

*Genome-wide association study reveals candidate genes controlling root system architecture under low phosphorus supply at seedling stage in Brassica napus*

Article

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1 **Genome-wide association study reveals candidate genes controlling**  
2 **root system architecture under low phosphorus supply at seedling**  
3 **stage in *Brassica napus***

4 **Running title: RSA of *B. napus* at LP**

5  
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17

18 **Keywords:** Oilseed rape (*B. napus* L.), root system architecture (RSA), low phosphorus  
19 supply, genome-wide association study (GWAS), haplotype analysis.

20

21 **Abstract**

22 Optimal root system architecture (RSA) is essential for vigorous growth and yield in  
23 crops. Plants have evolved adaptive mechanisms in response to low phosphorus (LP)  
24 stress, and one of those is changes in RSA. Here, more than five million single-  
25 nucleotide polymorphisms (SNPs) obtained from whole genome re-sequencing data  
26 (WGR) of an association panel of 370 oilseed rape (*Brassica napus* L.) were used to  
27 conduct genome-wide association study (GWAS) of RSA traits of the panel at LP in  
28 ‘pouch and wick’ system. Fifty-two SNPs were forcefully associated with lateral root  
29 length (LRL), total root length (TRL), lateral root density (LRD), root number (RN),  
30 mean lateral root length (MLRL) and root dry weight (RDW) at LP. There were

31 significant correlations between phenotypic variation and the number of favorable  
32 alleles of the associated loci on chromosomes A06 (chrA06\_20030601), C03  
33 (chrC03\_3535483) and C07 (chrC07\_42348561), respectively. Three candidate genes  
34 (*BnaA06g29270D*, *BnaC03g07130D* and *BnaC07g43230D*) were detected by  
35 combining transcriptome, candidate gene association analysis and haplotype analysis.  
36 Cultivar carrying “CCGC” at *BnaA06g29270DHap1*, “CAAT” at  
37 *BnaC03g07130DHap1* and “ATC” at *BnaC07g43230DHap1* had greater LRL, LRN  
38 and RDW than lines carrying other haplotypes at LP supply. The RSA of cultivar  
39 harboring the three favorable haplotypes was further confirmed by solution culture  
40 experiments. These findings define exquisite insights into genetic architectures  
41 underlying *B. napus* RSA at LP, and provide valuable gene resources for root breeding.

42

### 43 **Introduction**

44 Oilseed rape (*Brassica napus* L.; *B. napus*) is a globally important oil crop (Chalhoub  
45 et al. 2014). *B. napus* has high phosphorus (P) demand and P deficiency affects root  
46 development and reduces seed yield (Wang et al. 2017; Liu et al. 2021a). As populations  
47 expand around the world, the demand for edible oil is also growing. However, P  
48 fertilizers are derived from non-renewable P rock resources and over application of P  
49 fertilizers are associated with environmental issues, such as eutrophication (Vance et al.  
50 2003; Liu et al. 2015). Therefore, detecting the genetic mechanisms for tolerance to low  
51 P (LP) availability and breeding P-efficient *B. napus* cultivars to reduce P use in  
52 agricultural systems is crucial.

53 Root system architecture (RSA) is the spatial configuration of different types and  
54 ages of roots emerging from a single plant and is important for plant survival (Lynch et  
55 al. 1995). Traditional linkage analysis has identified abundant quantitative trait loci  
56 (QTLs) for root traits in *B. napus* in response to low P availability. A *B. napus* DH  
57 population (*BnaTNDH*) has been constructed from a cross between a P-efficient  
58 cultivar, ‘Ningyou7’, and a P-inefficient cultivar, ‘Tapidor’ (Shi et al. 2013). Thirty  
59 QTLs were detected for primary root length (PRL), total root length (TRL), root number  
60 (RN) and lateral root density (LRD) in an agar-based growth system with P-deficient

61 and P-sufficient supply in *BnaTNDH* population of *B. napus* (Shi et al. 2013). Among  
62 them, QTLs clustered on A03 chromosome associated with LRN and RDW at a low P  
63 supply. Altogether, 131 QTLs were detected for RSA traits using a high-density single-  
64 nucleotide polymorphism (SNP)-based genetic linkage map (Zhang et al. 2016). The  
65 intervals of QTLs for root and shoot biomass overlapped on chromosome A03, and  
66 QTLs for root biomass and lateral root emergence on chromosome A04 and C04, C08  
67 and C09 were co-located. A major QTL explained 18.0% of the phenotypic variation  
68 for lateral root density on chromosome C09 (Zhang et al. 2016).

69 Compared with traditional linkage analysis, GWAS (genome-wide association study)  
70 does not require construction of mapping populations, which can save time (Xiao et al.  
71 2017). For *B. napus*, a 60K SNP chip has been developed for genotyping line in GWAS  
72 studies and was used GWAS analysis of RSA at LP. Two hundred and eighty-five SNPs  
73 were significantly associated with RSA in response to LP by GWAS study involving  
74 405 accessions (Wang et al. 2017). Nine SNPs linked with RSA were consistent with  
75 the published QTL mapping of RSA in the *BnaTNDH* population (Wang et al. 2017;  
76 Zhang et al. 2016). Eleven SNPs were identified by a combined GWAS and QTL  
77 mapping approach, with the SNPs located on A06, A08 and C01 co-located with QTLs  
78 associated with root angle at LP (Duan et al. 2021).

79 GWAS of RSA of *B. napus* under nutrient conditions have also been reported. Thirty-  
80 one significant SNPs were detected for root traits by shovelomics approach in the field  
81 using GWAS analysis with 216 *B. napus* accessions (Arifuzzaman et al. 2019). Two  
82 association mapping panels were used to perform GWAS on root related traits in *B.*  
83 *napus*, and 27 significant SNPs were identified (He et al. 2019). Thirty-two SNPs are  
84 identified as significantly associated with root related traits at five vegetative stages by  
85 a GWAS analysis with 280 *B. napus* accessions, with *BnaA03g52990D*,  
86 *BnaA06g37280D*, and *BnaA09g07580D* identified as candidate genes (Li et al. 2021).

87 Compared with SNP chip genotyping, whole genome re-sequencing (WGR) provides  
88 higher accuracy in mapping the location of recombination events and is capable of  
89 detecting more genetic variants. Recently, WGR technology has been used to analyze  
90 the genetic structure of agronomic traits of *B. napus* including seed oil content (Tang et

91 al. 2021), glucosinolate content (Tan et al. 2022), phytate (Liu et al. 2021a) and low-  
92 temperature tolerance (Luo et al. 2021).

93 In this study, the genetic architecture of RSA at LP in the seeding stage was  
94 investigated by GWAS using a panel of 370 *B. napus* accessions and more than 5  
95 million SNPs. Here we report the (1) significant SNPs and candidate genes linked with  
96 RSA traits at LP and (2) identify the favourable haplotypes for breeding cultivars with  
97 optimal RSA in *B. napus*.

98

## 99 **Materials and methods**

### 100 **Plant materials**

101 The *B. napus* association panel contained 370 cultivars and inbred lines, comprising  
102 327 semi-winter, 37 spring, 4 winter and 2 vegetable types, converged from major *B.*  
103 *napus* breeding centers across China (Table S1).

104

### 105 **Phenotypic investigation**

106 The phenotype data for RSA of the association panel from the ‘pouch and wick’  
107 system of *B. napus* at low P (0 mM Pi) published by Wang et al. (2007) were used in  
108 this study. For individual genotypes, at least 16 replicates were sown. Fourteen days  
109 after sowing on free-P paper, seedling’s root system was detected using a digital single-  
110 lens reflex camera (Canon EOS 1100D, Canon Inc., Tokyo, Japan) with a marker at the  
111 base. Root images were analyzed using RootReader2D (RR2D) and automatically  
112 calculates PRL (primary root length), LRL (lateral root length) and LRN (lateral root  
113 number) (Clark et al. 2013). TRL (total root length) = PRL + LRL, LRD (lateral root  
114 density) = LRN / PRL and MLRL (mean lateral root length) = LRL/LRN as described  
115 by Wang et al. (2017). Root samples were oven-dried at 80°C for two days and dry  
116 biomass were collected. The mean, maximum, range, skewness and coefficient of  
117 variation were calculated using the psych package in R software ([https://cran.r-](https://cran.r-project.org/web/packages/psych/index.html)  
118 [project.org/web/packages/psych/index.html](https://cran.r-project.org/web/packages/psych/index.html)). The correlation coefficients between  
119 phenotypes were calculated by R language.

120 Cultivar L133 with three favourable haplotypes and cultivar L154 without these

121 haplotypes were used to investigate the difference in RSA between them. Seedlings  
122 were grown at sufficient P (SP: 250  $\mu\text{mol L}^{-1}$  P) and low P (LP: 5  $\mu\text{mol L}^{-1}$  P) in  
123 hydroponics in growth chamber (16 h light (7:00 am-23:00 pm)/ 8 h dark regime at  
124 22°C) (Wang et al. 2017). Twenty-four-day-old seedlings were harvested after the  
125 measurement of net photosynthetic rate (Pn). Pn of the youngest fully expanded leaf  
126 was measured between 10 am to 12 am using a portable photosynthesis system (Li6400;  
127 LI-COR, Lincoln, NE, USA). The root was scanned with a modified flatbed scanner  
128 (Epson V700, Nagano-ken, Japan) at 400 dpi. Total root length (TRL, cm), root volume  
129 (RV,  $\text{cm}^3$ ), root area (RA,  $\text{cm}^2$ ) and lateral root number (LRN, N) were determined with  
130 the WinRHIZO program (Regent Instruments Inc., Quebec, Canada). Leaves were  
131 scanned with the scanner and the whole area of leaves was measured using the Image J  
132 software (<http://rsbweb.nih.gov/ij/download.html>). Shoot and root samples were dried  
133 at 80°C for two days for dry biomass measurements.

134

### 135 **Genome-wide association study and candidate gene identification**

136 High-quality SNP markers (more than 10 million) across the *B. napus* association  
137 panel, comprising 403 distinct genotypes, cultivars and inbred lines were obtained  
138 previously (Tang et al. 2021). The SNPs filtered with minor-allele frequency (MAF)  
139 ( $>0.05$ ) and missing rate ( $<0.2$ ), 1.60 million SNPs were obtained for GWAS in this  
140 study. We used the factored spectrally transformed linear mixed models (Fast-LMM)  
141 (<https://www.softpedia.com/get/Science-CAD/FaST-LMM-Set.shtml>). The threshold  
142 for screening significant SNPs in this association panel is set to  $6.25 \times 10^{-07}$  (Liu et al.  
143 2021a; Liu et al. 2021b). Use GGplot2 package ([https://cran.r-](https://cran.r-project.org/web/packages/ggplot2/index.html)  
144 [project.org/web/packages/ggplot2/index.html](https://cran.r-project.org/web/packages/ggplot2/index.html)) in R software to draw Manhattan plot  
145 and CMplot package (<https://cran.r-project.org/web/packages/CMplot/>) to draw  
146 Quantile-Quantile plot. Population structure and kinship was calculated by Admixture  
147 software and Tassel 5.0 software, respectively (Bradbury et al. 2007; Alexander et al.  
148 2009). Analysis of molecular variance (AMOVA) was performed with the arlequin  
149 software (3.5.2.2) with 1000 permutations to statistically examine differences between  
150 populations (Excoffier et al. 2010). PopLDdecay software was used to calculate LD

151 decay value (Zhang et al. 2019). Genes located within 173 kb upstream and downstream  
152 of the peak SNPs were viewed as candidate genes according to the LD decay of the  
153 panel (173 kb).

154

#### 155 **Candidate gene association analysis and haplotype analysis**

156 The coding region and the upstream 2 kb promoter region of candidate genes were  
157 extracted using vcftools software (<https://vcftools.github.io/index.html>, Danecek et al.  
158 2011). Candidate gene association analysis of *BnaA06g29270D*, *BnaC03g07130D* and  
159 *BnaC07g43230D* were performed with Tassel 5.0 and LDBlockShow software  
160 (Bradbury et al. 2007; Dong et al. 2021). Haploview.4.2 software was used for  
161 haplotype analysis (Barrett et al. 2005). Further comparative analysis was conducted  
162 based on haplotypes containing at least 10 *B. napus* accessions. To compared the  
163 differences in the haplotypes of RSA, student's t-test was applied.

164

#### 165 **Expression profile of putative candidate genes in P efficient *B. napus* cultivar**

166 RNA was extracted using the EastepR super total RNA extraction kit (Promega,  
167 Madison, WI). RNA concentration was quantified using the NanoDropND-1000  
168 spectrophotometer (Thermo Scientific). One  $\mu\text{g}$  RNA was convert into cDNA with the  
169 ReverTra Ace qPCR RT master mix with gDNA remover (TOYOBO, Osaka, Japan).  
170 cDNA samples were diluted at 1:10 in ddH<sub>2</sub>O. RT-qPCR was performed in 10  $\mu\text{L}$   
171 reaction volumes with gene specific primers (Table S2), and conducted with a  
172 QuantStudio™ 6 Flex System (Applied Biosystems, Foster City, CA) using the SYBR  
173 Green Real-Time PCR Master Mix Kit (TOYOBO). The RT-qPCR was performed as  
174 following: 95°C for 5 min followed by 40 cycles of 95°C for 10 s, 55°C for 20 s, and  
175 72°C for 20 s, and a subsequent standard dissociation protocol to validate the presence  
176 of a unique PCR product. Each sample was analyzed in three technical replicates, and  
177 the  $2^{-\Delta\Delta\text{CT}}$  method was applied to calculate the relative expression from the normalizing  
178 of two housekeeping genes (*Tubulin* and *Actin2*). Primer sequences are listed in Table  
179 S2.

180



## 181 **Transcriptome sequencing**

182 The transcriptome data of P deficient *B. napus* cultivars 'Eyou changjia' at LP and SP  
183 conditions have been published (Du et al. 2017). Fifteen-day old seedlings were grown  
184 at 250  $\mu$ M Pi (SP) and P-free nutrient solution (LP, 0  $\mu$ M P) for 10 days. The roots and  
185 leaves were harvested with three biological replicates. Twelve RNA samples were  
186 subjected to the Illumina HiSeq 2000 platform (Illumina, USA). Data analysis was  
187 performed as described in Du et al. (2017).

188

## 189 **Statistical analysis**

190 Data were presented as means  $\pm$  SEM. Student's *t* test using Excel software  
191 (Microsoft, Redmond, WA) and SigmaPlot (SPSS Science Inc., IL) were applied for  
192 comparisons between samples. The asterisks indicate significant differences between  
193 samples at the \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.0001, \*\*\*\**P* < 0.0001 level using the *t*  
194 test. The phenotypic data of root traits were analyzed by restricted maximum likelihood  
195 (REML) programs to evaluate the line means and sources of variation (Shi et al. 2013).  
196 Means were approximated using the [Genotype] term as a fixed factor, retaining  
197 [(Replicate/Side of paper/Tray/Frame/Room)] as random factors. The sources of  
198 variation were estimated using the Random terms and no defined fixed factor. Statistical  
199 analysis was performed by GenStat15<sup>th</sup> (VSN International, Oxford, UK).

200

## 201 **Results**

### 202 **Phenotypic variation for root system architecture (RSA) of an association panel of** 203 ***B. napus* at LP supply**

204 The phenotype data for RSA of 405 genotypes in the association panel of *B. napus*  
205 at low P published by Wang et al. (2017) and the re-sequencing of 370 cultivars (Tang  
206 et al. 2021) from the 405 were used in this study. Extensive phenotypic variations for  
207 TRL, LRD, LRN, MLRL and RDW were detected in the association panel at LP in  
208 'pouch and wick' system (Fig. 1; Table S3). At LP, the LRL varied from 15.49 to 53.41  
209 cm with a coefficient of variation of 24.26% and a mean value of 33.72 cm (Table 1).  
210 The LRN varied from 7.5 to 24.8 per plant with a coefficient of variation of 23.27%

211 and a mean value of 16.2 per plant (Table 1). In addition, the skewness values of traits  
212 were close to 0, which indicates that these traits conform to a normal distribution and  
213 were suitable for GWAS analysis (Table 1). The correlation coefficient ( $r$ ) between LRL  
214 and TRL, TRL and LRN, TRL and RDW, LRN and RDW were 0.97, 0.85, 0.73 and  
215 0.77, respectively (Fig. S1).

216

### 217 **Population structure, relative kinship and LD decay**

218 To identify genes involved in RSA in response to low P availability, we used  
219 published genotype data consisting of more than 1.6 million high-quality SNPs for the  
220 370 accessions (Tang et al. 2021) to conduct a GWAS based on the six RSA traits at  
221 low P in the ‘pouch and wick’ system. When  $r^2$  was 0.1, the LD decay in this 370 *B.*  
222 *napus* association panel was 173 kb (Fig. S2). Quality control for the imputed data was  
223 conducted using Plink software, where SNPs were removed if they had a minor allele  
224 frequency lower than 0.2. The number of markers retained for subsequent analyses was  
225 417,787. Population was divided into four subgroups (Fig. S3). There were significant  
226 differences between the  $K = 4$  assigned populations ( $P < 0.01$ , Table S4). The kinship  
227 heatmap shows that most cultivars are distantly related, which showed that this  
228 association panel was suitable for GWAS analysis (Fig. S4). A total of 433,432 LD  
229 blocks were identified in the whole genome (Table S5), and the majority of LD blocks  
230 were less than 10 kb, and only 15 LD blocks were larger than 100 kb (Fig. S5). These  
231 data indicated that the genetic distance of the accessions in the association panel was  
232 appropriate for GWAS analysis.

233

### 234 **Genome-wide association study of RSA related traits of *B. napus* at LP supply**

235 GWAS for all six RSA traits was performed using the Fast-LMM. The quantile-  
236 quantile plots indicated that the model of the six RSA could be used to detect association  
237 signals (Fig. 2). Fifty-two SNPs were confirmed to be significantly associated with  
238 these RSA traits at LP with a  $P < 6.25 \times 10^{-07}$  in *B. napus* (Fig. 2; Table S6). Of the 52  
239 SNPs, 18 were located in the LD block interval (Table S7). Among them, 6, 4, 12, 6, 3  
240 and 21 SNPs were significantly associated with LRL, TRL, LRD, LRN, MLRL and

241 RDW, respectively, and were distributed on 12 chromosomes, explaining between 7.35  
242 and 12.37% of the phenotypic variance explained (PVE) (Fig. 2; Table S6).  
243 Chromosome C07 had the largest number of significant SNPs (11) and chromosome  
244 A01 had the least number of significant SNPs (1) (Fig. 2; Table S6). Four SNPs on  
245 chromosomes C03 and C07 (chrC03\_3535476, chrC03\_3535483, chrC07\_42348561  
246 and chrC07\_42348533) were significantly associated with RSA and were consistently  
247 associated with two RSA traits (Table S6). SNP chrC03\_3535476 was associated with  
248 LRL and TRL, and explained 9.82% and 9.09% of the PVE, respectively (Table S6).  
249 SNP chrC07\_42348561 was associated with LRN and RDW, and explained 8.99% and  
250 9.54% of the PVE, respectively (Table S6). In addition, 42 of the 52 significant SNPs  
251 were located in the 18 LD blocks in this study. These results indicated that RSA traits  
252 such as LRL were highly correlated with TRL, and LRN was highly correlated with  
253 RDW (Fig. S1).

254

#### 255 **Prediction of candidate genes for RSA at LP supply in *B. napus***

256 The lead SNP chrC03\_3535483 ( $P=2.37E-07$ ,  $PVE=10.19\%$ ) on C03 was  
257 significantly associated with LRL and TRL, and may be a major genetic locus  
258 responsible for RSA in *B. napus* at LP (Fig. 2; Table S6). LD decay was 173 kb for this  
259 370 *B. napus* association panel (Fig. S2). Based on the LD decay, 93 candidate genes  
260 were explored in the 173 kb up/down-stream of the significant SNP (chrC03\_3535483)  
261 (Table S8). The transcriptome data of P-efficient *B. napus* cultivars ‘Eyou changjia’  
262 under LP and SP conditions (Li et al. 2019) were used to determine differential  
263 expression of candidate genes identified by GWAS analysis. Transcript abundance of  
264 *BnaC03g07130D* was significantly increased at LP in both shoots and roots, and was  
265 also evidenced by qRT-PCR (Table 2; Fig. 3A). Lead SNP (chrC07\_42348561,  $P =$   
266  $1.82E-07$ ,  $PVE = 9.54\%$ ) on C07 was associated with both LRN and RDW at LP (Fig.  
267 2; Table S6). Based on the LD decay, 76 candidate genes were detected (Table S8).  
268 Transcriptome data and qRT-PCR analysis showed that the transcript of  
269 *BnaC07g43230D* was induced by P deficiency in shoots and roots (Table 2; Fig. 3B).

270 In addition, one region significantly associated with LRD on chromosome A06

271 ranged from 19.93 to 20.03 Mb (Fig. 2; Table S6). Seventy genes were detected  
272 underlying the region around lead SNP chrA06\_20030601, and two of the candidate  
273 genes were significantly expressed by LP (Table 2; Table S8). The expression levels of  
274 *BnaA06g29270D* in shoots and roots were significantly decreased at LP supply by qRT-  
275 PCR analysis (Table 2; Fig. 3C). Twenty-four SNPs were located within the 2 kb  
276 promoter region and the entire coding region of *BnaA06g29270D*. However, no SNPs  
277 were identified within the corresponding region of *BnaA06g29530D* (Table 2).  
278 Therefore, *BnaA06g29270D* was predicted to be the candidate gene for further study.

279

### 280 **Candidate gene association study and haplotype analysis of BnaA06g29270D,** 281 **BnaC03g07130D and BnaC07g43230D**

282 To further understand the intragenic variation affecting the phenotypic values and  
283 identify the favorable alleles and haplotypes, we extracted the SNPs within the entire  
284 coding regions and promoters (upstream, 2 kb) of three candidate genes  
285 *BnaC03g07130D*, *BnaA06g29270D* and *BnaC07g43230D*, respectively. Thirty-seven  
286 SNPs were identified in *BnaC03g07130D*, and 26 in *BnaC07g43230D* and 24  
287 *BnaA06g29270D* (Fig. 4-6).

288 Ten and four SNPs in *BnaC03g07130D* were significantly associated with LRL and  
289 TRL, respectively (Fig. 4A; 3G). Among the ten significant SNPs, four were located in  
290 the promoter, five in the exon and one in the intron region (Table 3). The five SNPs  
291 identified in the exons resulted in synonymous mutations, which were unlikely to  
292 influence the function of *BnaC03g07130D* protein (Table 3). Further, the ‘C’ allele of  
293 chrC03\_3396486, ‘A’ allele of chrC03\_3396712, ‘A’ allele of chrC03\_3396812 and ‘T’  
294 allele of chrC03\_3396932 located in the promoter of *BnaC03g07130D* were all  
295 identified as strong alleles associated with LRL and TRL (Fig. 4B-E, H-K). Two major  
296 haplotypes were detected, among which *BnaC03g07130Hap1* (CAAT) had higher LRL  
297 and TRL, while *BnaC03g07130Hap2* (“TGCC”) had lower LRL and TRL ( $P = 0.0002$ )  
298 (Fig. 4F, L).

299 For candidate gene *BnaC07g43230D* three SNPs were associated with the LRN and  
300 RDW (Fig. 5A, F), and the ‘A’ allele of chrC07\_42368601, ‘T’ allele of

301 chrC07\_42368638 and ‘C’ allele of chrC07\_42368650 were the positive alleles for root  
302 development at LP (Fig. 5B-D, G-I). Two major haplotypes were identified, with  
303 *BnaC07g43230Hap1* (“ATC”) having significantly greater LRN and RDW than  
304 *BnaC07g43230Hap2* (“TCT”) (Fig. 5E, J).

305 For candidate gene *BnaA06g29270D*, 11 SNPs were significantly associated with the  
306 LRD, and had strong LDs with each other (Fig. 6A; Table 3). Among the 11 significant  
307 SNPs, 3, 6 and 2 were located in the promoter, exon and intron region, respectively  
308 (Table 3). Five SNPs (chrA06\_19976766, chrA06\_19976785, chrA06\_19976887,  
309 chrA06\_19977025 and chrA06\_19977610) were in the exon region, but were  
310 synonymous mutations which resulted in no amino acid changes (Table 3). The SNP of  
311 chrA06\_19977004 (C/A) was located in the exon region of the gene *BnaA06g29270D*  
312 and resulted in amino acid change from cysteine to a stop codon, and might contribute  
313 to the phenotypic difference in LRD (Table 3). The ‘C’ allele of chrA06\_19975674, ‘C’  
314 allele of chrA06\_19975707, ‘G’ allele of chrA06\_19975749 and ‘C’ allele of  
315 chrA06\_19977004 were associated with higher LRD (Fig. 6B-E). Two major  
316 haplotypes were identified, with *BnaA06g29270Hap1* (“CCGC”) having significantly  
317 higher LRD than *BnaA06g29270Hap2* (“TACA”) ( $P < 0.0001$ ) (Fig. 6F).

318 A total of 73 *B. napus* cultivars in this association panel contained these three  
319 favorable haplotypes. As expected, RSA (such as, LRL, TRL, LRN and RDW) were  
320 significantly higher in the 73 cultivars than in the other 297 cultivars at low P (Fig. 7).  
321 To better understand the relative influence of the three favorable haplotypes on the root  
322 and shoot growth of *B. napus*, a cultivar harboring the three favorable haplotypes (L133)  
323 and a cultivar with none of these haplotypes (L154) with extremely different in root  
324 system architecture traits at low P (Figure 7) were chose to dissect root system  
325 architecture, biomass, whole leaf area and net photosynthesis at both sufficient P and  
326 low P (Fig. 8). TRL and WLA (whole leaf area) were higher in L133 cultivar than in  
327 L154 cultivar at SP (Fig. 8A, B, H). P deficiency decreased the RSA (TRL, RV, RA,  
328 LRN and RDW), shoot dry weight (SDW) and WLA significantly and resulted in  
329 decreased net photosynthesis rate. Moreover, L154 cultivar had lower RSA, SDW,  
330 WLA and NP than L133 cultivar at LP (Fig. 8). L154 was more sensitive to P deficiency

331 than L133.

332

### 333 **Discussion**

#### 334 **Advantages of WGR for genotyping in GWAS**

335 GWAS is a powerful method for connecting identified phenotypic differences and  
336 the underlying causative loci (Korte et al. 2013). Currently, SNP chips and WGR are  
337 widely employed genotyping methods to identify genetic variants within genotypes  
338 combined with GWAS. By far, more than half of GWAS in *B. napus* were performed  
339 based on 60K SNP chip (Liu et al. 2022). As the continuous development of sequencing  
340 technology and the continuous reduction in costs, WGR has become a routine method  
341 to identify genetic variants. Compared with SNP chip, WGR data have more genetic  
342 loci and cover the whole genome (Liu et al. 2022). In *B. napus*, a total of 24,403 SNPs  
343 are selected to perform association mapping of flowering time (Jun et al. 2019), and  
344 11,804 SNPs were used (Raman et al. 2019), which all the two panel were genotyped  
345 with a 60 K SNP array. However, 5.56 million SNPs were confirmed used for  
346 association analyses by WGR, and with identified 22 SNPs linked with 37 genes were  
347 associated with flowering time in *B. napus* (Wu et al. 2019). A total of 26,841 SNPs  
348 were selected by SNP array to perform association mapping of seed weight in *B. napus*,  
349 and detected a major QTL on chromosome A09 (Li et al. 2014). While, 690,953 SNPs  
350 high quality were used by WGR and 20 SNPs were detected to associate with seed  
351 weight in *B. napus* (Dong et al. 2018).

352 Previously, a total of 19,397 SNPs with minor allele frequency (MAF) > 0.05 were  
353 selected to conduct association analyses of RSA in *B. napus* at LP with the panel of *B.*  
354 *napus* lines genotyped with a 60 K *Brassica* Infinium SNP array(Wang et al. 2017).  
355 However, in this study, a total of 1.6 million higher quality SNPs (MAF > 0.2) identified  
356 by WGR were used for association analyses. As the 60 K SNP chip contains fewer SNPs  
357 than WGR, some SNPs that are significantly associated with the target traits could not  
358 be detected in the previous association analysis. A total of 52 significant SNPs  
359 associated with RSA at LP were identified on 12 of the 19 *B. napus* chromosomes,  
360 explaining a higher average of PVE (9.14%; Table S6) in this study than observed by

361 60 K SNP chip (an average of 6.09% PVE) (Wang et al. 2017). In this study, 12  
362 significant SNPs detected by WGR for RSA were adjacent to previously reported  
363 significant SNPs detected by 60 K SNP chip, proving the accuracy of the results of  
364 GWAS in this study (Table S9). For example, significant SNP chrA03\_26604503  
365 located at 26604503 bp on chromosome A03 was associated with RDW in this study,  
366 and SNP Bn-A03-p28127216 located at 26598176 bp on chromosome A03 was also  
367 associated with RDW by Wang et al. (2017) (Table S9).

### 368 **Favorable haplotype of candidate genes for RSA of *B. napus* at LP**

369 GWAS of six RSA traits (LRL, TRL, LRD, LRN, MLRL and RDW) identified a total  
370 of 52 significant SNPs in *B. napus* at LP (Fig. 2; Table S6), and a total of 184 candidate  
371 genes were assessed based on the lead SNPs (Table S8). We did not compare the SNPs  
372 for RSA identified at LP and SP as the RSA of the association panel of *B. napus* at high  
373 P was not investigated in Wang et al. (2017). Some significant SNPs, candidate genes  
374 and haplotypes identified at low P might also have a function under sufficient P. Using  
375 GWAS data in combination with transcriptome data to mine candidate genes has  
376 become a conventional method in *B. napus*, such as harvest index (Lu et al. 2017), seed  
377 oil content (Tang et al. 2021), flowering time (Huang et al. 2021) and glucosinolate  
378 content (Kittipol et al. 2019). Based on our previously published transcriptome  
379 sequencing of the root and shoot of P-efficient cultivar ('Eyouchangjia') at SP and LP,  
380 four significantly differentially expressed genes were detected at LP in close proximity  
381 to the significant SNPs associated with RSA traits (Table 2). Gene transcripts of  
382 *BnaC03g07130D* and *BnaC07g43230D* were significantly increased, and the transcript  
383 of *BnaA06g29270D* was significantly decreased by Pi deficiency (Table 2; Fig. 3).  
384 These candidate genes might play an important part in root development of *B. napus* at  
385 a deficiency P supply. In this study, *BnaA06g29270D* was located within the interval of  
386 the SNP chrA06\_20030601 for LRD (Fig. 2). *BnaA06g29270D* is homologous to  
387 *Arabidopsis At5g28770*, basic leucine zipper 63 (*bZIP63*), which was phosphorylated  
388 by Snf1-related-kinase1 (SnRK1) and then activates the promoter of AUXIN  
389 RESPONSE FACTOR 19 (ARF19), promoting LR emergence and subsequently LRD  
390 (Muralidhara et al. 2021). In *Arabidopsis*, *bzip63* mutants have increased PRL and

391 emerged LRD (defined as the number of LRs per primary root length) than wild type  
392 plants under control conditions (Muralidhara et al. 2021). These data support the role  
393 *BnaA06g29270D* in controlling LR density in *B. napus*.

394 *BnaC03g07130D*, a gene that encodes a reversibly glycosylated polypeptide 2  
395 (RGP2), was located within the interval of the SNP chrC03\_3535483 and linked with  
396 the trait for LRL and TRL (Fig. 2; Table S6). In castor oilseeds (*Ricinus communis*),  
397 sucrose synthase (SUS) interacts with RGP1 in roots, which suggests that these proteins  
398 interact to directly channel UDPG derived toward RGP glycosyl chain initiation and  
399 extension (Fedosejevs et al. 2017). Further study of the protein interaction between SUS  
400 and RGP in root development will be conducive to a better understanding of RSA and  
401 root carbon balance.

402 *BnaC07g43230D* was identified by significant SNP chrC07\_42348561, which was  
403 likely to influence the LRN and RDW at LP (Fig. 2; Table S6). *BnaC07g43230D* is an  
404 AIG2-like (avirulence induced gene) family protein and its function is unknown. In  
405 *Arabidopsis*, FIT (FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION  
406 FACTOR) is a key regulator of iron uptake in root. *AtAIG2*-like was detected by iron  
407 deficiency in the roots of proteomic and transcriptomic study of WT, *fit* knock-out  
408 mutant and FIT overexpression lines (Mai et al. 2015). Primary root extension is  
409 inhibited and lateral root formation is stimulated at Pi deprivation in members of the  
410 Brassicaceae family in agar system. Studies have confirmed external Pi sensing at the  
411 root tips depends on Fe availability by hormone and peptide signaling pathways (Ward  
412 et al., 2008; Ticconi et al., 2009; Müller et al., 2015; Singh et al., 2018). The function  
413 of this *BnaAIG2*-like protein may is likely to be involved in RSA at LP and should be  
414 examined in further studies.

415 Candidate gene association analysis is widely used to detect favorable haplotypes of  
416 candidate genes. Favorable haplotypes of candidate gene *BnaC02.GTR2* associated  
417 with seed glucosinolate content in *B. napus* (Tan et al. 2022) and the favorable  
418 haplotype of candidate gene *BnaA09.MRP5*, which influenced rapeseed phytate content  
419 (Liu et al. 2021b) have been reported previously. Additionally, overexpressing  
420 favorable haplotype of *BnaA03.NIP5* increased low boron tolerance in boron inefficient



421 cultivar of *B. napus* (He et al. 2021).

422 The favorable haplotypes of *BnaA06g29270D*, *BnaC03g07130D* and  
423 *BnaC07g43230D* were determined as “CCGC”, “CAAT” and “ATC” by candidate gene  
424 association analysis, respectively (Fig. 4-6). Furthermore, 73 *B. napus* cultivars  
425 containing three favorable haplotypes have higher LRL, LRN, RDW, and TRL than  
426 other *B. napus* cultivars (Fig. 7). L133, the cultivar containing three favorable  
427 haplotypes showed more tolerance to Pi starvation than L154, the cultivar which did  
428 not have these haplotypes confirm the root breeding value of these favorable haplotypes.

429

### 430 **Conclusions**

431 A total of 52 significant SNPs were significantly associated with RSA at LP by  
432 GWAS based on WGR of an association panel of *B. napus*. “CCGC”, “CAAT” and  
433 “ATC” were identified to be favorable haplotypes in candidate genes, which could be  
434 used for molecular marker-assisted breeding of optimal RSA in response to low P  
435 availability in *B. napus*. In addition, gene editing and modification can be applied to  
436 reveal the function and the underlying mechanism of these candidate genes.

437

### 438 **Data availability statement**

439 The original contributions presented in this study are included in the  
440 article/Supplementary Material, further inquiries can be directed to the corresponding  
441 author.

442

### 443 **Author contribution**

444 **Pan Yuan, Haijiang Liu and Lei Shi** designed research, reviewed the writing and  
445 drafted the manuscript. **Pan Yuan, Haijiang Liu and Xiaohua Wang** participated the  
446 experiments. **John P. Hammond** participated in manuscript revision.

447

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453

#### 454 **Data availability**

455 Raw sequencing data of genome re-sequencing are available in the Genome Sequence  
456 Archive (<https://bigd.big.ac.cn/gsa/>) with Bio-project IDs PRJCA002835 and  
457 PRJCA002836. All the materials in this study are available upon request.

458

#### 459 **Declarations**

460 **Ethics approval and consent to participate** Not applicable.

461 **Consent for publication** Not applicable.

462 **Competing interests** The authors declare no competing interests.

463

#### 464 **Figure legend**

465 **Fig. 1** Distribution of RSA (root system architecture) phenotypes in 370 *B. napus*  
466 accessions. Histograms of the distribution of LRL (A), TRL (B), LRD (C), RN (D),  
467 MLRL (E) and RDW (F) measured at low phosphorus supplies. LRL, lateral root length;  
468 TRL, total root length; LRD, lateral root density; RN, lateral root number; MLRL; mean  
469 lateral root length; RDW, root dry weight.

470 **Fig. 2** Quantile-quantile (QQ) and Manhattan plots for the RSA (root system  
471 architecture) by genome-wide association study (GWAS). GWAS of LRL (A), MLRL  
472 (B), TRL (C), RN (D), LRD (E) and RDW (F) in 370 *B. napus* at low phosphorus  
473 supplies by Fast-LMM model. LRL, lateral root length; MLRL, mean lateral root length;  
474 TRL, total root length; RN, lateral root number; LRD, lateral root density; RDW, root  
475 dry weight.

476 **Fig. 3** Relative expression level of three candidate genes (A, *BnaA06g29270D*; B,  
477 *BnaC03g07130D* and C, *BnaC07g43230D*) under low phosphorus and sufficient  
478 phosphorus conditions. LP, low phosphorus; SP, sufficient phosphorus. Data are means  
479  $\pm$  SEM (n=4). Asterisks indicate the significance of Student's t-test (\*P < 0.05, \*\*\*P  
480 < 0.0001, \*\*\*\*P < 0.0001).

481 **Fig. 4** Candidate gene association and haplotypes analysis of *BnaC03g07130D* with  
482 LRL (lateral root length) and TRL (total root length). (A) Candidate gene association  
483 analysis of *BnaC03g07130D* with LRL. (B-E) Association of the two alleles in  
484 chrC03\_3396486 (B), chrC03\_3396712 (C), chrC03\_3396812 (D) and  
485 chrC03\_3396932 (E) with LRL, respectively. (F) Two haplotypes of *BnaC03g07130D*.  
486 (G) Candidate gene association analysis of *BnaC03g07130D* with TRL. (H-K)  
487 Association of the two alleles in chrC03\_3396486 (H), chrC03\_3396712 (I),  
488 chrC03\_3396812 (J) and chrC03\_3396932 (K) with TRL, respectively. (L) Two  
489 haplotypes of *BnaC03g07130D*. The number of inbred lines harbouring the  
490 corresponding allele is shown in brackets at the bottom.

491 **Fig. 5** Candidate gene association and haplotypes analysis of *BnaC07g43230D* with  
492 LRN (lateral root number) and RDW (root dry weight). (A) Candidate gene association  
493 analysis of *BnaC07g43230D* with LRN. (B-D) Association of the three alleles in  
494 chrC07\_42368601 (B), chrC07\_42368638 (C) and chrC07\_42368650 (D) with LRN,  
495 respectively. (E) Two haplotypes of *BnaC07g43230D*. (F) Candidate gene association  
496 analysis of *BnaC07g43230D* with LRN. (G-I) Association of the three alleles in  
497 chrC07\_42368601 (G), chrC07\_42368638 (H) and chrC07\_42368650 (I) with RDW,  
498 respectively. (J) Two haplotypes of *BnaC07g43230D*. The number of inbred lines  
499 harbouring the corresponding allele is shown in brackets at the bottom.

500 **Fig. 6** Candidate gene association and haplotypes analysis of *BnaA06g29270D* with  
501 LRD (lateral root density). (A) Candidate gene association analysis of *BnaA06g29270D*  
502 with LRD. (B-E) Association of the four alleles in chrA06\_19975674 (B),  
503 chrA06\_19975707 (C), chrA06\_19975749 (D) and chrA06\_19977004 (E) with LRD,  
504 respectively. (F) Two haplotypes of *BnaA06g29270D*. The number of inbred lines  
505 harbouring the corresponding allele is shown in brackets at the bottom.

506 **Fig. 7** Differences in root system architecture at LP between *B. napus* cultivars with  
507 three favorable haplotypes (CCGC, CAAT and ATC) and *B. napus* cultivars without the  
508 three favorable haplotypes in the association panel. Favorable haplotypes of LRL (A),  
509 TRL (B), LRD (C), RN (D), MLRL (E) and RDW (F) were analysed. (73) represents  
510 73 cultivars with the three favorable haplotypes; (297) represents the rest of the

511 association panel after removing the above 73 cultivars. LRL, lateral root length; TRL,  
512 total root length; LRD, lateral root density; RN, lateral root number; MLRL, mean  
513 lateral root length; RDW, root dry weight.

514 **Fig. 8** Differences in root system architecture, biomass, whole leaf area and net  
515 photosynthesis between *B. napus* cultivar (L133) with three favorable haplotypes  
516 (CCGC, CAAT and ATC) and *B. napus* cultivar (L154) without the three favorable  
517 haplotypes (TGCC, TACA and TCT). (A) Root growth of L133 and L154 at LP for 14  
518 d in the 'pouch and wick' system. (B) TRL. (C) RV. (D) RA. (E) LRN. (F) RDW. (G)  
519 SDW. (H) WLA. (I) NP. LP, low phosphorus; TRL, total root length; RV, root volume;  
520 RA, root area; LRN, lateral root number; RDW, root dry weight; SDW, shoot dry weight;  
521 WLA, whole leaf area; NP, net photosynthesis. Data are means  $\pm$  SEM (n=6).  
522 Asterisks indicate the significance of Student's t-test (\*P < 0.05, \*\*P < 0.01, \*\*\*P <  
523 0.0001, \*\*\*\*P < 0.0001). Scale bar is 2 cm in (A).

524

## 525 **Supplementary material**

526 The following supplemental materials are available.

527 **Fig. S1.** Correlation of eight root related traits at low phosphorus supplies.

528 **Fig. S2.** The LD decay of an association panel of *B. napus*.

529 **Fig. S3.** Population structure of an association panel of *B. napus* with K from 2 to 8.

530 **Fig. S4.** The kinship of an association panel of 370 *B. napus* accessions.

531 **Fig. S5.** Distribution of linkage disequilibrium block sizes across all chromosomes.

532 **Table S1.** List of 370 accessions of *B. napus* used in the study.

533 **Table S2.** Primers used for qRT-PCR.

534 **Table S3.** Root related traits at low phosphorus supplies in an association panel of *B.*  
535 *napus*.

536 **Table S4.** AMOVA analysis between the K = 4 assigned populations in *Brassica napus*.

537 **Table S5.** Linkage disequilibrium block in this study.

538 **Table S6.** Significant SNP loci for root related traits of *B. napus* by genome wide  
539 association study at low phosphorus supplies.

540 **Table S7.** LD blocks harboring significant SNPs associated with RSA.

541 **Table S8.** Candidate genes within LD decay value up and down the lead SNPs  
542 (chrA06\_19934701, chrC03\_3535476 and chrC07\_42348526) for root related traits.

543 **Table S9.** Comparison of SNPs detected by WGR for RSA in this study with previously  
544 identified SNPs by 60 K SNP chip for RSA at a low phosphorus supply.

545

## 546 **References**

547 Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry  
548 in unrelated individuals. *Genome Res* 19 (9):1655-1664.

549 <https://doi.org/10.1101/gr.094052.109>

550 Arifuzzaman M, Oladzadabbasabadi A, McClean P et al (2019) Shovelomics for  
551 phenotyping root architectural traits of rapeseed/canola (*Brassica napus* L.) and  
552 genome-wide association mapping. *Mol Genet Genomics* 294 (4):985-1000.

553 <https://doi.org/10.1007/s00438-019-01563-x>

554 Barrett JC, Fry B, Maller J et al (2005) Haploview: analysis and visualization of LD  
555 and haplotype maps. *Bioinformatics* 21 (2):263-265.

556 <https://doi.org/10.1093/bioinformatics/bth457>

557 Bradbury PJ, Zhang Z, Kroon DE et al (2007) TASSEL: software for association  
558 mapping of complex traits in diverse samples. *Bioinformatics* 23 (19):2633-

559 2635. <https://doi.org/10.1093/bioinformatics/btm308>

560 Chalhoub B, Denoeud F, Liu SY et al (2014) Early allopolyploid evolution in the post-  
561 Neolithic *Brassica napus* oilseed genome. *Science* 345 (6199):950-953.

562 <https://doi.org/10.1126/science.1253435>

563 Clark RT, Famoso AN, Zhao K et al (2013) High-throughput two-dimensional root  
564 system phenotyping platform facilitates genetic analysis of root growth and  
565 development. *Plant Cell Environ* 36 (2):454-466.

566 <https://doi.org/10.1111/j.1365-3040.2012.02587.x>

567 Danecek P, Auton A, Abecasis G et al (2011) The variant call format and VCFtools.  
568 *Bioinformatics* 27 (15):2156-2158.

569 <https://doi.org/10.1093/bioinformatics/btr330>

570 Dong HL, Tan CD, Li YZ et al (2018) Genome-wide association study reveals both

571 overlapping and independent genetic loci to control seed weight and silique length  
572 in *Brassica napus*. Front Plant Sci 9:921. <https://doi.org/10.3389/fpls.2018.00921>  
573 Dong SS, He WM, Ji JJ et al (2021) LDBlockShow: a fast and convenient tool for  
574 visualizing linkage disequilibrium and haplotype blocks based on variant call  
575 format files. Brief Bioinform 22 (4). <https://doi.org/10.1093/bib/bbaa227>  
576 Du HY, Yang C, Ding GD (2017) Genome-wide identification and characterization of  
577 SPX domain-containing members and their responses to phosphate deficiency  
578 in *Brassica napus*. Front Plant Sci 8. <https://doi.org/10.3389/fpls.2017.00035>  
579 Duan XJ, Wang XH, Jin KM et al (2021) Genetic dissection of root angle of *Brassica*  
580 *napus* in response to low phosphorus. Front Plant Sci 12.  
581 <https://doi.org/10.3389/Fpls.2021.697872>  
582 Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to  
583 perform population genetics analyses under Linux and Windows. Mol Ecol  
584 Resour 10 (3):564-567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>  
585 Fedosejevs ET, Liu LNC, Abergel M et al (2017) Coimmunoprecipitation of reversibly  
586 glycosylated polypeptide with sucrose synthase from developing castor oilseeds.  
587 Febs Lett 591 (23):3872-3880. <https://doi.org/10.1002/1873-3468.12893>  
588 He ML, Wang SL, Zhang C et al (2021) Genetic variation of *BnaA3.NIP5;1* expressing  
589 in the lateral root cap contributes to boron deficiency tolerance in *Brassica*  
590 *napus*. Plos Genet 17 (7). <https://doi.org/10.1371/journal.pgen.1009661>  
591 He YJ, Hu DX, You JC et al (2019) Genome-wide association study and protein network  
592 analysis for understanding candidate genes involved in root development at the  
593 rapeseed seedling stage. Plant Physiol 137:42-52.  
594 <https://doi.org/10.1016/j.plaphy.2019.01.028>  
595 Huang LY, Min Y, Schiessl S et al (2021) Integrative analysis of GWAS and  
596 transcriptome to reveal novel loci regulation flowering time in semi-winter  
597 rapeseed. Plant Sci 310. <https://doi.org/10.1016/j.plantsci.2021.110980>  
598 Jan HU, Guan M, Yao M et al (2019) Genome-wide haplotype analysis improves trait  
599 predictions in *Brassica napus* hybrids. Plant Sci 283:157-164.  
600 <https://doi.org/10.1016/j.plantsci.2019.02.007>

601 Kittipol V, He ZS, Wang LH et al (2019) Genetic architecture of glucosinolate variation  
602 in *Brassica napus*. J Plant Physiol 240.  
603 <https://doi.org/10.1016/J.Jplph.2019.06.001>

604 Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS:  
605 a review. Plant Methods 9. <https://doi.org/10.1186/1746-4811-9-29>

606 Li F, Chen B, Xu K et al (2014) Genome-wide association study dissects the genetic  
607 architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.).  
608 DNA Res 21 (4):355-367. <https://doi.org/10.1093/dnares/dsu002>

609 Li KQ, Wang J, Kuang LQ et al (2021) Genome-wide association study and  
610 transcriptome analysis reveal key genes affecting root growth dynamics in  
611 rapeseed. Biotechnol Biofuels 14 (1). [https://doi.org/10.1186/s13068-021-](https://doi.org/10.1186/s13068-021-02032-7)  
612 [02032-7](https://doi.org/10.1186/s13068-021-02032-7)

613 Li Y, Wang X, Zhang H et al (2019) Molecular identification of the phosphate  
614 transporter family 1 (PHT1) genes and their expression profiles in response to  
615 phosphorus deprivation and other abiotic stresses in *Brassica napus*. PLoS One  
616 14 (7). <https://doi.org/10.1371/journal.pone.0220374>

617 Liu HJ, Wang JC, Zhang BB et al (2021a) Genome-wide association study dissects the  
618 genetic control of plant height and branch number in response to low-  
619 phosphorus stress in *Brassica napus*. Ann Bot 128 (7):919-929.  
620 <https://doi.org/10.1093/aob/mcab115>

621 Liu HJ, Li XJ, Zhang QW et al (2021b) Integrating a genome-wide association study  
622 with transcriptomic data to predict candidate genes and favourable haplotypes  
623 influencing *Brassica napus* seed phytate. DNA Res 28 (5).  
624 <https://doi.org/10.1093/dnares/dsab011>

625 Liu HJ, Wang W, Yang M et al (2022) Genome-wide association studies of important  
626 agronomic traits in *Brassica napus*: what we have learned and where we are  
627 headed. Annual Plant Reviews 5:1-30.

628 Liu YX, Wang L, Deng M et al (2015) Genome-wide association study of phosphorus-  
629 deficiency-tolerance traits in *Aegilops tauschii*. Theor Appl Genet 128  
630 (11):2203-2212. <https://doi.org/10.1007/s00122-015-2578-x>

631 Lu K, Peng L, Zhang C et al (2017) Genome-wide association and transcriptome  
632 analyses reveal candidate genes underlying yield-determining traits in *Brassica*  
633 *napus*. Front Plant Sci 8. <https://doi.org/10.3389/Fpls.2017.00206>

634 Luo T, Zhang YT, Zhang CN et al (2021) Genome-wide association mapping unravels  
635 the genetic control of seed vigor under low-temperature conditions in rapeseed  
636 (*Brassica napus* L.). Plants (Basel) 10 (3).  
637 <https://doi.org/10.3390/Plants10030426>

638 Lynch J (1995) Root Architecture and Plant Productivity. Plant Physiol 109 (1):7-13.  
639 <https://doi.org/10.1104/Pp.109.1.7>

640 Mai HJ, Lindermayr C, von Toerne C et al (2015) Iron and FER-LIKE IRON  
641 DEFICIENCY-INDUCED TRANSCRIPTION FACTOR-dependent regulation  
642 of proteins and genes in *Arabidopsis thaliana* roots. Proteomics 15 (17):3030-  
643 3047. <https://doi.org/10.1002/pmic.201400351>

644 Müller J, Toev T, Heisters M. et al (2015). Iron-dependent callose deposition adjusts  
645 root meristem maintenance to phosphate availability. Dev Cell 33 (2): 216-230.  
646 <https://doi.org/10.1016/j.devcel.2015.02.007>

647 Muralidhara P, Weiste C, Collani S et al (2021) Perturbations in plant energy  
648 homeostasis prime lateral root initiation via SnRK1-bZIP63-ARF19 signaling.  
649 Proc Natl Acad Sci USA 118 (37). <https://doi.org/10.1073/pnas.2106961118>

650 Raman H, Raman R, Qiu Y et al (2019) GWAS hints at pleiotropic roles for  
651 FLOWERING LOCUS T in flowering time and yield-related traits in canola. BMC  
652 Genomics 20 (1):636. <https://doi.org/10.1186/s12864-019-5964-y>

653 Shi L, Shi TX, Broadley MR et al (2013) High-throughput root phenotyping screens  
654 identify genetic loci associated with root architectural traits in *Brassica napus*  
655 under contrasting phosphate availabilities. Ann Bot 112 (2):381-389.  
656 <https://doi.org/10.1093/aob/mcs245>

657 Singh AP, Fridman Y, Holland N et al (2018). Interdependent nutrient availability and  
658 steroid hormone signals facilitate root growth plasticity. Dev Cell 46 (1):59-  
659 72.e4. <https://doi.org/10.1016/j.devcel.2018.06.002>

660 Tan ZD, Xie ZQ, Dai LH et al (2022) Genome- and transcriptome-wide association



661 studies reveal the genetic basis and the breeding history of seed glucosinolate  
662 content in *Brassica napus*. Plant Biotechnol J 20 (1):211-225.  
663 <https://doi.org/10.1111/pbi.13707>

664 Tang S, Zhao H, Lu SP et al (2021) Genome- and transcriptome-wide association  
665 studies provide insights into the genetic basis of natural variation of seed oil  
666 content in *Brassica napus*. Mol Plant 14 (3):470-487.  
667 <https://doi.org/10.1016/j.molp.2020.12.003>

668 Ticconi CA, Lucero RD, Sakhonwasee S et al (2009) ER-resident proteins PDR2 and  
669 LPR1 mediate the developmental response of root meristems to phosphate  
670 availability. Proc Natl Acad Sci U S A. 106 (33):14174-14179.  
671 <https://doi.org/10.1073/pnas.0901778106>

672 Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical  
673 adaptations by plants for securing a nonrenewable resource. New Phytol 157  
674 (3):423-447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>

675 Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG (2008) The effect of iron  
676 on the primary root elongation of *Arabidopsis* during phosphate deficiency.  
677 Plant Physiol 147:1181-1191. <https://doi.org/10.1104/pp.108.118562>

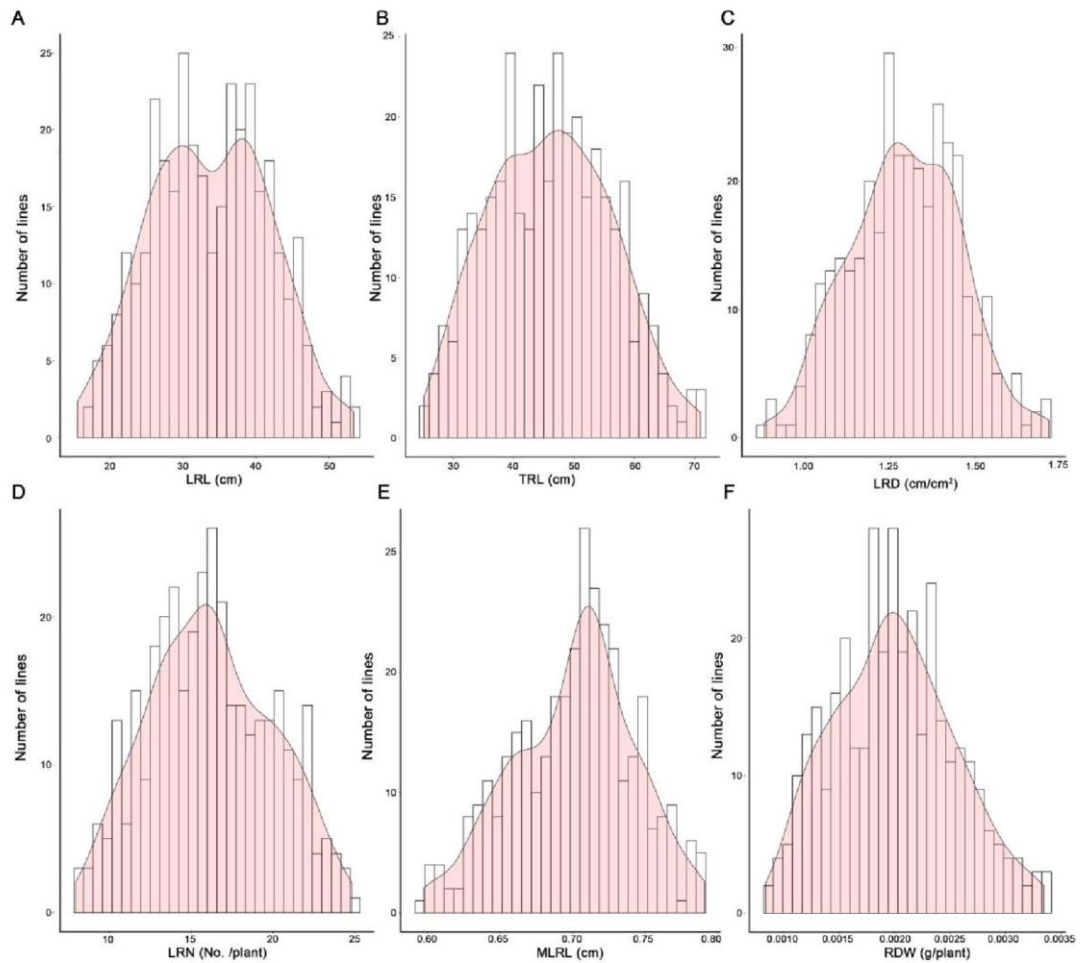
678 Wang XH, Chen YL, Thomas CL et al (2017) Genetic variants associated with the root  
679 system architecture of oilseed rape (*Brassica napus* L.) under contrasting  
680 phosphate supply. DNA Res 24 (4):407-417.  
681 <https://doi.org/10.1093/dnares/dsx013>

682 Wu D, Liang Z, Yan T et al (2019) Whole-Genome Resequencing of a Worldwide  
683 Collection of Rapeseed Accessions Reveals the Genetic Basis of Ecotype  
684 Divergence. Mol Plant 12 (1):30-43.  
685 <https://doi.org/10.1016/j.molp.2018.11.007>

686 Xiao YJ, Liu HJ, Wu LJ et al (2017) Genome-wide Association Studies in Maize: Praise  
687 and Stargaze. Mol Plant 10 (3):359-374.  
688 <https://doi.org/10.1016/j.molp.2016.12.008>

689 Zhang C, Dong SS, Xu JY et al (2019) PopLDdecay: a fast and effective tool for linkage  
690 disequilibrium decay analysis based on variant call format files. Bioinformatics

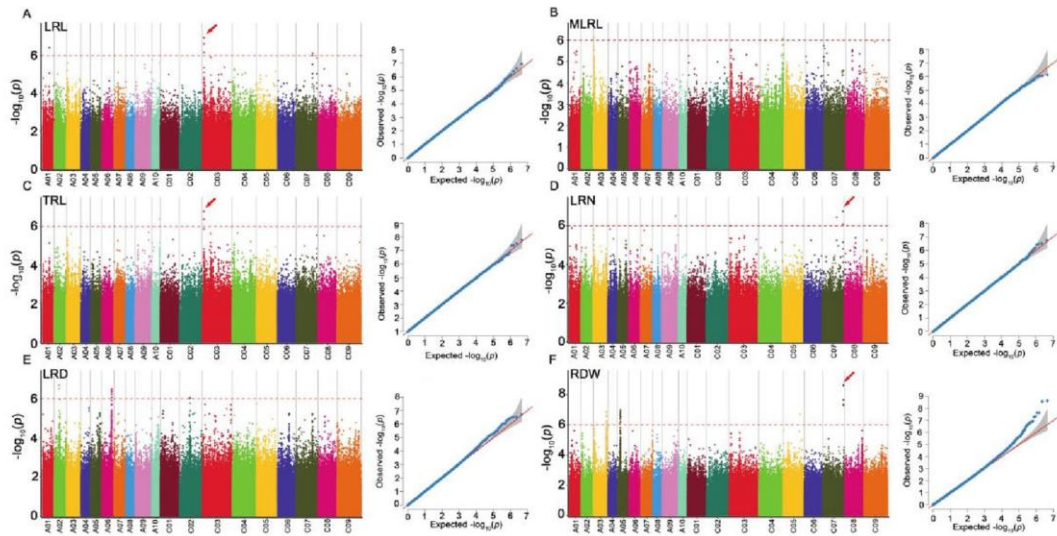
691 35 (10):1786-1788. <https://doi.org/10.1093/bioinformatics/bty875>  
692 Zhang Y, Thomas CL, Xiang JX et al (2016) QTL meta-analysis of root traits in  
693 *Brassica napus* under contrasting phosphorus supply in two growth systems.  
694 Sci Rep 6. <https://doi.org/10.1038/Srep33113>  
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698 **Fig. 1** Distribution of RSA (root system architecture) phenotypes in 370 *B. napus*  
 699 accessions. Histograms of the distribution of LRL (A), TRL (B), LRD (C), RN (D),  
 700 MLRL (E) and RDW (F) measured at low phosphorus supplies. LRL, lateral root length;  
 701 TRL, total root length; LRD, lateral root density; RN, lateral root number; MLRL; mean  
 702 lateral root length; RDW, root dry weight.

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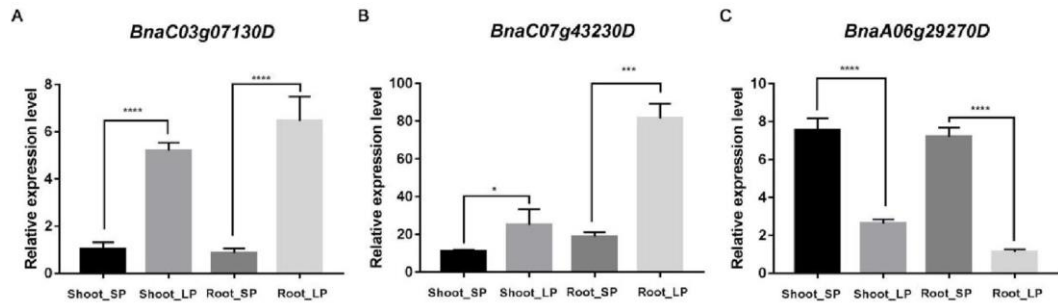


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705 **Fig. 2** Quantile-quantile (QQ) and Manhattan plots for the RSA (root system  
 706 architecture) by genome-wide association study (GWAS). GWAS of LRL (A), MLRL  
 707 (B), TRL (C), RN (D), LRD (E) and RDW (F) in 370 *B. napus* at low phosphorus  
 708 supplies by Fast-LMM model. LRL, lateral root length; MLRL, mean lateral root length;  
 709 TRL, total root length; RN, lateral root number; LRD, lateral root density; RDW, root  
 710 dry weight.

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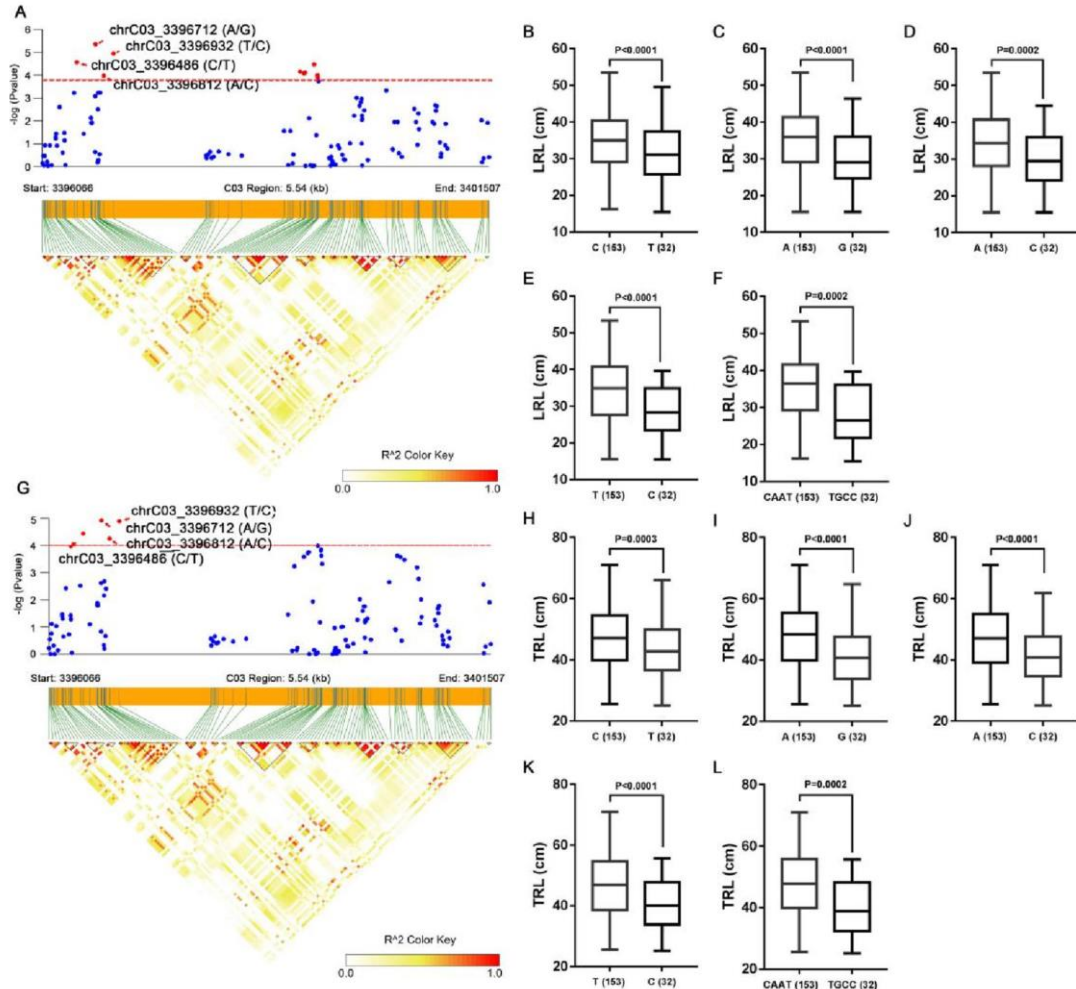
714 **Fig. 3** Relative expression level of three candidate genes (A, *BnaA06g29270D*; B,  
 715 *BnaC03g07130D* and C, *BnaC07g43230D*) under low phosphorus and sufficient  
 716 phosphorus conditions. LP, low phosphorus; SP, sufficient phosphorus. Data are means  
 717  $\pm$  SEM (n=4). Asterisks indicate the significance of Student's t-test (\*P < 0.05, \*\*\*P  
 718 < 0.0001, \*\*\*\*P < 0.0001).

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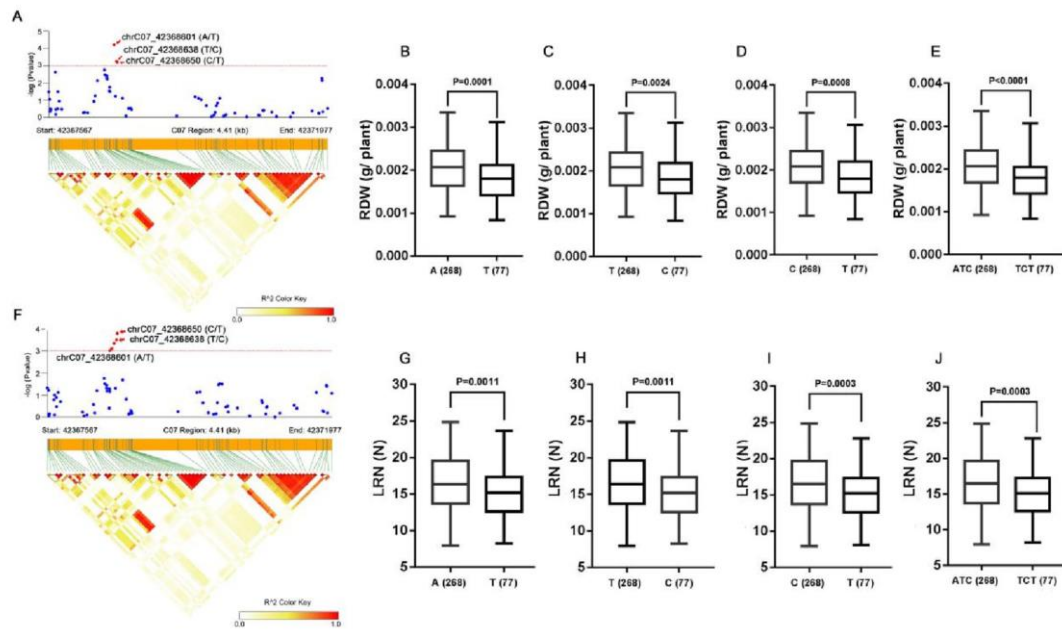
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724 **Fig. 4** Candidate gene association and haplotypes analysis of *BnaC03g07130D* with  
 725 LRL (lateral root length) and TRL (total root length). (A) Candidate gene association  
 726 analysis of *BnaC03g07130D* with LRL. (B-E) Association of the two alleles in  
 727 chrC03\_3396486 (B), chrC03\_3396712 (C), chrC03\_3396812 (D) and  
 728 chrC03\_3396932 (E) with LRL, respectively. (F) Two haplotypes of *BnaC03g07130D*.  
 729 (G) Candidate gene association analysis of *BnaC03g07130D* with TRL. (H-K)  
 730 Association of the two alleles in chrC03\_3396486 (H), chrC03\_3396712 (I),  
 731 chrC03\_3396812 (J) and chrC03\_3396932 (K) with TRL, respectively. (L) Two  
 732 haplotypes of *BnaC03g07130D*. The number of inbred lines harbouring the  
 733 corresponding allele is shown in brackets at the bottom.

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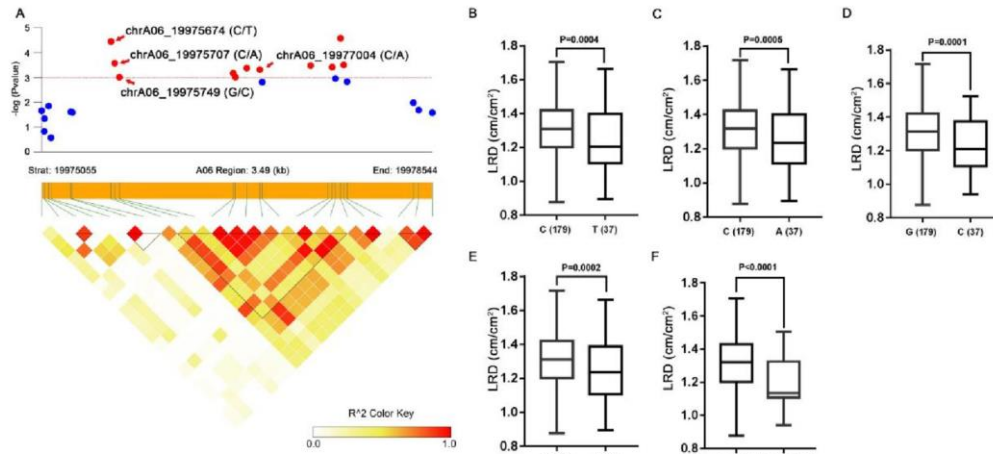


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738 **Fig. 5** Candidate gene association and haplotypes analysis of *BnaC07g43230D* with  
 739 LRN (lateral root number) and RDW (root dry weight). (A) Candidate gene association  
 740 analysis of *BnaC07g43230D* with LRN. (B-D) Association of the three alleles in  
 741 chrC07\_42368601 (B), chrC07\_42368638 (C) and chrC07\_42368650 (D) with LRN,  
 742 respectively. (E) Two haplotypes of *BnaC07g43230D*. (F) Candidate gene association  
 743 analysis of *BnaC07g43230D* with LRN. (G-I) Association of the three alleles in  
 744 chrC07\_42368601 (G), chrC07\_42368638 (H) and chrC07\_42368650 (I) with RDW,  
 745 respectively. (J) Two haplotypes of *BnaC07g43230D*. The number of inbred lines  
 746 harbouring the corresponding allele is shown in brackets at the bottom.

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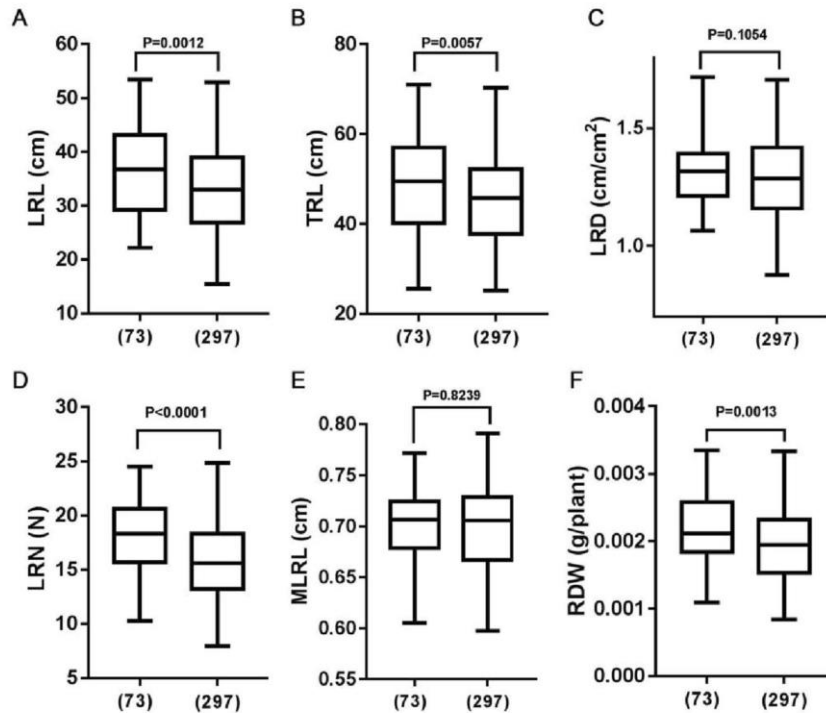
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 750 **Fig. 6** Candidate gene association and haplotypes analysis of *BnaA06g29270D* with  
 751 LRD (lateral root density). (A) Candidate gene association analysis of *BnaA06g29270D*  
 752 with LRD. (B-E) Association of the four alleles in chrA06\_19975674 (B),  
 753 chrA06\_19975707 (C), chrA06\_19975749 (D) and chrA06\_19977004 (E) with LRD,  
 754 respectively. (F) Two haplotypes of *BnaA06g29270D*. The number of inbred lines  
 755 harbouring the corresponding allele is shown in brackets at the bottom.

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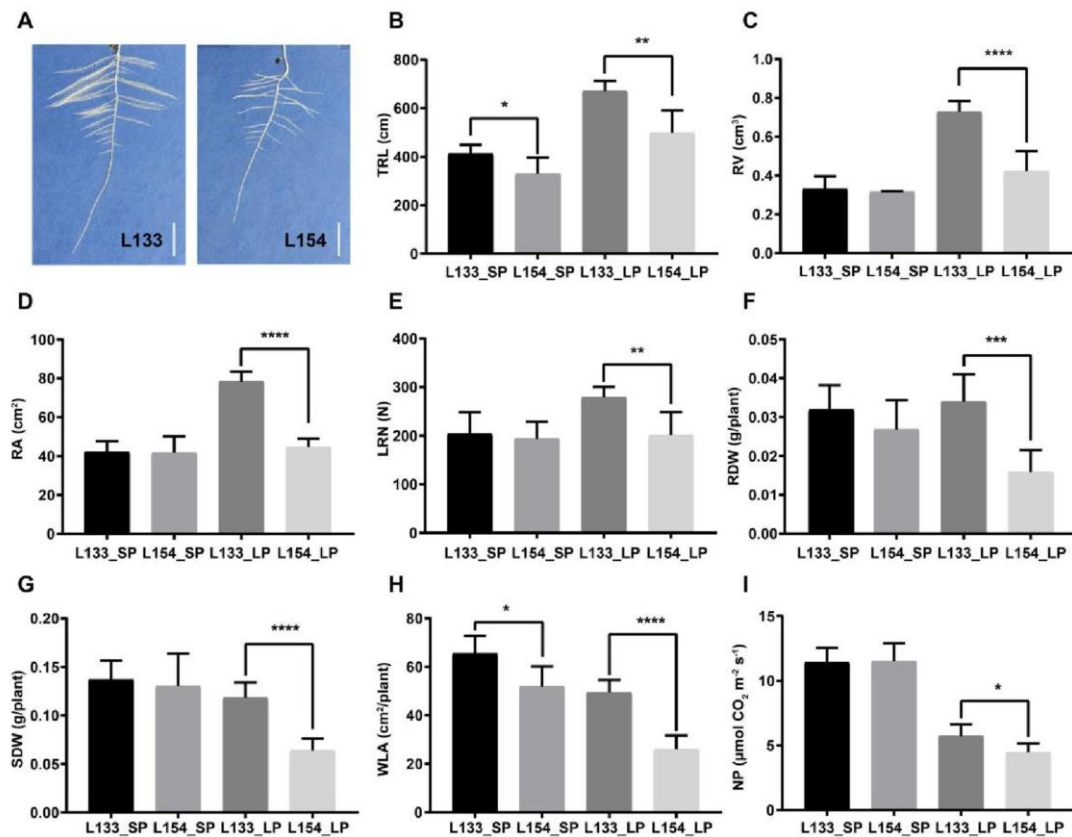


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759 **Fig. 7** Differences in root system architecture at LP between *B. napus* cultivars with  
 760 three favorable haplotypes (CCGC, CAAT and ATC) and *B. napus* cultivars without the  
 761 three favorable haplotypes in the association panel. Favorable haplotypes of LRL (A),  
 762 TRL (B), LRD (C), RN (D), MLRL (E) and RDW (F) were analysed. (73) represents  
 763 73 cultivars with the three favorable haplotypes; (297) represents the rest of the  
 764 association panel after removing the above 73 cultivars. LRL, lateral root length; TRL,  
 765 total root length; LRD, lateral root density; RN, lateral root number; MLRL, mean  
 766 lateral root length; RDW, root dry weight.

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770 **Fig. 8** Differences in root system architecture, biomass, whole leaf area and net  
 771 photosynthesis between *B. napus* cultivar (L133) with three favorable haplotypes  
 772 (CCGC, CAAT and ATC) and *B. napus* cultivar (L154) without the three favorable  
 773 haplotypes (TGCC, TACA and TCT). (A) Root growth of L133 and L154 at LP for 14  
 774 d in the 'pouch and wick' system. (B) TRL. (C) RV. (D) RA. (E) LRN. (F) RDW. (G)  
 775 SDW. (H) WLA. (I) NP. LP, low phosphorus; TRL, total root length; RV, root volume;  
 776 RA, root area; LRN, lateral root number; RDW, root dry weight; SDW, shoot dry weight;  
 777 WLA, whole leaf area; NP, net photosynthesis. Data are means  $\pm$  SEM (n=6).  
 778 Asterisks indicate the significance of Student's t-test (\*P < 0.05, \*\*P < 0.01, \*\*\*P <  
 779 0.0001, \*\*\*\*P < 0.0001). Scale bar is 2 cm in (A).

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