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Bi-directional selection in upland rice leads to its adaptive differentiation from lowland rice on drought resistance and productivity

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1	Running title: Evolution of drought resistance in rice
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ding au.

Abstract: Drought resistance is required in rice breeding to address the challenge of frequent 17 18 droughts. However, the evolution of rice drought resistance is not fully understood. We 19 investigated the genetic differentiation between upland and lowland rice domesticated in 20 agro-ecosystems of contrasting water-soil conditions by high-throughput SNPs. We estimated their morphological differences in drought resistance and productivity through common 21 22 garden experiments. Upland rice possessed better drought resistance but poorer productivity. 23 Negative correlations between traits of drought resistance and productivity were observed. These negative correlations are attributed to genetic tradeoffs between drought resistance and 24 productivity by tight linkages (e.g. DCA1 and OsCesA7) or pleiotropic effects (e.g. LAX1). 25 The genetic tradeoff is common and greatly shapes the evolution of drought resistance in 26 upland rice. Signs of balancing selection detected in upland rice while signs of directional 27 selection detected in lowland rice, on genomic regions associated with both productivity and 28 drought resistance, lead to their adaptive differentiation. Signs of balancing selection in 29 30 upland rice resulted from bi-directional selection during its domestication in drought-prone 31 upland agro-ecosystem. Bi-directional selection, applied in breeding water-saving and drought resistance rice (WDR), breaks tight linkages by accumulating recombination events. Using 32 genome-wide association analysis, we identified several valuable QTLs associated with 33 34 drought resistance, in which highly differentiated genes should be candidates.

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36 Key words: drought resistance, upland rice, ecological adaptation, ecotype, tradeoff,

37 domestication

38 Introduction

39 Drought is one of the most disastrous stressors for rice cultivation. It causes serious yield loss 40 to annual rice production (Farooq et al., 2009; Luo, 2010). Thus, it is necessary to identify and 41 utilize genetic resources of drought resistance in rice breeding (Luo, 2010). However, drought resistance is a complicated trait composed of several mechanisms (e.g. drought-avoidance, 42 drought-tolerance, and drought-recovery) (Bernier et al., 2010; Fang and Xiong, 2015) and 43 hundreds of genes with minor effects (Fang and Xiong, 2015). Interactions with environments 44 substantially affect drought resistance as well (Farooq et al., 2009; Hu and Xiong, 2014). 45 Therefore, the molecular mechanism of rice drought resistance and its evolutionary process in 46 47 rice remain unknown.

Given its complicated nature, drought resistance is considered an integrated trait that is 48 associated with other agronomic traits (Farooq et al., 2009; Bernier et al., 2010; Fletcher et al., 49 50 2015). It is typically assumed that a balance exists between drought resistance and 51 productivity (Fletcher et al., 2015; Vikram et al., 2015). At the gene scale, a yield penalty 52 detected along with a drought resistance gene is not rare (e.g. SNAC2, OsIAA6, OsABF1, etc.) (Hu et al., 2008; Jung et al., 2015; Zhang et al., 2016). At the physiological scale, a plant 53 always inhibits its photosynthetic capacity and many other life activities during drought to 54 55 ensure better survival. However, these acclimation responses may delay the growth and 56 decrease the productivity of a plant (Yordanov et al., 2000; Harb et al., 2010; Pandey et al., 2015). At the individual scale, elite irrigated rice varieties bred for high productivity and good 57 quality are very sensitive to drought (Luo, 2010; Vikram et al., 2015). These observations 58 59 indicate a potential tradeoff between drought resistance and productivity. If the tradeoff exists, 60 it will substantially shape the adaptive evolution of drought resistance in rice and should be overcome in breeding for drought resistant cultivars (Vikram et al., 2015). However, 61 62 knowledge is still limited about the genomic tradeoff and its influences on the evolution of drought resistance in rice. 63

Fortunately, there are two rice ecotypes domesticated in agro-ecosystems with 64 contrasting soil-water conditions, which allows them to be differentiated in drought resistance. 65 Upland rice is domesticated in rain-fed unbunded fields. It has been reported to accumulate 66 genetic variance during its adaptation to drought-prone mountain areas, resulting in improved 67 68 drought resistance (Gupta and O'Toole, 1986; Bernier et al., 2010). However, upland rice 69 exhibits generally poor productivity (Baños, 1975; Gupta and O'Toole, 1986; Xia et al., 2014; Lyu et al., 2014). In contrast, lowland rice is commonly grown in fields with 70 71 water-maintenance and even irrigation facilities. Lowland rice encounters relatively less 72 drought risk during its domestication (Bernier et al., 2010). Consequently, lowland rice is 73 more productive and of better quality than upland rice (Baños, 1975; Gupta and O'Toole, 74 1986). These two ecotypes provide us with a good opportunity to study the adaptive

75 differentiation of the two rice ecotypes in drought resistance under human selection.

76 In this study, we investigated performances of 112 upland and 103 lowland rice landraces 77 in regularly managed paddy fields, drip-irrigated fields, and naturally dried fields for their productivity and drought resistance. These rice landraces, as well as some common wild rice 78 accessions, were further genotyped by high-throughput technologies (SNP array and 79 resequencing) to study the genomic differentiation between upland and lowland ecotypes, 80 81 particularly for drought resistance. We aimed to address the following questions: (1) Is upland rice differentiated from lowland rice for drought resistance? (2) If it is, how are upland and 82 lowland rice adaptively differentiated for drought resistance? The knowledge gained from this 83 84 study can deepen our understandings of drought resistance and provide informative cues for 85 breeding drought resistant cultivars.

86

87 **Results**

Morphological differences in drought resistance and productivity between upland and lowland rice ecotypes

Based on the field performance evaluated under drought conditions, upland rice demonstrated 90 91 significantly higher drought resistance than lowland rice did through several key drought 92 resistant traits. For example, it possessed a higher ratio of deep-rooting (RDR), a lower rate of 93 water loss (RWL) in excised leaves, higher relative water content (RWC) under drought, 94 higher relative fecundity (RF), and higher relative grain weight (RGW) (Table 1). In contrast, lowland rice exhibited better performance in growth, development, and productivity (GDP, 95 the abbreviation for growth, development, and productivity) under well-watered (W) and 96 drip-irrigated (CK) conditions, which was representing as ed through more panicles, greater 97 biomass, and higher grain yield. Negative correlations between the traits of drought resistance 98 99 and GDP were frequently detected in our experiments (Figure 1). For example, RDR, RWC, and RGW were negatively correlated with the number of tillers (NT) (Figure 1). These 100 101 negative correlations reflected certain tradeoffs between drought resistance and productivity.

102 A comparison of Q_{ST} to the neutral F_{ST} was conducted to detect the potential adaptive 103 evolution for drought resistant or agronomic traits. In this study, the neutral genomic F_{ST} 104 (calculated using intergenic SNPs) was 0.097 ± 0.001 between upland and lowland rice 105 ecotypes. Surprisingly, none of estimated drought resistant traits had significantly higher Q_{ST} 106 than the neutral genomic F_{ST} between the typical upland and lowland rice ecotype. (Figure 107 S1).

108

109 Genomic differentiation, linkage disequilibrium, and genetic diversity between upland110 and lowland rice

111 Based on >30,000 informative SNPs from the rice SNP array, the Geng (japonica) upland and

112 lowland rice could be generally separated *via* cluster analysis. This indicated there was a 113 considerable level of genetic differentiation (mean $F_{ST}=0.171\pm0.002$) between the two rice 114 ecotypes (Figure 2b). Patterns of linkage disequilibrium (LD) across the genome were 115 generally similar between the two ecotypes and decayed quickly within 200K bp. However, 116 the upland ecotype represented a slightly quicker LD decay than the lowland rice (Figure 2c).

Across the genome, we detected 184 highly differentiated windows (F_{sT} >0.379, beyond 117 the 95% confidence interval) out of total 3,684 available windows, and 21.7% of the windows 118 contained at least one gene relevant to drought resistance (Figure 3a). As expected, windows 119 containing genes relevant to drought resistance exhibited significantly higher mean F_{ST} values 120 than other regions (Figure 3d), whereas windows containing GDP genes had the equivalent 121 mean F_{ST} to the genomic average (Table S1). This outcome indicates that upland and lowland 122 rice are genetically differentiated in regions relevant to drought resistance. Highly 123 124 differentiated regions (HDR) thus could be potentially associated with drought resistance.

Estimated by $\prod_{upland}/\prod_{lowland}$, upland rice possesses higher relative genetic diversity 125 (mean $\prod_{upland}/\prod_{lowland}=1.158$) in general (Figure 3b). Surprisingly, windows possessing higher 126 $\prod_{upland}/\prod_{lowland}$ ratios were generally highly differentiated (Figure 3e). This indicates that 127 128 genetic regions relevant to drought resistance possess higher genetic diversity in upland rice. At the gene scale, highly differentiated genes (HDE) (F_{ST} >0.455, beyond the 95% confidence 129 130 interval) of $\prod_{upland}/\prod_{lowland}$ ratio>2.0 were relevant to plant responses to various stressors 131 (Figure S2). This result also confirmed that genes potentially associated with drought resistance had higher genetic diversity in upland rice. Higher genetic diversity detected in 132 drought resistant genes indicated there was no strong directional selection imposed on upland 133 rice for drought resistance. It was also noteworthy that upland rice shared great 134 ecotype-private alleles with common wild rice (Figure S3). 135

136

Balancing selection in upland rice and directional selection in lowland rice detected by Tajima's *D* test and selective sweep detection

As mentioned above, highly differentiated regions relevant to drought resistance were not 139 under directional selection in upland rice; thus, there must be other causes for promoting 140 differentiation between upland and lowland rice for drought resistance. To uncover potential 141 142 explanations, we performed Tajima's D test (Figure 4a) and analysis of selective sweep (Figure 4b, c) in the two rice ecotypes. We detected 18 regions with balancing selection in 143 upland rice and two regions with balancing selection in lowland rice (Figure 4a, Table S2). 144 145 Those regions detected in upland rice possessed high F_{ST} values between upland and lowland rice, as well as higher $\prod_{upland}/\prod_{lowland}$ ratios (Table S2). The results obtained from SweeD 146 suggested that highly differentiated regions represented signs of selective sweep only in 147 148 lowland rice (Figure 4b, c; Table S3). Hence, by integrating all these results, we speculated

that these highly differentiated regions were derived by directional selection occurred in
lowland rice while balancing selection occurred in upland rice. Noticeably, most of these
highly differentiated regions contain genes/QTLs of both drought resistance and GDP (Table
S2).

153

Genomic tradeoffs between drought resistance and productivity by tandem linkage andpleiotropy

Based on the Ricedata (up to 30th, September), 285 and 356 genes, were relevant to drought 156 resistance and GDP, respectively (Table S4). They were distributed in 918 (517 for drought 157 resistance and 613 for GDP) 200 kb-windows (sliding in 100 Kb steps) across the genome. 158 Among these windows, 212 (5.67% of total windows) contained genes relevant to both 159 drought resistance and GDP (Figure 3a), which was significantly beyond the ratio by chance 160 (2.27% of total windows, p<0.001 by γ^2 test). Thus, tight linkages between genes of drought 161 resistance and productivity are very common. We further calculated the frequencies of 162 163 recombinant genotypes within windows containing genes of both drought resistance and GDP in cultivated rice genotypes and wild rice accessions (Table S5). Interestingly, upland rice 164 possessed more windows containing ecotype-specific (37 vs. 10) and ecotype-preferential (11 165 166 vs. 4) recombinant genotypes than lowland rice did (Figure S4, Table S5). Meanwhile, typical 167 upland and lowland rice could be separated by recombination within windows containing 168 genes of both drought resistance and GDP (Figure S4). Some rare, but ecotype-specific, 169 recombinant genotypes could only be detected in upland rice. This could partially explain the 170 slightly slower LD decay detected in upland rice and suggest a role for recombination in the adaptation of upland rice to a drought-prone environment. Meanwhile, 148 genes of drought 171 resistance have been functionally studied. Among these function-studied drought resistant 172 173 genes, 28 genes were reported to have unwanted pleiotropic effects (Table S6). This result indicates the unwanted pleiotropic effect of a drought resistant gene on GDP is another 174 potential cause for the genetic tradeoff between drought resistance and productivity. 175

176

177 Genome-wide associations for drought resistance and agronomic traits

We detected 53 QTLs for 27 measured traits among experiments by GWAS (Figure 5). Many known genes, including *OsGS3* for 100GW-CK/D, *OsCOW1* for FLW-D, *OsPUP7* for GW-CK, *OsGI, YL1*, and *OsHIGD* for PH-W, *Ghd7* for HI-D, *DCA1* for RWC, *OsGL1-10* for RB, and *OsSIK1* for RF, were located within these QTLs (Figure 5) and were considered to be corresponding candidates. These results indicated our GWAS had good efficiency for identifying candidate genes of drought resistance.

Among all the detected QTLs, the QTLs for RWC and PH (Chr10:16600000–16800000)
were noteworthy (Figure 5). Signals of balancing selection in upland rice and selective sweep

186 in lowland rice were detected at this region (Table S2, Table S3). A known drought resistant 187 gene (DCA1, LOC_Os10g31850), which enhances rice drought resistance by controlling 188 stomatal aperture, was located within its region. It could generally separate upland and lowland ecotypes based on its sequence (Figure 6a). However, DCA1 is reported to have no 189 obvious impact on productivity and only a minor effect on plant height (Figure S5). 190 Interestingly, we found *Ehd1* (controlling heading date and panicle development) and 191 192 OsCesA7 (having significant impacts on the number of tillers, plant height, and productivity) were also located nearby (distance of \sim 500kb). The two genes are tightly linked with DCA1, 193 which was revealed by high correlation coefficients (r) among SNPs in DCA1, Ehd1, and 194 OsCesA7 (Figure 6b). Meanwhile, they possess high F_{ST} values and $\prod_{uvland}/\prod_{lowland}$ ratios 195 (Figure 6b). This region provides a good example of the genetic tradeoffs caused by tight 196 linkages. Additionally, we detected several recombination events between DCA1 and 197 198 OsCesA7 that occurred only in upland rice (Figure S6).

Another notable QTL of RWL located at the region of Chr1:35500000–35700000 (Figure 199 200 5). A well-known GDP gene, LAX1 (LOC_Os01g61480) is found within this region. It had the highest F_{ST} value (Figure S7a) between upland and lowland rice ecotypes and could generally 201 separate the two ecotypes in the cluster analysis (Figure 6c). Interestingly, its transgenic lines 202 203 of VP64-TF fusion type (activated form) represented the typical morphological features of 204 upland rice compared to lowland rice, such as taller height, fewer tillers, wider flag leaves, 205 and poorer productivity (Figure S7c-h). Once it had any impacts on drought resistance, 206 tolerant genotypes should contain the advantageous allele for drought-resistance, whereas 207 susceptible genotypes should contain the disadvantageous allele. As expected, it possesses the higher F_{ST} between the drought-tolerant and drought-susceptible groups (Figure S7b), and 208 209 LAX1 should have pleiotropic effects on drought-resistance. This assumption was supported 210 by lower RWL (Figure 6d) and better RWC observed in VP64-TF transgenic lines at the seedling stage under osmotic stress (Figure 6e). LAX1 thus provided an ideal example of a 211 drought-resistant gene having unwanted pleiotropic effects on GDP that was adaptively 212 213 differentiated between rice ecotypes.

For potential drought-resistant candidates, the QTL of RDR (peak signal at Chr08: 214 8640319) should be paid to particular attentions (Figure 5). It is located at regions 215 representing signs of selective sweep in upland rice. The OsWOX12A (LOC_Os08g14400) 216 217 was considered to be the candidate gene as it was highly differentiated between ecotypes $(F_{ST}=0.576)$ (Figure S8a) and reported to be related with root primordia initiation. Meanwhile, 218 219 the QTL for RGW (peak signal at Chr11: 24507478) were detected repeatedly in 2016 and 220 2017 (Figure 5). As RGW exhibited significant differences between upland and lowland 221 ecotypes, three highly differentiated (F_{ST} >0.25) drought-responsive genes (LOC Os11g41410, 222 LOC_Os11g41600, LOC_Os11g41710) and the drought-responsive gene (LOC_Os11g41610)

containing the SNP of peak signal were good candidates for further validation (Figure S8b).

224

225 Discussion

The upland and lowland rice are morphologically and genetically differentiated on bothdrought resistance and productivity

Investigated by International Rice Research Institute, many typical upland rice 228 229 varieties/landraces exhibit good drought resistance and have some morphological differences compared to lowland/irrigate rice such as wider leaves, increased height, fewer tillers, and 230 deeper roots (Gupta and O'Toole 1986; Baños1975). After examining hundreds of upland and 231 232 lowland rice landraces covering most areas in China, we found that upland rice confers better drought-avoidance (e.g. higher RDR and lower RWL) and drought-tolerance (e.g. RWC, 233 relative fecundity, and relative grain weight measured in the field with shallow soil-layers). 234 235 However, upland rice generally presents poor performances compared to lowland rice in both regularly managed paddy fields and drip-irrigated fields. The significant differences detected 236 237 in this study indicate upland and lowland rice are somewhat differentiated in terms of drought 238 resistance and productivity.

In addition to morphological differences, we also detected a considerable level of genetic 239 240 differentiation between upland and lowland rice. This result is consistent with previous 241 studies using genomic SSR (Zhang et al., 2009), EST-SSR (Xia et al., 2014), and 242 resequencing (Lyu et al., 2014) data. We further found that F_{ST} values for regions relevant to 243 drought resistance went beyond the genomic average, which provided solid evidence that upland and lowland rice are adaptively differentiated for drought resistance. However, we did 244 not detect significant differences in the FST values between GDP-relevant regions and the 245 genomic average, although many agronomic traits also represent morphological differences. 246 247 This outcome indicates that selection for agronomic traits in upland and lowland rice are 248 generally similar.

The comparison of quantitative genetic divergence (Q_{ST}) to the neutral genetic 249 250 divergence (F_{ST}) can be used to detect adaptive evolution. If the Q_{ST} is significantly higher than the neutral F_{ST} , it means that the directional selection drives phenotypic divergence and 251 results in ecological adaptation (Miller et al., 2008; Leinonen et al., 2013). Surprisingly, no 252 253 drought resistant traits have significantly higher Q_{ST} values than the neutral genomic F_{ST} . This 254 result indicates that directional selection on drought resistance may not be the primary force leading to the adaptive differentiation between upland and lowland rice ecotypes for drought 255 256 resistance.

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258 Genomic tradeoffs between drought resistance and productivity

259 The tradeoff between drought resistance and GDP has been previously discussed for many

260 plant species (Harb et al., 2010; Koziol et al., 2012; Fletcher et al., 2015; Vikram et al., 2015; 261 Denison, 2015). It is considered to be caused by the contradiction between the biological and 262 agricultural requirements of drought resistance. For a wild species, a plant activates acclimation responses, such as inhibiting normal metabolic processes, slowing growth and 263 development, and reducing productivity, to ensure better survival under drought conditions 264 (Yordanov et al., 2000; Harb et al., 2010; Pandey et al., 2015). In contrast, humans require a 265 266 stable yield for a crop under a drought, in which manner drought resistance is defined for the crop. Evidently, many genes that can enhance the survival rate of rice seedlings under 267 simulated drought stress have been reported to have penalties on GDP (Hu et al., 2008; Jung 268 et al., 2015; Zhang et al., 2016). In this study, negative correlations were observed between 269 many drought resistant traits and GDP traits. This finding reflects certain tradeoffs between 270 271 drought resistance and productivity during rice domestication. Two genetic mechanisms are the causes of the observed tradeoff: (1) tight linkages between genes of drought resistance and 272 productivity (Vikram et al., 2015), and (2) unwanted pleiotropic effects of drought resistant 273 274 genes on GDP (Hu et al., 2008; Jung et al., 2015; Zhang et al., 2016). Genomic tradeoffs between drought resistance and productivity may substantially block the utilization of genetic 275 resources for drought resistance in breeding (Vikram et al., 2015). 276

277 Tight linkages between genes of drought resistance and productivity are very common, 278 indicated at a frequency of 23.1% across the rice genome in this study. We provide an 279 example of the tight linkage between DCA1 and OsCesA7. DCA1 is a gene that enhances rice 280 drought resistance by controlling the stomatal aperture (Cui et al., 2015). It is tightly linked 281 with OsCesA7, which controls many important agronomic traits (e.g., plant height, number of tillers, and fecundity) (Huang et al., 2015; Wang et al., 2016). The tight linkage between the 282 two genes may be the cause of negative correlations between the RWC and plant height 283 284 observed in our experiments, because QTLs for both traits were identified within the same region by GWAS. If we want to obtain the maximum benefit from DCA1 in breeding, its tight 285 linkage with OsCesA7 should be broken. 286

287 Meanwhile, a proportion of drought resistant genes (18.9%) have negative pleiotropic effects on productivity, which results in yield penalties. In this study, we found LAX1 288 (LOC_Os01g61480), which controls plant height, number of tillers, and grain weight 289 (Komatsu et al., 2003), also has opposite impacts on drought resistance. The selection on 290 291 LAX1 alleles for drought resistance may result in typical morphological features (wider leaves, 292 taller, and less tillers) in upland rice. These results mean we could not obtain both advantages 293 on drought resistance and productivity from major alleles of LAX1. To overcome the 294 unwanted pleiotropic effect of a drought resistant gene, utilizing ideal alleles in natural 295 variants is an effective way (Kumar et al., 2010; Tao et al., 2011). Upland rice shares great 296 ecotype-specific alleles with common wild rice and retains higher genetic diversity due to

- balancing selection during its domestication in upland environments. It is thus a good genetic
 resource for drought resistance and could be used for collecting rare but ideal alleles for
 drought resistance (Lyu et al., 2013).
- 300

Bi-directional selection in upland rice promotes adaptive differentiation between upland and lowland rice ecotypes

303 The primary hypothesis, which is accepted by many researchers, is that drought resistance is under directional selection in upland rice, which differentiated upland rice from lowland rice 304 (Lyu et al., 2013, 2014; Xia et al., 2014). However, most of the molecular evidence from the 305 present study does not support this hypothesis. Reductions of genetic diversity and signs of 306 selective sweep are always caused by directional selection (Clark, et al., 2004; Wright and 307 Gaut, 2005; Doebley et al., 2006). In this study, upland rice confers higher genetic diversity 308 on highly differentiated regions and drought-responsive genes than lowland rice does. 309 Meanwhile, it is very surprising that balancing selection is always detected in upland rice 310 311 while selective sweep was uniquely detected in lowland rice for their highly differentiated regions. By integrating these results, we generated a model for adaptive differentiation 312 between upland and lowland rice during domestication: (a) Genetic tradeoffs exist between 313 314 drought resistance and GDP. (b) Domesticating lowland rice in paddy fields focused on 315 improving its productivity, which results in directional selection on GDP. (c) Domesticating 316 upland rice in drought-prone upland environments took into account both yield potential in 317 ideal environments (productivity) and yield stability under drought conditions (drought resistance) (Baños, 1975; Bernier et al., 2010; Hu and Xiong, 2014). This required a 318 bi-directional selection on GDP during rain-sufficient years and on drought resistance during 319 drought years. This pattern of selection results in signs of balancing selection for some 320 321 genomic regions in upland rice. (d) Divergent patterns of human selection (bi-directional selection between drought resistance and GDP in upland rice while directional selection for 322 GDP in lowland rice) led to adaptively differentiated ecotypes, particularly for drought 323 324 resistance. This model is feasible and supported by our results.

Furthermore, other patterns, such as the possibility for a gene of drought resistance being 325 directionally selected in upland rice (Lyu et al., 2013; 2014), may also exist. Many genetic 326 327 regions in upland rice also receives directional selection, which is represented through low 328 relative genetic diversity and signs of selective sweep. For example, we detected a selective sweep at a highly differentiated region of Chr7:1-600000 in upland rice. It contains two 329 330 potential drought resistant genes, including ARAG1 (LOC_Os07g01070) and OsGL1-8 331 (LOC_Os07g01150), which may be directionally selected in upland rice. Meanwhile, a QTL 332 of drought-avoidance (position of associated SNP for RDR, Chr08:8640319) is also located at 333 the genomic regions which receives directional selection in upland rice. The highly

differentiated gene, *OsWOX12A*, should be the candidate gene according to its function for root primordia initiation (Cheng et al., 2014; Hu and Xu, 2016). These results mean some highly differentiated regions relevant to drought resistance are resulted from directional selection on drought resistant genes in upland rice. This pattern of selection and its roles in rice adaptive differentiation during domestication should be further investigated.

339

340 Implications from evolution of drought resistance in upland rice for breeding

Bi-directional selection and its subsequent evolutionary results provide informative cues for 341 overcoming the tradeoff arising from the tight linkage between genes of drought resistance 342 and productivity. For example, recombinant genotypes between drought resistant and GDP 343 genes (e.g. DCA1 and OsCesA7) could be accumulated in upland rice landraces. Additionally, 344 some upland rice possesses rare ecotype-specific recombinant genotypes. A recombinant 345 event may break the tradeoff caused by the tight linkage and is meaningful for breeding. 346 Recombinant genotypes may confer both good drought resistance and high productivity 347 348 (Vikram et al., 2015). In fact, the strategy of bi-directional selection between drought resistance and productivity season by season has been applied in rice breeding. Many WDR 349 varieties (derived from upland rice \times lowland rice) with both advantages in drought resistance 350 351 and productivity have been developed by rotating selection on yields and drought resistance 352 season by season (Luo, 2010).

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356 Methods

357 Plant materials and genotyping

358 China is the origin and domestication center for Asian cultivated rice (particularly for Geng (*japonica*) subspecies) and has a long history of rice culture (Kovach et al., 2011; Wang et al., 359 2018). Studying adaptive differentiation between upland and lowland rice from China can 360 build the general model of rice ecotypes adaptation to different agro-ecosystems under human 361 selection. Two hundred and fifteen rice genotypes, including 113 upland and 102 lowland 362 landraces, were involved in this study (Figure 2a, Table S7). These upland and lowland 363 364 landraces were all Geng (japonica) subspecies. It is because ecotypes of Geng (japonica) subspecies exhibit considerable level of differentiation revealed by previous studies (Xia et al., 365 2014; Lyu et al., 2014). These rice landraces were collected from seven provinces (Hebei, 366 367 Henan, Jiangsu, Hunan, Guangxi, Guizhou, and Yunnan) in China (Figure 1), in which most 368 landraces are of the subgroup of East Asian temperate (Wang et al., 2018). These provinces 369 cultivate 79.7% of the total Geng (japonica) upland rice in China, according to Agrodata 370 (http://crop.agridata.cn/A010110.asp). We selected equivalent numbers of upland and lowland

371 landraces within each province to make them balance in geography. Thus, upland landraces 372 from Shandong and Guangdong Provinces were not included in this study as there was no 373 *Geng (japonica)* lowland rice grown in these two provinces. This sampling strategy could 374 avoid statistical bias from isolation by distance. Meanwhile, these landraces were preselected 375 to ensure their complete life histories in Shanghai. Sixty-five accessions of common wild rice 376 (*Oryza rufipogon*) were also used in this study as the reference (Table S7).

The 215 rice landraces and 20 accessions of common wild rice were genotyped using the 60K rice SNP array after the extraction of entire, high-quality DNA from green leaves using a routine protocol. To study the genetic differentiation between ecotypes at the gene scale, 112 (52 upland and 60 lowland) typical landraces and 45 accessions of common wild rice were sent for resequencing by Illumina X Ten at Shanghai MajorbioBiopharm Technology Co. Ltd. (Shanghai, China). A total of 921.6 Gb pair-end sequence data were generated, which covered an average depth of ~15× for each sample (Table S8).

384

385 Measurements of important agronomic and drought resistant traits

Eight important agronomic traits (Table 1) were measured for two growing seasons (2014.11-386 387 2015.4 and 2015.11–2016.4) in regularly managed paddy fields at the Lingshui Experimental Station, Hainan Province. Rice seedlings were transplanted into fields 25 days after 388 389 germination. Each landrace was planted in a plot of 7 rows \times 7 hills with 18 cm intervals. Six 390 traits of drought-avoidance, including the ratio of deep-rooting (RDR), number of deep roots 391 (NDR), number of shallow roots (NSR), total number of roots (NTR), number of roots per 392 panicle (NRP), and the rate of water loss (RWL) in excised leaves after two hours were measured for one season (2014.11-2015.4) at the Lingshui Experimental Station in Hainan. 393 394 The measurements of root traits were from four biological replicates for each landrace grown 395 in the paddy field using the 'basket' method (Uga, 2012) with minor modifications (Lou et al., 2015). The RWL was measured from three biological replicates containing two mature 396 flag-leaves two hours after the leaf was sampled from the paddy field and naturally dried at 397 398 room temperature. Measurements of drought-tolerance were conducted in the drought resistance screening facility for two seasons (2016.5-2016.10 and 2017.5-2017.10) at Baihe 399 Experimental Station in Shanghai. The canopy of the facility was normally opened and could 400 401 be closed on rainy days to enable continuous drought conditions. The depth of the soil-layer 402 in the experimental field was limited to 30cm, which enabled the separation of 403 drought-tolerance from drought-avoidance (Ma et al., 2016). With the shallow soil-layer, root 404 development was restricted and equalized among genotypes. Therefore, the differences in 405 drought-avoidance by roots could be largely mitigated. The design of sallow soil-layers also 406 permitted homogenous levels of soil-water content across the drought-treated field (~5% C.V.) (Ma et al. 2016). As crop drought-tolerance was empirically quantified by its relative 407

performances under drought to well-watered conditions, rice plants of each genotype were 408 409 planted in two nearby fields: one that was treated with mid to late (from the tillering stage to 410 the heading stage) drought conditions (D) and the other remained drip-irrigated as the control (CK). Rice seedlings were transplanted into plots with 8 rows \times 8 hills with 18 cm intervals 411 30 days after germination. Both D and CK fields were normally irrigated as paddy fields 412 during the first 20 days after transplanting. After the seedlings began tillering, water was 413 414 pumped out of the D field, and it was allowed to dry naturally. Meanwhile, the CK field was drip irrigated to make the soil-oxygen conditions closer to those of the D field. After 415 approximately 40 days of drought treatment, when all landraces had flowered and the 416 soil-water content at a depth of 30cm dropped severely (~12.6% in 2016 and ~8.4% in 2017), 417 the drought treatment was stopped and both fields were reirrigated. Nine important agronomic 418 traits were measured under D and CK fields (Table 1). Five drought-tolerance related traits, 419 including relative content under drought on 30 days after drought-treated (RWC), relative 420 100-grain weight, relative fecundity, relative biomass, and relative grain weight, were also 421 422 measured or calculated (Table 1). RWC was measured from three individuals while other drought-tolerant traits were measured from six individuals. The comparison of each measured 423 trait between upland and lowland rice ecotypes was conducted by independent t-test by 424 425 SPSS15.0.

426

427 Data analysis

428 SNP genotyping by SNP array and re-sequencing

In total, 235 plant materials were genotyped by the rice SNP array "Rice60K" (Patent no. CN201380056318.5), which was developed by China National Seed Group Co., Ltd. and contains ~60,000 SNPs based on MSU6.1 genome assembly. Detailed information on this array and the genotyping procedure are described in detail in a previous study (Chen et al., 2014). The original genotype data was provided as Supplementary Dataset 1.

For the resequencing, the raw paired-end reads were first filtered into clean data using 434 Fastp v0.6.0 (Chen et al., 2018) (https://github.com/OpenGene/fastp). Clean reads of each 435 accession were mapped to the *japonica* rice reference genome MSU v6.1 436 (ftp://ftp.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudo 437 **BWA** 438 molecules/version_6.1/all.dir/) using v0.7.16 with default settings (http://bio-bwa.sourceforge.net) (Li and Durbin, 2009). GATK v3.8.0was applied for variant 439 detection (McKenna et al., 2010). Raw variants were called based on the realigned bam file. 440 441 Using the called variants as known sites, 'Base Recalibrator' and 'Print Reads' in the GATK 442 were applied for base-pair scores recalibration. The proceeded BAM files for each sample 443 were used for the multi-sample variant genotyping. 'Unified Genotyper' in GATK was 444 applied to generate the raw variant calls with parameters '-stand_call_conf 30,

- -stand_emit_conf 10'. To reduce the variants discovery rate, the SNP calls were filtered
 according to the following threshold: QUAL<30, DP<5, QD<2, MQ<20, FS>60, Haplotype
 Score>13, and Read Pos Rank Sum<-8.
- 448

449 **Population structure inference**

450 Based on the total SNPs from 235 plant materials genotyped by the SNP array, a phylogenetic

tree was constructed using Fasttree2.1 (Price et al., 2010) with 1,000 replicates for bootstrapconfidence analysis. MEGA v5.1 was applied to draw the constructed tree.

453

454 Estimation of population parameters

A sliding-window approach (200 Kb windows sliding in 100 Kb steps) was applied to 455 estimate genetic diversity (π), genetic differentiation (F_{ST}), and selection statistics (Tajima's D) 456 between upland and lowland rice ecotypes based on SNP data gained from the SNP array. 457 $\prod_{upland}/\prod_{lowland}$ was calculated to estimate the relative diversity of the upland ecotype to the 458 459 lowland ecotype. Meanwhile, π and F_{ST} were also estimated for each window using resequencing data between selected upland and lowland landraces by VCFtools (Danecek et 460 al., 2011). MAF>0.05 were used to filter uninformative SNPs before calculating above 461 parameters. The F_{ST} and π estimated by the data from the SNP array and the data from 462 463 re-sequencing were significantly correlated (Figure S9), indicating that the selected plant 464 materials for resequencing were typical. Meanwhile, the outcome also indicated that our results obtained from the high-density SNP array were convincing. Finally, the relative π ratio 465 $(\prod_{upland}/\prod_{lowland})$ and F_{ST} for each gene (upstream 2000bp + genebody + downstream 200bp) 466 between upland and lowland ecotypes were calculated based on the re-sequencing data of 112 467 468 genotypes.

469

470 LD analysis

To evaluate LD decay across the genome, the squared correlation (r^2) between any two loci 471 was calculated using VCFtools based on data from the SNP array. The average r^2 value was 472 calculated for pairwise SNPs in a 500 Kb region and averaged across the whole genome. To 473 investigate the linkage status of DCA1 with other genes in the region of Chr10: 15400000-474 17600000, the squared correlation (r^2) between any two SNPs (one SNP of *DCA1* and one 475 SNP in the other gene) was calculated. The linkage status between a gene and DCA1 was 476 determined by their highest r^2 calculated from any two SNPs (one SNP of *DCA1* and one SNP 477 478 in the other gene). This analysis was based on resequencing data.

479

480 Analysis of the ecotype-private allele

481 The ecotype-private allele was defined as a SNP allele detected uniquely in upland or lowland

rice ecotypes. If an ecotype-private allele could be detected in common wild rice, it was considered to have been inherited from the wild ancestor. In contrary, if an ecotype-private allele could not be detected in common wild rice, it may have been newly mutated during rice domestication.

486

487 Detection of potential recombinant genotypes within genomic regions conferring both 488 drought resistant and GDP genes

We detected recombinant genotypes within 126 genomic regions (200K windows, listed in Table S5) conferring both drought resistant and GDP genes in upland, lowland, and common wild rice. First, we determined the major genotype (frequency>0.50) by SNPs within the analyzed window and scored it as "0". Any other genotypes containing the successive five different SNP alleles were determined as recombinant genotypes and scored as "1". If a genotype was uniquely or majorly (frequency >0.70) detected in one ecotype, it was defined as an ecotype-specific or ecotype-preferential recombinant genotype.

496

497 **Detection of genomic differentiation**

The comparison of quantitative genetic divergence (Q_{ST}) and neutral genetic divergence (F_{ST}) was applied to detect adaptive evolution (Miller et al. 2008). The Q_{ST} of each trait was calculated as: $Q_{ST} = V_{pop}/(V_{pop}+2V_{ind})$, where V_{pop} was the variance among populations and V_{ind} was the variance within a population. The neutral F_{ST} was calculated from SNPs from intergenic regions. Any significant differences between the QST and FST at the p <0.05 level was determined when $|Q_{ST}-F_{ST}| > 2SQRT$ ($SE_{Qst}^2+SE_{Fst}^2$).

Based on the Ricedata (http://www.ricedata.cn/gene/), genes belong to trait ontology (TO) 504 of plant height (TO:0000207), no. of panicles (TO:0000432), seed-setting rate (TO:0000448), 505 506 seed production (TO:0000396 and TO:0002759), biomass (TO:0000327), and 1,000-seed weight (TO:0000592) were categorized as GDP (growth, development, and productivity) 507 relevant genes (Table S4). Genes belong to trait ontology (TO) and gene ontology (GO) of 508 509 drought-tolerance (TO:0000277), water channel activity (GO:0015250), and response to osmotic stress (GO:0006970) were categorized as drought resistance (DR) relevant genes 510 (Table S4). If a 200Kb window contained at least one GDP or DR gene, it was then 511 determined as a GDP- or DR-related window. Mean FST values of GDP- and DR-related 512 windows were compared with the genomic average by independent *t*-test via SPSS15.0. There 513 were 517 DR-related (517/3738=13.8% of total windows) and 613 GDP-related 514 (613/3738=16.4% of total windows) windows, respectively. It is therefore, the random 515 516 ratio for a window to became a window of both drought resistance and GDP is about 2.27% (=16.4%*13.8%). We conducted χ^2 test to test whether the actual ratio 517

(5.67%=212/3738) for a window related to both drought resistance and GDP was beyond the
ratio by chance *via* SPSS15.0..

For the detection of genomic regions receiving potential balancing selection during upland-lowland differentiation, the genomic windows with the top 5% Tajima's D were selected in upland or lowland ecotypes. Some continuous windows were further combined, to form larger genomic regions. Meanwhile, the regions with an average Tajima's D>1 or containing windows of high Tajima's D values (>2) in corresponding lowland or upland ecotypes were filtered further, as suggested by Qiu et al. (2017)

In addition, we also applied SweeD to detected signs of selective sweeps in the genomes of upland and lowland rice ecotypes. This method uses CLR statistics and identified signals of selective sweep by detecting significant deviations from the neutral site frequency spectrum (Pavlidis et al. 2013). In this study, ecotype-unique (with the top 1% CLR values uniquely in one ecotype) and shared regions (with the top 1% CLR values in both ecotypes) with signs of selective sweep were defined.

532

533 Enrichment of Gene Ontology (GO) for different categories of highly different genes

534 Based on SNPs generated from resequencing, the F_{ST} between ecotypes and $\Pi_{upland}/\Pi_{lowland}$ ratios were calculated for each annotated gene on the reference genome (ver. MSU 6.1). 535 Genes with high F_{ST} values beyond the 95% confidence interval (F_{ST} >0.455) were determined 536 as highly differentiated genes (HDGs). We defined three categories of HDGs by their relative 537 π ratio: (1) $\prod_{\text{upland}}/\prod_{\text{lowland}} < 0.5$, (2) $0.5 \leq \prod_{\text{upland}}/\prod_{\text{lowland}} \leq 2.0$, and (3) $\prod_{\text{upland}}/\prod$ 538 lowland >2.0. We conducted analyses of GO enrichment for the three categories of HDGs using 539 the software GOatools (https://github.com/tanghaibao/GOatools). Top 15 (by p value) GO 540 terms of biological processes were listed and compared among different categories of HDGs. 541

542

543 Genome-wide association analysis (GWAS) for GDP- and DR-related traits

The GWAS was conducted via the efficient mixed-model association (EMMA) method using 544 545 the R package of Genomic Association and Prediction Integrated Tool (GAPIT) (Lipka et al., 546 2012). The kinship (K) matrix was calculated among genotypes with default settings before applying GWAS. Meanwhile, all landraces in this study are of *Gene (Japonica)* subspecies 547 from China, the mixed model without inferred population structure as cofactor was applied. 548 The observed $-\log_{10}^{(p)}$ fit the expected $\log_{10}^{(p)}$ well in the QQ-plot for our traits by this model. 549 A total of ~24,656 available SNP markers (MAF>5%) were used in GWAS. The 550 genome-wide threshold was set at 1/n (n=total number of SNPs), which was widely used in 551 plant GWAS (Wen et al., 2015; Wang et al., 2015), particularly for rice drought resistance (Ma 552 et al., 2016). The original phenotype data for GWAS was provided in Table S9. 553

555 Function validation of the candidate drought resistant genes (*DCA1* and *LAX1*) 556 identified by GWAS

557 Transgenic mutants of two candidate genes associated with RWL (LAX1) and RWC (DAC1) were used to ascertain their functions in drought resistance and/or GDP. LAX1 transgenic lines 558 of the activated (coded as XE16 and XE18) and inactivated (coded as XE19 and XE20) forms 559 560 were kindly provided by Dr. Lin Chentao. Detailed information for the transgenic lines has been described in Zhao et al. (2015). The RWL was measured from matured leaves of the 561 mutants and the wild-type plant. The RWC was measured from 20-day-old seedlings after 48 562 hours of treatment with osmotic stress stimulated by 20% PEG6000. Three replicates were 563 designed. Their morphological appearances (e.g. plant height, number of tillers, flag leaf 564 width, 1,000-seed weight, and grain weight) were measured from eight individuals in the 565 regularly managed paddy field at the Baihe Experimental Station in 2017. DCA1 was located 566 within the QTL associated with RWC and plant height. As the effect of DCA1on 567 568 drought-tolerance has been elaborated by a previous study (Cui et al. 2015), only its effect on the plant height was estimated using its function-loss (dca1) and over-expression 569 (35S::DCA1-7) mutants. These two transgenic lines and their wild types were kindly provided 570 571 by Dr. Lin Hongxuan and detailed information can be found in Cui et al. (2015). The plant 572 height was measured from eight individual plants grown in the regularly managed paddy 573 fields at the Baihe Experimental Station in 2017.

574

554

575 Evolutionary analysis of *LAX1* and *DCA1* between upland and lowland rice ecotypes

576 Consensus sequences (reference sequence with SNPs called from each genotype) of *LAX1* and 577 *DCA1* (-2000 to 2000 bp) were extracted from 112 re-sequenced genotypes using in house 578 Perl. MEGA v5.1 was used for the phylogenetic analysis and to draw the constructed 579 phylogenetic tree.

580

581 SNP validation by Sanger sequencing

For genotyping validation, approximately a 1,000bp portion of one gene (LOC_Os01g61480, *LAX1*) was selected to be Sanger sequenced in all 112 typical re-sequenced rice landraces.
Five SNPs called from re-sequencing data within this PCR-amplified segment were well
validated by the Sanger method (Table S10). The primers for PCR-amplification are listed in
Table S11.

587

588 Data availability

The SNP data from SNP array is provided as a supplementary dataset and the re-sequence
data included in this study would been deposited into the NCBI Sequence Read Archive (SRA)

under the accession number PRJNA260762.

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606 Contributions

H.X., L.C. and L.J.L. designed the experiments. H.X., Z.L., J.X., and X.S.M. conducted most
of the field and molecular experiments. Q.J.L. and L.C. evaluated root-related traits. H.X.,
Z.L., X.S.M., J.Q. and L.J.F. analyzed the data. H.Y. and G.L.L. collected plant materials in

- 610 this study. H.X., L.J.F. and L.J.L. wrote the manuscript.
- 611

612 **Competing interests**

- 613 The authors declare no competing financial interests.
- 614

615 **References**

- Cheng S, Huang Y, Zhu N, Zhao Y. 2014. The rice WUSCHEL-related homeobox genes are
 involved in reproductive organ development, hormone signaling and abiotic stress
 response. Gene 549: 266-274.
- Chen H, Xie W, He H, Yu H, Chen W, Li J, Yu R, Yao Y, Zhang W, He Y, et al. 2014. A
 high-density snp genotyping array for rice biology and molecular breeding. *Mol Plant*7:541–553.
- 622 Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor.
 623 *bioRxiv*:274100.
- Clark RM, Linton E, Messing J, Doebley JF. 2004. Pattern of diversity in the genomic region
 near the maize domestication gene tb1. *Proc Natl Acad Sci.* 101:700–707.
- Cui LG, Shan JX, Shi M, Gao JP, Lin HX. 2015. DCA1 Acts as a Transcriptional Co-activator
 of DST and contributes to drought and salt tolerance in rice. *PLoS Genet*. 11:e1005617.

- Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter
 G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. *Bioinformatics* 27:2156–2158.
- Denison RF. 2015. Evolutionary tradeoffs as opportunities to improve yield potential. *Field Crop Res.* 182:3–8.
- Doebley JF, Gaut BS, Smith B. 2006. The molecular genetics of crop domestication. *Cell*127:1309–1321.
- Fang Y, Xiong L. 2015. General mechanisms of drought response and their application in
 drought resistance improvement in plants. *Cell Mol Life Sci.* 72:673–689.
- Fang Y, Xiong L. 2015. General mechanisms of drought response and their application in
 drought resistance improvement in plants. *Cell Mol Life Sci.* 72:673–689.
- Farooq M, Wahid A, Lee DJ, Ito O, Siddique KHM. 2009. Advances in Drought Resistance of
 Rice. *Crit Rev Plant Sci.* 28:199–217.
- Fletcher RS, Mullen JL, Heiliger A, Mckay JK. 2015. QTL analysis of root morphology,
 flowering time, and yield reveals trade-offs in response to drought in *Brassica napus*. J *Exp Bot*. 66:245-256.
- Gupta PC, O'Toole JC. 1986. Upland rice : a global perspective. International Rice Research
 Institute, Los Baños, Philippines.
- Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD. 2009. Inferring the joint
 demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genet.* 5: e1000695
- Harb A, Krishnan A, Ambavaram MMR, Pereira A. 2010. Molecular and Physiological
 Analysis of Drought Stress in Arabidopsis Reveals Early Responses Leading to
 Acclimation in Plant Growth. *Plant Physiol.* 154:1254–1271.
- Hu H, Xiong L. 2014. Genetic engineering and breeding of drought-resistant crops. *Annu Rev Plant Biol.* 65:715–741.
- Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L. 2008. Characterization of transcription factor
 gene *SNAC2* conferring cold and salt tolerance in rice. *Plant Mol Biol.* 67:169–181.
- Hu XM, Xu L. 2016. Transcription factors WOX11/12 directly activate WOX5/7 to promote
 root primordia initiation and organogenesis. *Plant Physiol.* 172: 2363.
- Huang D, Wang S, Zhang B, Shang-Guan K, Shi Y, Zhang D, Liu X, Wu K, Xu Z, Fu X, et al.
 2015. A Gibberellin-mediated DELLA-NAC signaling cascade regulates cellulose
 synthesis in rice. *Plant Cell* 27:1681–1696.
- Jérôme B, Garyn A, Rachid S, Arvind K, Dean S. 2010. Breeding upland rice for drought
 resistance. *J Sci Food Agric*. 88:927–939.
- Jung H, Lee DK, Choi Y Do, Kim JK. 2015. *OsIAA6*, a member of the rice Aux/IAA gene
 family, is involved in drought tolerance and tiller outgrowth. *Plant Sci.* 236:304–312.

- 665 Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, Okamoto H, Shimamoto K, Kyozuka 666 J. 2003. LAX and SPA: major regulators of shoot branching in rice. Proc Natl Acad Sci. 667 100:11765-11770. Koziol L, Rieseberg LH, Kane N, Bever JD. 2012. Reduced drought tolerance during 668 domestication and the evolution of weediness results from tolerance-growth trade-offs. 669 Evolution 66:3803-3814. 670 Kumar GR, Sakthivel K, Sundaram RM, Neeraja CN, Balachandran SM, Rani NS, 671 Viraktamath BC, Madhav MS. 2010. Allele mining in crops: Prospects and potentials. 672 673 Biotechnol Adv. 28:451-461. Leinonen T, McCairns RJS, O'Hara RB, Merilä J. 2013. OST-FST comparisons: Evolutionary 674 and ecological insights from genomic heterogeneity. Nat Rev Genet. 14:179-190. 675 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler 676 transform. Bioinformatics 25:1754-1760. 677 Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z. 678 679 2012. GAPIT: Genome association and prediction integrated tool. Bioinformatics 28:2397-2399. 680 Lou Q, Chen L, Mei H, Wei H, Feng F, Wang P, Xia H, Li T, Luo L. 2015. Quantitative trait 681 682 locus mapping of deep rooting by linkage and association analysis in rice. J Exp Bot. 683 66:4749-4757. 684 Lyu J, Li B, He W, Zhang S, Gou Z, Zhang J, Meng L, Li X, Tao D, Huang W, et al. 2014. A genomic perspective on the important genetic mechanisms of upland adaptation of rice. 685 BMC Plant Biol. 14:160. 686 Lyu J, Zhang S, Dong Y, He W, Zhang J, Deng X, Zhang Y, Li X, Li B, Huang W, et al. 2013. 687 Analysis of elite variety tag SNPs reveals an important allele in upland rice. Nat 688 689 *Commun.* 4:1–9. Ma XS, Feng FJ, Wei HB, Mei HW, Xu K, Chen SJ, Li TM, Liang XH, Liu HY, Luo LJ. 690 (2016) Genome-wide association study for plant height and grain yield in rice under 691 contrasting moisture regimes. Front Plant Sci. 7:1801. 692 Ma X, Xia H, Liu Y, Wei H, Zheng X, Song C, Chen L, Liu H, Luo L. 2016. Transcriptomic 693 and metabolomic studies disclose key metabolism pathways contributing to 694 well-maintained photosynthesis under the drought and the consequent drought-tolerance 695 in rice. Front Plant Sci. 7:1-18. 696 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, 697 698 Altshuler D, Gabriel S, Daly M, et al. 2010. The Genome Analysis Toolkit: A 699 MapReduce framework for analyzing next-generation DNA sequencing data. Genome
- 700 *Res.* 20:1297–1303.
- 701 Miller JR, Wood BP, Hamilton MB. 2008. FST and QST under neutrality. Genetics 180:1023-

702 103

- Pandey V, Shukla A. 2015. Acclimation and tolerance strategies of rice under drought stress.
 Rice Sci. 22:147–161.
- Pavlidis P, Živković D, Stamatakis A, Alachiotis N. 2013. SweeD: Likelihood-Based
 detection of selective sweeps in thousands of genomes. *Mol Biol Evol.* 30:2224.
- Price MN, Dehal PS, Arkin AP. (2010) FastTree 2 -- Approximately maximum-likelihood
 trees for large alignments. *PLoS ONE*. 5(3): e9490.
- 709 International Rice Research Institute. 1975. Major research in upland rice. International Rice
 710 Research Institute, Los Baños, Philippines.
- Qiu J, Zhou Y, Mao L, Ye C, Wang W, Zhang J, Yu Y, Fu F, Wang Y, Qian F, et al. 2017.
 Genomic variation associated with local adaptation of weedy rice during
 de-domestication. *Nat Commun.* 8:15323.
- Tao Z, Kou Y, Liu H, Li X, Xiao J, Wang S. 2011. *OsWRKY45* alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. *J Exp Bot.* 62:4863–4874.
- 717 Uga Y. 2012. Quantitative measurement of root growth angle by using the basket method. In:
 718 Shashidhar HE, Henry A, Hardy B, eds. Methodologies for root drought studies in rice.
 719 The Philippines: International Rice Research Institute, 22–26.
- Vikram P, Swamy BPM, Dixit S, Singh R, Singh BP, Miro B, Kohli A, Henry A, Singh NK,
 Kumar A. 2015. Drought susceptibility of modern rice varieties: An effect of linkage of
 drought tolerance with undesirable traits. *Sci Rep.* 5:1–18.
- Wang D, Qin Y, Fang J, Yuan S, Peng L, Zhao J, Li X. 2016. A missense mutation in the zinc
 finger domain of *OsCESA7* deleteriously affects cellulose biosynthesis and plant growth
 in rice. *PLoS One* 11:1–16.
- Wang Q, Xie W, Xing H, Yan J, Meng X, Li X, Fu X, Xu J, Lian X, Yu S, et al. 2015. Genetic
 architecture of natural variation in rice chlorophyll content revealed by a genome-wide
 association study. *Mol Plant* 8:946–957.
- Wen W, Li D, Li X, Gao Y, Li W, Li H, Liu J, Liu H, Chen W, Luo J, et al. 2014.
 Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights. *Nat Commun.* 5:1–10.
- Wang W, Mauleon R, Hu Z, Chebotarov D, Tai S, Wu Z, Li M, Zheng T, et al. 2018. Genomic
 variation in 3,010 diverse accessions of Asian cultivated rice. *Nature* 557: 43-49.
- Wright SI. 2005. The Effects of Artificial Selection on the Maize Genome. *Science* 308:1310–
 1314.
- Xia H, Zheng X, Chen L, Gao H, Yang H, Long P, Rong J, Lu B, Li J, Luo L. 2014. Genetic
 differentiation revealed by selective loci of drought-responding EST-SSRs between
 upland and lowland rice in China. *PLoS ONE* 9:e106352.

- Yordanov I, Velikova V, Tsonev T. 2003. Plant responses to drought and stress tolerance. *Photosynthetica* 38:187–206.
- 741 Zhang C, Liu J, Zhao T, Gomez A, Li C, Yu C, Li H, Lin J, Yang Y, Liu B, et al. 2016. A
 742 drought-inducible transcription factor delays reproductive timing in rice. *Plant Physiol.*743 171:334–343.
- Zhang D, Zhang H, Wang M, Sun J, Qi Y, Wang F, Wei X, Han L, Wang X, Li Z. 2009.
 Genetic structure and differentiation of *Oryza sativa* L. in China revealed by
 microsatellites. *Theor Appl Genet*. 119:1105–1117.
- Zhao T, Liu J, Li HY, Lin J-Z, Bian MD, Zhang CY, Zhang YX, Peng YC, Liu B, Lin CT.
 2015. Using hybrid transcription factors to study gene function in rice. *Sci China Life Sci.* 58:1160–1162.

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751 Figure legends

Figure 1. Matrix of Pearson's correlation coefficients (*r*) (left triangle) and *p*-values (right triangle) among drought-resistant and agronomic traits. Abbreviations: NT, no. of tillers; PH, plant height; FLL, flag leaf length; FLW, flag leaf width; RWC, relative water content; RWL, ratio of water loss; 100GW, 100-grain weight; GW, grain weight; HI, harvest index; RF, relative fecundity; R100GW, relative 100-grain weight; RB, relative biomass; RGW, relative grain weight; RDR, ratio of deep-rooting; NDR, no. of deep root; NSR, no. of shallow root; NTR, no. of total root; NRP no. of root per tiller.

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Figure 2. Upland and lowland rice collected from China, their phylogenetic relationships, and the decay of linkage disequilibrium (LD) across the genome. (a) Geographic distributions of upland rice in China. Numbers in red indicate provinces where equivalent numbers of upland and lowland rice landraces were collected. 1 Hebei, 2 Henan, 3 Jiangsu, 4 Hunan, 5 Guangxi, 6 Guizhou, 7 Yunnan. (b) A phylogenetic tree of upland, lowland, and common wild rice. (c) LD decay across the genome in upland and lowland rice.

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Figure 3. Genomic differentiation and selection detected between rice ecotypes. (a) The 767 genome-wide manhattan plot of F_{ST} estimated in a 200Kb sliding window with 100Kb 768 769 step-size. Bars under the manhattan plot describes distributions of genes relevant to 770 drought-resistance and GDP (growth, development, and productivity). Blue bars indicate 771 windows containing genes of drought-resistance. Green bars indicate windows containing genes of GDP. Red bars indicate windows containing genes of both drought- resistance and 772 GDP. (b) The genome-wide manhattan plot of Log₂^([]upland/[]lowland) estimated in a 200Kb sliding 773 window with 100Kb step-size. (c) The genome-wide manhattan plot of number of SNPs in the 774 200Kb sliding window with 100Kb step-size. (d) Mean F_{ST} of DR, GDP relevant, and neutral 775 windows. The bar indicates SE. "***" indicates significance at p < 0.001 by independent *t*-test 776 in comparison with neutral windows. (e) Mean F_{ST} of windows with different gradients of 777 $\prod_{\text{upland}}/\prod_{\text{lowland}}$ ratios. The bar indicates SE. "***" indicates significance at p<0.001 by 778 779 independent *t*-test in comparison with the genomic average.

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Figure 4. Signs of selection detected in upland and lowland rice. (a)Tajima's *D* estimated in upland (blue) and lowland rice (orange). A region receiving balancing selection in upland or lowland ecotype is labeled in the green or red shade. (b) Composite likelihood ratio (CLR) estimated by SweeD in upland rice. (c) Composite likelihood ratio (CLR) estimated by SweeD in lowland rice.

786

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QTLs (annotated by red arrows) are particularly discussed in the manuscript.

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Figure 6. A drought-resistant gene (DCA1) in tight linkage with other agronomic genes 791 and a drought-resistant gene (LAXI) possessing pleiotropic effects. (a) A phylogenetic tree 792 793 of upland, lowland, and common wild rice based on the DCA1 sequence. (b) F_{ST} (max value=0.734, outside circle), \prod upland/ \prod lowland ratio (max value=30.1, middle circle), and r^2 794 of each gene to DCA1 within the region of Chr10: 15400000–17600000. r^2 is the max 795 coefficient of correlation for SNPs within each gene with SNPs of DCA1. (c) A phylogenetic 796 tree of upland, lowland, and common wild rice based on the DCA1 sequence. (d) Rate of water 797 loss (RWL) at two hours in matured leaves of transgenic lines and the wild type (WT). (e) 798 799 Relative water content (RWC) measured in osmotic-stressed seedlings. XE16 and XE18 are transgenic lines of activated form of LAX1. XE19 and XE20 are transgenic lines of 800 inactivated forms of LAX1. Bars indicate SE. *, **, and †indicate significances at p<0.05, 801 802 p<0.01, and p<0.1 by independent *t*-test in comparisons between transgenic lines and WT.

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Table 1. Agronomic and drought-resistant traits measured in well-watered paddy, drought-stressed (D), and control (CK) fields. \dagger , *, **, and *** indicate significance at levels of p<0.1, P<0.05, p<0.01, and p<0.001 by independent *t*-test between upland and lowland rice ecotypes. NS indicates no significance detected. RWL is the abbreviation for ratio of water loss in excised-leaves.

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Cotogory	Trait	2014-2015 (Hainan)					2015-2016 (Hainan)					
Category		N	upland	N	lowland	sig	N	upland	N	lowland	Sig.	
Agronomic	Plant height (cm)	112	125.3±2.3	101	120.2±2.4	NS	Ĵ		Not evalu	iated		
traits in	No. of panicle	112	7.73±0.33	101	12.31±0.51	***	109	7.67±0.24	95	9.68±0.30	***	
paddy field	Biomass (g)	112	31.83±1.00	101	39.41±1.23	***	99	28.45 ± 0.68	92	31.66±0.82	**	
	Grain weight (g)	112	15.08±0.48	101	18.64±0.67	***	98	10.45±0.34	92	11.63±0.46	*	
	100-grain weight (g)	112	2.91±0.03	101	2.77±0.03	**			Not evalu	iated		
	Harvest index	112	0.469 ± 0.009	101	0.468 ± 0.010	NS	99	0.350 ± 0.008	92	0.359 ± 0.008	NS	
	Length of flag leaf (cm)	112	27.4±0.66	101	27.8±0.66	NS	108	27.9±0.5	97	25.6±0.5	*	
	Width of flag leaf (cm)	112	1.61 ± 0.02	101	1.43±0.03	***	108	1.56±0.03	97	1.31±0.03	***	
Traits of	RWL at 2 hours	112	0.346 ± 0.008	101	0.372±0.009	*						
drought	Ratio of deep rooting	112	0.346±0.012	101	0.277 ± 0.010	***						
avoidance	No. of deep root	112	58.4±2.39	101	46.6±2.31	***						
	No. of sallow root	112	118.9±5.9	101	126.3±5.1	NS			Not evalu	lated		
	No. of total root	112	177.3±7.0	101	172.9±6.6	NS						
	No. of root per panicle	112	9.66±0.29	101	7.71±0.30	***						
		1	201	6 (Shar	nghai)			2	017 (Sha	nghai)		
Agronomic	Plant height-CK (cm)	104	136.3±2.57	89	128.8±2.94	†	106	126.2±1.6	98	120.7±1.9	*	
traits in	No. of panicle-CK	102	4.51±0.11	91	5.38±0.15	***	106	5.81±0.21	98	7.64±0.24	***	

813 detected. RWL is the abbreviation for ratio of water loss in excised-leaves. N indicates number of samples.

control	Biomass-CK (g)	102	21.58±1.25	91	22.61±0.99	NS	100	23.36±1.07	96	27.05±1.11	*
field	Grain weight-CK (g)	102	6.83±0.40	91	8.94±0.45	***	94	3.94±0.23	97	5.28±0.32	**
	Fecundity -CK	102	0.621±0.019	91	0.704±0.016	**	94	0.385±0.017	97	0.456±0.186	**
	100-grain weight-CK (g)	102	2.68 ± 0.04	91	2.61±0.04	NS	94	2.26±0.04	97	2.21±0.03	NS
	Harvest index-CK	102	0.336±0.013	91	0.401±0.010	***	94	0.176±0.009	97	0.194±0.009	NS
	Length of flag leaf-CK (cm)	104	31.4±0.65	89	30.8±0.64	NS		Not	- a valı	atad	
	Width of flag leaf-CK (cm)	104	1.41 ± 0.03	89	1.21±0.03	***		not evaluated			
Agronomic	Plant height-D (cm)	103	119.2±2.58	90	109.0±2.95	**	106	105.3±1.1	98	101.3±1.8	NS
traits in	No. of panicle-D	102	3.86±0.10	91	5.03±0.16	***	106	4.11±0.12	98	5.20±0.17	***
drought	Biomass-D (g)	104	14.4±0.63	89	15.0±0.60	NS	101	11.73±0.44	96	12.45±0.35	NS
stressed	Grain weight-D (g)	104	4.22±0.21	89	4.18±0.22	NS	100	0.92 ± 0.09	96	0.73 ± 0.08	NS
field	Fecundity-D	102	0.457 ± 0.018	90	0.379±0.022	**	100	0.262 ± 0.022	96	0.192±0.019	*
	100-grain weight-D (g)	102	2.35 ± 0.04	90	2.18±0.04	**	95	1.96±0.05	84	1.87 ± 0.04	NS
	Harvest index-D	102	0.231±0.009	89	0.196±0.012	*	100	0.085 ± 0.007	96	0.055 ± 0.005	**
	Length of flag leaf-D (cm)	103	26.1±0.60	90	23.0±0.59	***		Not	Not one loot of		
	Width of flag leaf-D (cm)	102	1.40±0.03	91	1.16±0.03	***		Not evaluated			
Traits of	Relative water content	91	0.789±0.010	83	0.713±0.010	***	101	0.762 ± 0.006	90	0.745 ± 0.005	*
drought	Relative fecundity	102	0.800 ± 0.043	89	0.533 ± 0.044	***	90	0.709 ± 0.075	95	0.480 ± 0.068	*
tolerance	Relative 100-grain weight	102	0.884 ± 0.012	90	0.837±0.013	*	85	0.860 ± 0.017	83	0.843 ± 0.017	NS
	Relative biomass	102	0.758±0.036	89	0.730±0.031	NS	95	0.558 ± 0.028	96	0.524±0.021	NS
	Relative grain weight	102	0.628±0.055	90	0.398 ± 0.044	**	90	0.280 ± 0.037	95	0.199±0.029	†

815 Supplemental Information

816 Supplemental Dataset 1. Original dataset from SNP array for genotyped rice materials.

817

818 Supplementary Figures and Tables

819 Figure S1. Q_{ST} values of measured drought-resistant and agronomic traits and their comparisons to the neutral genomic F_{ST} . (a) Agronomic traits measured in well-watered 820 821 paddy fields at Hainan in season 2014-2015. (b) Agronomic traits measured in well-watered 822 paddy fields at Hainan in 2015-2016. (c) Traits of drought-avoidance measured at Hainan in 823 season 2014-2015. (d) Agronomic traits measured in control (drip-irrigated) fields at 824 Shanghai in season 2016. (e) Agronomic traits measured in drought-treated fields at Shanghai in season 2016. (f) Drought-tolerance estimated at Shanghai in season 2016. (g) Agronomic 825 traits measured in control (drip-irrigated) fields at Shanghai in season 2017. (h) Agronomic 826 827 traits measured in drought-treated fields at Shanghai in season 2017. (i) Drought-tolerance estimated at Shanghai in season 2017. "*", "**", and "***" indicate differences in means at 828 829 significances of p < 0.05, p < 0.01, and p < 0.001 between upland and lowland rice by independent t test. The column in black indicates the Q_{ST} is significantly higher than the 830 831 neutral genomic F_{ST}. NA is the abbreviation for "not available".

832

Figure S2. Gene Ontology (GO) terms of biological process (BP) enriched by highly 833 differentiated genes of different gradients of $\prod^{\text{upland}}/\prod^{\text{lowland}}$ ratios. (a) Venn diagram of 834 enriched GOBPs by highly differentiated genes of different gradients of $\Pi^{upland}/\Pi^{lowland}$ ratios. 835 (b) Top fifteen (by p values) GOBPs enriched by highly differentiated genes of 836 $\prod^{\text{upland}}/\prod^{\text{lowland}} > 2.0.$ (c) Top fifteen GOBPs (by p values) enriched by highly differentiated 837 genes of $0.5 < \prod^{\text{upland}} / \prod^{\text{lowland}} < 2.0$. (d) Top fifteen (by p values) GOBPs enriched by highly 838 differentiated genes of $0.5 < \prod^{\text{upland}} / \prod^{\text{lowland}} < 0.5$. "***", and "*" indicate Bonferroni 839 corrected p values <0.001, <0.01, and <0.05. GOBPs in red indicate responses to various 840 stimuli. GOBPs in green indicate various transporters. 841

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Figure S3. Venn diagram of private alleles detected in upland rice, lowland rice, and common wild rice.

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Figure S4. Recombinant genotypes detected in upland, lowland, and common wild rice in regions containing both genes of drought-resistance and productivity. Red (1) and brown (0) in the heatmap indicate recombinant and major genotypes. The red arrow indicates some rare upland-specific recombination. Accessions (right) in red, green, and blue are of upland, lowland, and wild rice, respectively. Regions in red, orange, green, and blue indicate upland-preferential, upland-specific, lowland-preferential and lowland-specific recombination

genotypes. The genomic position and corresponding information for each region are listed inTable S5.

854

Figure S5. A minor pleiotropy effect on the plant height detected in transgenic lines of *DCA1*. 35S::*DCA1*-7 is an over-expression line at genetic background of Zhonghua11. *dca1* is
a *DCA1* knockdown mutant by *Tos17* fragment insertion. Bar indicates SE. "*" and "†"

- 858 indicate significances at p < 0.05 and p < 0.1 by independent t test in comparison with WT.
- 859

Figure S6. Genotypes of *DCA1*, *Ehd1*, and *OsCesA7* in resequenced landraces and common
wild rice. Blue indicates the allele of reference type. Yellow indicates the allele of alternated
type. Red indicates SNP in hybrid status.

863

Figure S7. Genetic differentiation of *LAX1* and its impacts on agronomic traits. (a) F_{ST} values of *LAX1* and genes nearby between upland and lowland ecotypes. (b) F_{ST} values of *LAX1* and genes nearby between tolerant and susceptible groups. (c-h) Impacts of *LAX1* on some agronomic traits evaluated by transgenic rice lines. XE16 and XE18 are transgenic lines of activated form of *LAX1*. XE19 and XE20 are transgenic lines of inactivated form of *LAX1*. "***", "**", and "*" indicate significances at *p*<0.001, *p*<0.01, and *p*<0.05.

870

Figure S8. F_{ST} of each gene within the QTL region (-200Kb to 200Kb from the peak signal) and potential candidate genes. (a) The QTL for ratio of deep-rooting. (b) The QTL for relative grain weight. The green arrow indicates gene of peak signal. The red arrow indicates potential candidates. The yellow column indicates a drought-responsive gene from our unpublished transcriptomic data.

876

Figure S9. Correlations of $F_{ST}(a)$ and $\prod_{upland}/\prod_{lowland}$ ratios (b) calculated by data from SNP array and re-sequencing.

879

Table S1. F_{ST} and $\prod_{upland}/\prod_{lowland}$ ratios (mean \pm SE) of windows containing genes relevant to growth, development, and productivity (GDP). "*" indicates significant differences between GDP and neutral windows at the level of p<0.05 by independent *t* test. The description of trait ontology and genes involved in could be find at the database of Ricedata (http://www.ricedata.cn/gene/)

885

Table S2. Genomic regions receiving balancing selections in upland (U) or lowland rice (L) determined by Tajima's D test, their F_{ST} values, and relative diversity ($\Pi_{upland}/\Pi_{lowland}$).

888	Genes/QTLs relevant to drought-resistance (DR) and rice growth, development, and
889	productivity (GDP) within this region were listed. The information of the QTL could be found
890	in Gramene (<u>http://www.gramene.org/</u>).
891	
892	Table S3. Genomic regions detecting signs of selective sweeps in upland/lowland rice and
893	their mean FST values and relative diversity (Π upland/ Π lowland).
894	
895	Table S4. Genes relevant to GDP (growth, development, and productivity) and
896	drought-resistance based on database of Ricedata (http://www.ricedata.cn/gene/) and their
897	FST and relative Pi ratio (upland/lowland).
898	
899	Table S5. Information of windows included in the analysis of recombinant genotypes.
900	
901	Table S6. Function-proven drought-resistant genes, their FST values and relative diversities,
902	and type of pleiotropy effects. "0" indicates no pleiotropy effect on GDP (growth,
903	development, and productivity) has been reported. "1" indicates it has positive impacts on
904	GDP. "2" indicates opposite impacts of this gene on DR and GDP.
905	
906	Table S7. Plant materials involved in this study and their basic information.
907	
908	Table S8. Basic information of resequenced rice landraces and wild rice accessions.
909	
910	Table S9. Phenotype data of for each rice genotype.
911	
912	Table S10. Four SNPs at LAX1 validated by the Sanger method.
913	
914	Table S11. Information of PCR primers used for Sanger sequencing.
915	



Figure 1. Matrix of Pearson's correlation coefficients (*r*) (left triangle) and *p*-values (right triangle) among drought-resistant and agronomic traits. Abbreviations: NT, no. of tillers; PH, plant height; FLL, flag leaf length; FLW, flag leaf width; RWC, relative water content; RWL, ratio of water loss; 100GW, 100-grain weight; GW, grain weight; HI, harvest index; RF, relative fecundity; R100GW, relative 100-grain weight; RB, relative biomass; RGW, relative grain weight; RDR, ratio of deep-rooting; NDR, no. of deep root; NSR, no. of shallow root; NTR, no. of total root; NRP no. of root per tiller.

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Figure 2. Upland and lowland rice collected from China, their phylogenetic relationships, and the decay of linkage disequilibrium (LD) across the genome. (a) Geographic distributions of upland rice in China. Numbers in red indicate provinces where equivalent numbers of upland and lowland rice landraces were collected. 1 Hebei, 2 Henan, 3 Jiangsu, 4 Hunan, 5 Guangxi, 6 Guizhou, 7 Yunnan. (b) A phylogenetic tree of upland, lowland, and common wild rice. (c) LD decay across the genome in upland and lowland rice.



Figure 3. Genomic differentiation and selection detected between rice ecotypes. (a) The genome-wide manhattan plot of F_{ST} estimated in a 200Kb sliding window with 100Kb stepsize. Bars under the manhattan plot describes distributions of genes relevant to drought-resistance and GDP (growth, development, and productivity). Blue bars indicate windows containing genes of drought-resistance. Green bars indicate windows containing genes of GDP. Red bars indicate windows containing genes of both drought- resistance and GDP. (b) The genome-wide manhattan plot of $Log_2^{([lupland/]lowland)}$ estimated in a 200Kb sliding window with 100Kb step-size. (c) The genome-wide manhattan plot of number of SNPs in the 200Kb sliding window with 100Kb step-size. (d) Mean F_{ST} of DR, GDP relevant, and neutral windows. The bar indicates SE. "***" indicates significance at p<0.001 by independent *t*-test in comparison with neutral windows. (e) Mean F_{ST} of windows with different gradients of $\prod_{upland}/\prod_{lowland}$ ratios. The bar indicates SE. "***" indicates significance at p<0.001 by independent *t*-test in comparison with the genomic average.



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