4	34.1	21.5	7.4		18.6
	I5	63.6	44.6	18.0	39.2	

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Table 3 XP-CLR scores and summary on the regions under selection in each subpopulation

Individual comparisons are shown in Supplementary Table S2

	mean xp_clr score	cutoff (99 percentile)	filtered regions >80000bp	mean length	total length	% of genome 373,245,519 bp	number of genes
J1	10.5	136	28	576,707	16,147,785	4.3	2427
J2	25.9	256	23	726,689	16,713,841	4.5	2439
J3	16.1	228	24	577,089	13,850,139	3.7	2007
J4	27.1	297	25	731,341	18,283,522	4.9	2643
I1	7.6	161	44	453,570	19,957,065	5.3	3077
I2	17.2	275	41	550,836	22,584,270	6.1	3346
I3	27.9	401	42	474,009	19,908,387	5.3	2993
I4	20.4	306	38	619,404	23,537,343	6.3	3465
I5	41.4	440	52	583,706	30,352,734	8.1	4576

Table 4: 14 of the 52 regions under selection in the Indica I5 subpopulation, which overlap with QTLs. Detailing the overlap of selected regions with published QTLs for Vietnamese rice populations, selected regions in Indica and Japonica subpopulations, and published selected regions (Xie et al. 2015; Lyu et al. 2014; Cui et al. 2019)

Region	Chrom	Segment position (bp)	ΔF_{ST} I5 vs I2,I3,I4	number of genes per region	Overlap with regions selected in other subpopulations	Overlap with regions in other studies *	Overlap with QTLs, plotted in Fig.4 (c)
I5_1	1	5,563,164 - 6,569,946	0.28	138	I2, I4, J1, J3, J4	1 (39)	Root mass (Phung et al. 2016) panicle morphology (Ta et al. 2018) (a)
I5_5	1	37,850,965 - 38,378,420	0.64	84	I1		Leaf mass (Hoang, Gantet, et al. 2019) Relative phosphate uptake efficiency (To et al. 2020) (b)
I5_16	2	28,191,142 - 29,329,745	0.24	168	I3		Jasmonate RTL (To et al. 2019) (c)
I5_30	5	386,347 - 1,563,159	0.28	190		3 (2)	9_PL (d)
I5_31	6	6,640,258 - 7,189,250	0.17	80	I1, I2, I4	1 (7), 3 (39)	12_GL (e)
I5_32	6	7,860,166 - 8,418,475	0.38	70	I3, I4, J3	1 (3), 3 (34)	Leaf length (Phung et al. 2016) (f)
I5_33	6	19,470,641 - 20,499,968	0.58	165	I1		Panicle length (Ta et al. 2018) root length and number (Phung et al. 2016) (g)
I5_34	7	19,443,608 - 19,825,988	0.19	54	I1, I4		Water content after drought (Hoang, Van Dinh, et al. 2019) (h)
I5_35	7	29,030,233 - 29,677,525	0.76	97	I3		Root depth (Phung et al. 2016) (i)
I5_36	8	3,484,045 - 3,758,632	0.35	39	I3, I4		Jasmonate SHL (To et al. 2019) (j)
I5_37	8	5,052,017 - 5,809,093	0.38	127	I3, I4		Panicle branches (Ta et al. 2018) (k)
I5_39	8	24,300,313 - 24,859,863	0.23	92			Response of crown roots to phosphate (Mai et al. 2020) (l)
I5_48	11	2,510,079 - 3,239,747	0.38	109	I1, I4	1 (56)	Water content after drought (Hoang, Van Dinh, et al. 2019) (m)
I5_49	11	4,590,276 - 5,937,318	0.35	200	J1	1 (3), 2 (14)	Root number (Phung et al. 2016) (n)

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666 ^ FST per region between the 43 samples in subpopulation I5 and the 190 samples in subpopulations I2, I3 and I4.

667 Full list of regions and further details are available in Supplementary Table S7

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669 * number of genes in brackets

670 1 tall (Ind1) [Xie 2015]

671 2 semi-dwarf (IndII) [Xie 2015]

672 3 Cui 2018

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677 **Table 5: Candidate genes under selection in the Indica I5 subpopulation.** Functional annotation of the 65 candidate genes and overlap with genes selected in previous
678 studies *1. ecotype differentiated genes (Lyu et al. 2014)x, 2. tall (Ind1) (Xie et al. 2015)3. semi-dwarf (IndII) (Xie et al. 2015) 4. domestication(Cui et al. 2019)

679 ^ FST per gene between the 43 samples in subpopulation I5 and the 190 samples in subpopulations I2, I3 and I4.

680 Further details are available in Supplementary Table S13

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Region	Gene (MSU)	^ FST I5 vs I2,I3,I4	Gene function	Symbol	* Selected in	impact	Ref
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I5_1	LOC_Os01g11860	0.300	DJ-1 family protein, putative, expressed		2		
I5_5	LOC_Os01g65670	0.909	amino acid transporter, putative, expressed	OsAAP6 qPC1			(Peng et al. 2014; Abbai et al. 2019)
I5_5	LOC_Os01g65770	0.936	expressed protein - rice specific			start_lost	
I5_5	LOC_Os01g65904	0.788	expressed protein - rice specific			stop_gained	
I5_5	LOC_Os01g66030	0.651	OsMADS2 - MADS-box family gene with MIKCC type-box, expressed	OsMADS2			(Lombardo et al. 2017)
I5_5	LOC_Os01g66070	0.445	PHD-finger domain containing protein, putative				(To et al. 2019)
I5_16	LOC_Os02g47310	0.564	Cyclopropane-fatty-acyl-phospholipid synthase, putative, expressed	VTE4			(To et al. 2019)
I5_16	LOC_Os02g47350	0.666	oxidoreductase, short chain dehydrogenase/reductase family, putative, expressed				(To et al. 2019)
I5_16	LOC_Os02g47400	0.501	pectinacetyltransferase domain containing protein, expressed				(To et al. 2019)
I5_16	LOC_Os02g47410	0.522	protein kinase, putative, expressed				(To et al. 2019)
I5_16	LOC_Os02g47420	0.572	ATROPGEF7/ROPGEF7, putative, expressed	OSROPGEF			(To et al. 2019)
I5_16	LOC_Os02g47440	0.536	syntaxin, putative, expressed				(To et al. 2019)
I5_16	LOC_Os02g47590	0.637	ornithine carbamoyltransferase,				(To et al. 2019)

			putative, expressed				
I5_16	LOC_Os02g47660	0.372	basic helix-loop-helix, putative, expressed	OsBLR1			(Wang et al. 2020)
I5_17	LOC_Os03g12840	0.477	Inositol 1, 3, 4-trisphosphate 5/6-kinase, putative, expressed	DSM3 OsITPK2		stop gained	(Du et al. 2011)
I5_17	LOC_Os03g13010	0.837	U-box domain containing protein, expressed	TUD1 DSG1 ELF1			(Sakamoto, Kitano, and Fujioka 2017)
I5_17	LOC_Os03g13140	0.879	non-symbiotic hemoglobin 2, putative, expressed	Hb1			(Lira-Ruan, Ruiz-Kubli, and Arredondo-Peter 2011)
I5_17	LOC_Os03g14669	0.918	core histone H2A/H2B/H3/H4, putative, expressed	OsHAP5C			(Kim et al. 2016)
I5_23	LOC_Os03g49500	0.719	ethylene receptor, putative, expressed	Os-ERS1			(Yu, Yau, and Yip 2017)
I5_23	LOC_Os03g51050	0.660	peptide transporter PTR2, putative, expressed	PTR8	1,3		(Ouyang et al. 2010)
I5_25	LOC_Os03g58600	0.844	PAZ domain containing protein, putative, expressed	MEL1			(Yi et al. 2012)
I5_25	LOC_Os03g58630	0.886	thioredoxin, putative, expressed	OsTrxh4			(Ying et al. 2017)
I5_29	LOC_Os04g58740	0.818	expressed protein - rice specific		2	start_lost	
I5_29	LOC_Os04g58750	0.815	protein kinase family protein, putative, expressed	OsBSK3	2		(Zhang et al. 2016)
I5_29	LOC_Os04g58780	0.806	pentatricopeptide repeat protein, putative, expressed	WSL5 OsPPR4	2		(Liu et al. 2018)

I5_29	LOC_Os04g58870	0.813	exo70 exocyst complex subunit, putative, expressed			splice_acceptor_variant&intron_variant	(Tu et al. 2015)
I5_29	LOC_Os04g58880	0.826	exo70 exocyst complex subunit, putative, expressed	RLS2 OsEXO70A1			(Tu et al. 2015)
I5_30	LOC_Os05g02260	0.617	interacts with OsMPK1	bip130		stop_gained	(Zhou et al. 2019)
I5_31	LOC_Os06g12450	0.360	soluble starch synthase 2-3, chloroplast precursor, putative, expressed	ALK SSIIa	4		(Zhang et al. 2011)
I5_32	LOC_Os06g14620	0.471	ribonucleoside-diphosphate reductase small chain, putative, expressed	<i>SDL/RNRSI</i>	4		(Qin et al. 2017)
I5_33	LOC_Os06g34360	0.959	zinc finger, C3HC4 type domain containing protein, expressed				(Zang et al. 2016)
I5_33	LOC_Os06g34650	0.948	zinc finger, C3HC4 type domain containing protein, expressed				(Zang et al. 2016)
I5_33	LOC_Os06g33520	0.509	DEAD/DEAH box helicase, putative, expressed	OsABP			(Macovei et al. 2012)
I5_35	LOC_Os07g48560	0.927	homeobox domain containing protein, expressed	WOX11			(Zhang et al. 2018)
I5_35	LOC_Os07g48640	0.953	short-chain dehydrogenase/reductase, putative, expressed	OsSDR			(Kim et al. 2009)
I5_35	LOC_Os07g48680	0.955	zinc finger, C3HC4 type domain containing protein, expressed				(Zang et al. 2016)
I5_35	LOC_Os07g48750	0.920	alpha-N-arabinofuranosidase, putative, expressed	OsARAF1			(Sumiyoshi et al. 2013)

I5_35	LOC_Os07g48780	0.907	OsCam1-2 - Calmodulin, expressed	OsCam1-2 OsCam1			(Saeng-ngam et al. 2012), (Yuenyong et al. 2018)
I5_35	LOC_Os07g48820	0.901	transcription factor, putative, expressed	OsZIP63 OsNIF1			(Delteil et al. 2012), (Vemanna et al. 2019)
I5_35	LOC_Os07g48830	0.931	glycosyl transferase 8 domain containing protein, putative, expressed	OsGolS2 wsi76			(Mukherjee et al. 2019)
I5_35	LOC_Os07g48920	0.916	aldehyde dehydrogenase, putative, expressed	OsALDH22			(Yang et al. 2012)
I5_36	LOC_Os08g06370	0.014	MYB family transcription factor, putative, expressed				(To et al. 2019)
I5_37	LOC_Os08g09110	0.904	NB-ARC domain containing protein, expressed			stop_gained	
I5_37	LOC_Os08g09190	0.286	auxin efflux carrier component, putative, expressed	OsPILS2			(Ta et al. 2018)
I5_39	LOC_Os08g39100	0.239	protein phosphatase 2C, putative, expressed	OsPP2C66			(Mai et al. 2020)
I5_39	LOC_Os08g38990	0.202	WRKY30, expressed	OsWRKY30			(Mai et al. 2020)
I5_41	LOC_Os09g28280	0.654	gibberellin receptor GID1L2, putative, expressed		4		
I5_41	LOC_Os09g28840	0.654	OsSCP43 - Putative Serine Carboxypeptidase homologue, expressed				
I5_42	LOC_Os09g30340	0.971	photosystem I reaction center subunit, chloroplast precursor, putative, expressed	PSAG			(Park et al. 2012)
I5_42	LOC_Os09g30360	0.973	caffeoyl-CoA O-methyltransferase,				

			putative, expressed				
I5_42	LOC_Os09g30380	0.966	AP005392-AK108636 - NBS/LRR genes that are S-rich, divergent TIR, divergent NBS, expressed				
I5_42	LOC_Os09g30400	0.954	WRKY90, expressed	OsWRKY80			(Peng et al. 2016)
I5_42	LOC_Os09g30410	0.961	expressed protein				
I5_42	LOC_Os09g31019	0.942	ubiquitin fusion protein, putative, expressed				(Chen et al. 2017)
I5_47	LOC_Os10g35260	0.703	Rf1, mitochondrial precursor, putative, expressed		3		
I5_47	LOC_Os10g35540	0.783	hydrolase, alpha/beta fold family domain containing protein, expressed		3		
I5_47	LOC_Os10g35560	0.692	expressed protein	OsSFR6	3		(de Freitas et al. 2019)
I5_47	LOC_Os10g35604	0.661	expressed protein		3	stop_gained	
I5_47	LOC_Os10g35640	0.700	Rf1, mitochondrial precursor, putative, expressed	Rf1b	3		
I5_48	LOC_Os11g05640	0.367	bZIP transcription factor domain containing protein, expressed	OsZIP-2a OsbZIP80	2		(Nijhawan et al. 2008)
I5_48	LOC_Os11g06390	0.746	actin, putative, expressed	OsACTIN2	2		
I5_48	LOC_Os11g06410	0.841	homeodomain, putative, expressed	SAB18	2		

I5_48	LOC_Os11g06490	0.715	ribosome inactivating protein, putative, expressed				
I5_49	LOC_Os11g09360	0.919	OsFBX398 - F-box domain containing protein, expressed	OsFBX398		splice_donor_variant&intron_variant	(Jain et al. 2007)
I5_49	LOC_Os11g10070	0.721	transcriptional corepressor SEUSS, putative, expressed	OsSEU2	3	splice_donor_variant&intron_variant	(Tanaka, Toriba, and Hirano 2017)

Fig. 1 XP-CLR scores and regions under selection.

(a) Selected regions for the five Indica subpopulations covering 5.4%, 6.1%, 5.3%, 6.3% and 8.1% of the genome for I1, I2, I3, I4 and I5 respectively. Centromeric regions are shown as 100 kb regions in dark grey.
(b) Selected region for the four Japonica subpopulations covering 4.3%, 4.5%, 3.7% and 4.9% of the genome for J1, J2, J3 and J4 respectively.

Fig. 2 Gene Ontology overrepresentation

Fig. 3 Selection sweeps in the Indica I5 subpopulation compared to the other Vietnamese subpopulations
XP-CLR scores in 100,000 bp sliding windows are plotted along the 12 chromosomes, showing selection in the I5 subpopulation compared to (a) I2, (b) I2, (c) I3, (d) I4. The horizontal dashed line indicates the threshold XP-CLR score of 440 for determining selected regions. (e) F_{ST} in 100,000 bp sliding windows for the 43 samples in the I5 subpopulation compared to the 190 samples in the I2, I3 and I4 subpopulations. (f) Whole genome genetic diversity (Π) in 100,000 bp sliding windows for the 43 samples in the I5 subpopulation. The vertical lines show the position of the 52 selected regions.

Fig. 4 Vietnamese QTLs and their overlap with selected regions in the I5 subpopulation. QTLs from 8 published studies (Phung et al. 2016; Ta et al. 2018; To et al. 2019; Hoang, Gantet, et al. 2019; Hoang, Van Dinh, et al. 2019; Mai et al. 2020; To et al. 2020) (Higgins et al.) are plotted along each chromosome together with the 52 regions selected in the I5 subpopulation. The fourteen selected regions which overlap with at least one QTL are highlighted, the letters refer to the details shown in Table 2

Fig. 5 Allele Plots for “High impact” SNPs within eight candidate genes. Bar plots showing the base count for each subpopulation. A=adenine, T=thymine G = guanine, C = cytosine. Heterozygous calls are shown using IUPAC ambiguity codes.

Supplementary tables

Supplementary Table S1 Reciprocal difference between comparisons for each subpopulation pair

719 Supplementary Table S2. Summary of XP-CLR comparisons for the Indica and Japonica subpopulations.
720 Detailing the XP-CLR mean, cut off and number of regions for each comparison.
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722 Supplementary Table S3. List of genes selected in each subpopulation.
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724 Supplementary Table S4. Overlap of the selected regions in Indica subpopulations with QTL found in
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734 Supplementary Table S8. Annotation of I5 selected regions using PhytoMine. Enrichment analysis for protein
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739 Supplementary Table S10. List of all genes selected in I5 subpopulation. Detailing MSU and RAP gene ID,
740 annotation and enrichment in Phytomine, high impact SNPs and mean F^{st}
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746 Supplementary Table S13. Details of 64 candidate genes.
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748 Supplementary Table S14. Details of “*High impact*” SNPs within eight candidate genes.

Supplementary Figures

Fig. S1 Chromosome plots of regions selected in each Indica subpopulation showing the regions selected against each individual subpopulation and the shaded final selected regions which were selected against three subpopulations.

a) 44 regions selected in I1, b) 41 regions selected in I2, c) 42 regions selected in I3, d) 38 regions selected in I4, e) 52 regions selected in I5

Fig. S2 Chromosome plots of regions selected in each Japonica subpopulation showing the regions selected against each individual subpopulation and the shaded final selected regions which were selected against two subpopulations.

a) 28 regions selected in J1, b) 23 regions selected in J2, c) 24 regions selected in J3, d) 25 regions selected in J4

Fig. S3 Range of sizes for selected regions in (a) five Indica subpopulations and (b) four Japonica subpopulations

Fig. S4 Upset plots for overlap of genes in selected regions for (a) all nine subpopulations, (b) five Indica subpopulations and (c) four Japonica subpopulations

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