

Genome-wide association study dissects the genetic control of plant height and branch number in response to low-phosphorus stress in Brassica napus

Article

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1 Original Article

2 **Genome-wide association study dissects the genetic control of plant**
3 **height and branch number in response to low-phosphorus stress in**
4 ***Brassica napus***

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17

18 Running title: Genetic control of PH and BN of *B. napus* at LP

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1 **Background and Aims** Oilseed rape (*Brassica napus*) is one of the most important oil
2 crops worldwide. Phosphorus (P) deficiency severely decreases the plant height (PH)
3 and branch number (BN) of *B. napus*. However, the genetic bases controlling PH and
4 BN in *B. napus* under P deficiency remain largely unknown. This study aims to mine
5 candidate genes for PH and BN by genome-wide association study (GWAS) and
6 determine low-P tolerance haplotypes.

7 **Methods** An association panel of *B. napus* were grown in the field with a low P supply
8 (P, 0 kg/ha) and a sufficient P supply (P, 40 kg/ha) across two years and PH and BN
9 were investigated. More than five million single-nucleotide polymorphisms (SNPs)
10 were used to conduct GWAS of PH and BN at two contrasting P supplies.

11 **Key Results** A total of 2127 SNPs were strongly associated ($P < 6.25 \times 10^{-07}$) with PH
12 and BN at two P supplies. There was significant correlation between phenotypic
13 variation and the number of favorable alleles of associated loci on chromosomes A10
14 (chrA10_821671) and C08 (chrC08_27999846), which will contribute to breeding
15 improvement by aggregating these SNPs. *BnaA10g09290D* and *BnaC08g26640D* were
16 identified to be associated with the chrA10_821671 and chrC08_27999846,
17 respectively. Candidate gene association analysis and haplotype analysis showed that
18 the inbred lines carrying ATT at '*BnaA10g09290HapI*' and AAT at
19 '*BnaC08g26640HapI*' had higher PH than lines carrying other haplotype alleles at low
20 P supply.

21 **Conclusion** Our results demonstrate the power of GWAS in identifying genes of
22 interest in *B. napus* and provided insights into the genetic basis of PH and BN at low P

1 supply in *B. napus*. Candidate genes and favorable haplotypes may facilitate marker-
2 based breeding efforts aimed at improving P use efficiency in *B. napus*.

3

4 **Keywords:** oilseed rape; genome wide association study; plant height; branch number;
5 low phosphorus supply; haplotype analysis

6

1 INTRODUCTION

2 Oilseed rape (*B. napus*) is one of the most important sources of vegetable oil globally.
3 The global production of vegetable oil reached 75 million tons in 2020
4 (<http://www.fao.org/faostat/zh/#data/QC/visualize>). Oilseed rape canopy architecture is
5 determined by plant height, the length of the main inflorescence and the number and
6 distribution of ancillary branches (Li et al 2016). Canopy architecture indirectly
7 influences cultivar yield potential by significantly influencing the number of siliques
8 per plant. It has previously been shown that plant height negatively correlates with
9 siliques per plant, owing to the greater lodging risk of taller plants and branch number
10 positively correlates with the number of siliques per plant (Qiu *et al.*, 2006; Chen *et al.*,
11 2014).

12 Phosphorus (P) is an essential macro-element for plant growth and development
13 (Kochian, 2012). Oilseed rape has a high P requirement and P deficiency reduces PH
14 and BN, and the subsequent seed yield of *B. napus* (Shi *et al.*, 2013). P in the soil is
15 easily fixed by metal cations to form insoluble compounds or bound in organic forms,
16 which are difficult to be directly absorbed and used by plants (Holford, 1997). Therefore,
17 P fertilizers must be supplied to ensure the normal growth and development of crops.
18 The use of P fertilizer increased from 34.5 million tons in 2001 to 45.4 million tons in
19 2017 (<http://www.fao.org/faostat/zh/#data/QC/visualize>). However, long-term excess P
20 fertilizer is problematic because of its limited bioavailability and potential
21 environmental problems such as eutrophication, and the depletion of non-renewable
22 rock phosphate resources (Vance *et al.*, 2003). Therefore, uncovering the genetic

1 mechanisms of *B. napus* tolerance to low P, and breeding high P-efficient varieties is
2 an important way to reduce P use in agricultural systems.

3 Linkage mapping analysis has been widely used to study the genetic basis of P
4 tolerance in *B. napus* (Yang *et al.*, 2010a; Yang *et al.*, 2010b; Shi *et al.*, 2013; Ding *et*
5 *al.*, 2012). Sixty-two significant quantitative trait locus (QTLs) were associated with
6 plant P uptake, total root surface area, root length, root volume and total dry weight
7 under high and low P conditions in three experiments, explaining 12.7 %~31.9% of the
8 phenotypic variation (Yang *et al.*, 2010a). Ding *et al.* (2012) detected 21 significant
9 QTLs associated with PH and BN under high and low P conditions, explaining
10 8.5%~19.8% of the phenotypic variation. One hundred and fifty-five significant QTLs
11 were associated with seed yield and yield-related traits from three different trials, and
12 79 QTLs detected at LP (a deficient P supply) and 76 under SP (a sufficient P supply)
13 (Shi *et al.* 2013). Among them, 16 QTLs were associated with PH and 5 QTLs were
14 associated with BN (Shi *et al.*, 2013). However, due to the limited number of genetic
15 markers and the frequency of recombination in mapping populations, the physical
16 interval for these QTL are usually large, which makes it difficult to determine the
17 candidate genes (Xiao *et al.*, 2017).

18 Compared with QTL mapping, genome wide association study (GWAS) can achieve
19 a higher resolution of the underlying genetic loci, which greatly improves the efficiency
20 of gene mapping (Nordborg and Weigel, 2008). GWAS has been widely applied to the
21 genetic dissection of complex traits in crops, such as grain size in rice (Si *et al.*, 2016),
22 PH in maize (Zhang *et al.*, 2019) and silique number in *B. napus* (Li *et al.*, 2020).

1 GWAS combined with QTL analysis has been used successfully to mine candidate
2 genes in *B. napus*, such as root related traits (Wang et al., 2017) and clubroot resistance
3 (Laperche et al., 2017) in *B. napus*, and capsaicinoid content in *Capsicum* (Han et al.,
4 2018).

5 GWAS of the PH and BN of *B. napus* under sufficient P conditions has been
6 conducted in several studies (Li et al., 2016; Sun et al., 2016; Zheng et al., 2017).
7 However, the genetic bases controlling PH and BN in *B. napus* under P deficiency
8 remain unknown. In this study, we investigated the PH and BN of a *B. napus* association
9 panel at low and sufficient P supplies across two years. GWAS of PH and BN was
10 performed using high-quality SNPs by whole-genome resequencing (Tang *et al.*, 2020).
11 We aimed to identify the (i) genetic diversity of the population, (ii) significant SNPs
12 and candidate genes associated with PH and BN at contrasting P supplies (iii) and reveal
13 the favourable haplotypes for breeding P-efficient *B. napus* cultivars.

14

15 MATERIALS AND METHODS

16 *Plant materials and growth conditions*

17 The association panel of *B. napus* comprises 403 cultivars and inbred lines, including
18 350 semi-winter, 44 spring, 8 winter and 1 unknown type, collected from major *B.*
19 *napus* breeding centers across China (Supplementary Data Table S1). Of them, 361
20 lines originated in China, 21 from Europe, 8 from Japan, 5 from Canada, 4 from
21 Australia, 3 from Korea and 1 unknown (Supplementary Data Table S1). The panel
22 were grown in the field with a low P supply (P, 0 kg/ha) and a sufficient P supply (P, 40

1 kg/ha) with three replications at Meichuan Town, Wuxue city, Hubei province, China
2 (E 115.55, N 29.85°) from 2018 to 2019 (Trial 1) and from 2019 to 2020 (Trial 2). The
3 soil was sandy loam soil. The top soil (0-30 cm) were collected before sowing (before
4 fertilization) for determination of the available nutrients concentrations (Table 1). All
5 the plots received basal fertilizer, and the application rate was as follows (per hectare),
6 108 kg of N (supplied as urea), 0 or 40 kg of P (supplied as calcium superphosphate),
7 87 kg of K (supplied as potassium chloride) and 6 kg of B (supplied as borax). These
8 fertilizers were thoroughly mixed and applied in bands near the crop rows. The
9 remaining N (72 kg/ha) was top dressed as urea in equal amounts at the four to five-
10 leaf stage. Each accession had 4 rows and each plot had 8 plants each row. At the mature
11 stage, six plants each plot were selected to measure the PH and BN. PH was the length
12 of the plant from the base of the stem to the tip of the main inflorescence, and BN was
13 calculated by the number of primary branches arising from main shoot. PHr and BNr
14 were defined as the ratio of PH_LP to PH_SP, and that of BN_LP to BN_SP,
15 respectively.

16

17 *Genome-wide association analysis and candidate gene identification*

18 More than 10 million high-quality SNP markers of the association panel of *B. napus*
19 were derived from previous studie (Tang et al., 2020). After filtering the SNPs with
20 minor-allele frequency (MAF) > 0.05 and missing rate < 0.2, we obtained 5.58 million
21 SNPs for GWAS. GWAS for PH was carried out using general linear models (GLM)
22 and mixed linear models (MLM) by the Tassel 5.0 software (Bradbury *et al.*, 2007). In

1 order to minimize the contribution from regions of extensive strong LD, we scanned
2 the whole genome with a sliding window of 500 kb (in steps of 100 SNPs), and used
3 Plink software to remove SNPs related with other SNPs within the window with
4 correlation coefficient (R^2) > 0.2. Finally, we obtain 497761 SNPs. Admixture software
5 was used to calculate the Q matrix and Tassel 5.0 software was used to calculate the K
6 matrix. The Manhattan plot was drawn by ggplot2 ([https://cran.r-](https://cran.r-project.org/web/packages/ggplot2/index.html)
7 [project.org/web/packages/ggplot2/index.html](https://cran.r-project.org/web/packages/ggplot2/index.html)) software and the Quantile-Quantile plot
8 was drawn by the CMplot software (<https://github.com/YinLiLin/CMplot>). The
9 threshold for the significance of associations between SNPs and traits was $P < 6.25 \times 10$
10 $^{-07}$. The linkage disequilibrium (LD) statistic was calculated by PopLDdecay software
11 (Zhang *et al.*, 2019). According to the LD decay (238kb) of the panel, the genes located
12 within 300 kb upstream and downstream of the peak SNPs were considered as candidate
13 genes. GO enrichment analysis of the candidate genes were performed with the
14 omicshare online platform (<https://www.omicshare.com/quote.php>). The genotypes of
15 *BnaA02g33340D*, *BnaA10g09290D* and *BnaC08g26640D* in the association panel of
16 *B. napus* were obtained by bcftools software
17 (<http://samtools.github.io/bcftools/bcftools.html>). Candidate gene association analysis
18 of *BnaA02g33340D*, *BnaA10g09290D* and *BnaC08g26640D* were performed with
19 Tassel 5.0 software (Bradbury *et al.*, 2007). The SNP markers from 2 kb up-stream of
20 the gene to 2 kb down-stream of the gene were used to conduct association analysis
21 with the PH of the association panel of *B. napus* at both P supplies.

22

1 *Haplotype analysis*

2 The haplotype analysis was predicted using the HaploView software (Barrett *et al.*,
3 2005). One-way ANOVA and Student's *t*-test were employed to compare the differences
4 in PH among the haplotypes. Haplotypes containing at least twenty *B. napus* cultivars
5 were used for further comparative analysis. Student's *t*-test was used to compare the
6 differences in PH among the haplotypes.

8 *Statistical Analysis of Phenotypic Data*

9 The mean values of PH and BN of six plants of each replication were calculated using
10 Excel 2007. Best linear unbiased prediction (BLUP) of PH and BN at a deficient P
11 supply (LP) and a sufficient P supply (SP) for each line was calculated using the R
12 package 'lme4' (<https://cran.r-project.org/web/packages/lme4/index.html>).
13 Trial1_BLUP and Trial2_BLUP were calculated with the phenotypic values of three
14 replications in Trial 1 and Trial 2, respectively (Supplementary Data Table S2). R
15 language was used to calculate the correlation coefficients between phenotypes. The
16 broad-sense heritability was calculated as: $H^2 = V_G / (V_G + V_E / nr)$, and among them
17 V_G is genetic variance, V_E is environmental variance, n is the number of environments
18 and r is the number of replicates.

19

20 **RESULTS**

21 *Phenotypic variation for PH and BN of an association panel of B. napus at low and sufficient P*
22 *supplies*

1 From the seedling stage to the silique stage under LP, the old leaves turned dark and
 2 purple, and the inhibition of root and shoot growth was observed in most lines
 3 (Supplementary Data Fig. S1A-E). In addition, the PH and BN of *B. napus* decreased
 4 under LP compared to SP (Supplementary Data Fig. S1F-I). Extensive phenotypic
 5 variations for PH and BN were observed in the association panel of *B. napus* at LP and
 6 SP (Supplementary Data Fig. S1G; S2, Table 2, Supplementary Data Table S2). Under
 7 LP, PH varied from 57.5 to 179.3 cm (Supplementary Data Fig. S2, Table 2,
 8 Supplementary Data Table S2) and BN varied from 0 to 11 per plant across the 2 years
 9 (Supplementary Data Fig. S2, Table 2, Supplementary Data Table S2). Under SP, PH
 10 varied from 84.0 to 235.0 cm (Supplementary Data Fig. S2, Table 2, Supplementary
 11 Data Table S2) and BN varied from 1 to 16 per plant across the 2 years (Supplementary
 12 Data Fig. S2, Table 2, Supplementary Data Table S2). PHr varied from 0.40 to 1.00 and
 13 BNr varied from 0.10 to 1.00 across the 2 years (Supplementary Data Fig. S2, Table 2,
 14 Supplementary Data Table S2). High h^2 values were observed for all traits (Table 2).
 15 The correlation coefficients of PH_SP, BN_SP, PH_LP, BN_LP, PHr and BNr between
 16 Trial 1 and Trial 2 were 0.57, 0.28, 0.27, 0.09, 0.19 and 0.09, respectively
 17 (Supplementary Data Fig. S3). Thus, best linear unbiased prediction (BLUP) analysis
 18 was employed to deal with multi- year and field phenotypic values of PH and BN.

19

20 *Population structure, relative kinship, and LD decay*

21 A total of 5.58 million SNP markers were identified for this *B. napus* association
 22 panel (Supplementary Data Table S3). SNP number on each chromosome ranged from

1 171159 on A08 to 475529 on C03 (Supplementary Data Table S3). LD decay on each
2 chromosome ranged from 1996 kb on C07 to 45 kb on A03, when r^2 was 0.1
3 (Supplementary Data Table S3). The LD decay on the whole genome was 238 kb
4 (Supplementary Data Fig. S4, Supplementary Data Table S3). A total of 497761 SNPs
5 were selected to assess the population structure, relative kinship and LD. The
6 population could be divided into five subgroups based on the cross validation (CV)
7 errors (Supplementary Data Fig. S5). The r pairwise relative kinship is close to 0
8 (Supplementary Data Fig. S6, Supplementary Data Table S4). For example, the values
9 of the relative kinships were 0 to 129976 pairs and 0.1 to 148803 pairs, and the ratios
10 to the total value were 80.03% and 91.62%, respectively (Supplementary Data Table
11 S4). These results showed that the genetic distance of the majority of the accessions in
12 the association panel were large enough for the GWAS analysis.

13

14 *Genome wide association mapping of PH and BN of B. napus at low and sufficient P* 15 *supplies*

16 We performed GWAS with GLM and MLM approaches to identify SNPs associated
17 with PH and BN at LP and SP in *B. napus*. A total of 1289 SNPs were identified to be
18 significantly associated with PH of *B. napus* at LP and SP across two years ($P < 6.25 \times 10$
19 $^{-07}$) (Supplementary Data Fig. S7 A-D, Supplementary Data Table S5-S6). Among them,
20 133, 685 and 471 SNPs were associated with PH_LP, PH_SP and PHr, respectively. The
21 GLM analysis detected a total of 1275 significant SNPs at both P supplies. Among them,
22 131, 678 and 466 SNPs were associated with PH_LP, PH_SP and PHr, respectively, and

1 were distributed on all chromosomes, explaining between 6.03% and 14.01% of the
2 phenotypic variation (Supplementary Data Table S5). Chromosome C03 had the largest
3 number of significant SNPs (244) and chromosome A07 had the least number of
4 significant SNPs (7 SNPs) (Supplementary Data Table S5). MLM analysis detected 106
5 significant SNPs on 18 of the 19 *B. napus* chromosomes (excluding C02) at both P
6 supplies. Among them, 15, 74 and 17 SNPs were associated with PH_LP, PH_SP and
7 PHr, respectively, and explained between 7.85% and 14.23% of the phenotypic
8 variation (Supplementary Data Table S6). Among them, 93 significant SNPs were
9 simultaneously identified by the two models (Supplementary Data Table S5-S6).

10 For BN, the GLM analysis detected a total of 837 significant SNPs at both P supplies.
11 Among them, 34, 770 and 33 SNPs were associated with BN_LP, BN_SP and BNr,
12 respectively, which were distributed on all chromosomes and explained between 6.12%
13 and 15.83% of the phenotypic variation (Supplementary Data Fig. S7 E-H,
14 Supplementary Data Table S5-S6). MLM analysis detected 93 significant SNPs at both
15 P supplies. Among them, three and 90 SNPs were associated with BN_LP and BN_SP,
16 respectively, which explained between 6.92 % and 14.47% of the phenotypic variation.
17 Of them, 90 SNPs were detected by both the GLM and MLM analyses (Supplementary
18 Data Table S5-S6).

19

20 *Candidate genes for PH and BN at low P supply in B. napus*

21 We identified 1289 significant SNPs significantly associated with PH_LP and PHr.
22 2, 19, 3, 9, 88, 5 and 5 significant SNPs were associated with PH_LP in Trial 1_R1,

1 Trial 1_R2, Trial 1_R3, Trial 1_BLUP, Trial 2_R1, Trial 2_R2 and Trial 2_BLUP,
2 respectively (Supplementary Data Fig. S7 A-D, Supplementary Data Table S5-S6). 15,
3 17, 12, 23, 250, 1 and 158 significant SNPs were associated with PHr in Trial 1_R1,
4 Trial 1_R2, Trial 1_R3, Trial 1_BLUP, Trial 2_R1, Trial 2_R2 and Trial 2_BLUP,
5 respectively (Supplementary Data Fig. S7 A-D, Supplementary Data Table S5-S6). The
6 SNP of chrA10_8216711 on chromosome A10 was associated with PH_LP and PHr in
7 both Trial 2_R2 and Trial 2_BLUP (Fig 1A, B). In this study, the LD decay was 238 kb
8 for this association panel (Supplementary Data Table S3). Based on the LD decay, 300
9 kb up/downstream of the significant SNPs were selected to identify candidate genes on
10 A10 and 76 candidate genes were detected.

11 The significant SNP associated with PH_LP and PHr identified at the 8216711 bp
12 position on chromosome A10 was found located in the genetic region of
13 *BnaA10g09290D*, whose function was unknown. Candidate gene association analysis
14 of *BnaA10g09290D* with SNP markers from 2 kb promoter region and the entire coding
15 region showed that three SNPs in *BnaA10g09290D* were significantly associated with
16 the PH_LP and PHr, and had strong LD with each other (Fig. 1C, H). Further analysis
17 demonstrated that A allele of chrA10_8216680, T allele of chrA10_8216711 and T
18 allele of chrA10_8216756 were the P deficiency tolerant alleles (Fig. 1). Two major
19 haplotypes based on the three significant SNPs were detected in this association panel,
20 among which '*BnaA10g09290Hap2*' (TCA) was P deficiency sensitive, while
21 '*BnaA10g09290Hap1*' (ATT) were P deficiency tolerant (Fig. 1G, L). Notably,
22 chrA10_8216680 (T/A) located in the exon region of the gene *BnaA10g09290D* and

1 resulted in amino acid changes from isoleucine to asparagine and might contribute to
2 the phenotypic difference in PH_LP and PHr (Table 3).

3 The region significantly associated with PHr in both Trial 1_R2, Trial 1_R3, Trial
4 1_BLUP and Trial 2_BLUP on chromosome C08 ranged from 27.95 Mb to 28.13 Mb
5 (Fig. 2A, Fig. 2B). Based on the LD decay (238kb), 300 kb upstream and downstream
6 regions of the significant SNP (chrC08_27999846, $P = 8.91E-08$, PVE = 12.46%) were
7 selected and found to contain 101 genes. The lead SNP of chrC08_27999846 was
8 located within *BnaC08g26640D*. Three SNPs in *BnaC08g26640D* were detected to be
9 significantly associated with the PHr (Fig. 2C), and A allele of chrC08_27999709, A
10 allele of chrC08_27999778 and T allele of chrC08_27999846 were the P deficiency -
11 tolerant alleles (Fig. 2D, E, F). These three significant SNPs revealed two major
12 haplotypes, and '*BnaC08g26640Hap1*' (AAT) had significantly greater PHr value than
13 '*BnaC08g26640Hap2*' (GTC) ($P = 1.51E-15$) (Fig. 2G). The SNP 'chrC08_27999709'
14 (A/G) was located in the intron region, and did not result in any amino acid change
15 (Table 3). The SNP of chrC08_27999846 (T/C) was in the exon region, and it was a
16 synonymous mutation that did not result in any amino acid change (Table 3). The SNP
17 of chrC08_27999778 (A/T) located in the exon region of the gene *BnaC08g26640D*
18 and resulted in amino acid changes from isoleucine to asparagine and might contribute
19 to the phenotypic difference in PHr (Table 3).

20 Three SNPs associated with BN_LP, chrA01_13846343, chrA03_20898013 and
21 chrC07_521008, were detected simultaneously by GLM and MLM (Supplementary
22 Data Table S5-S6). On chromosome A01, 40 candidate genes were detected underlying

1 the region around chrA01_13846343 (Supplementary Data Table S7). The ATP
2 phosphoribosyl transferase 1 (*BnaA01g21560D*) and low temperature and salt
3 responsive protein (*BnaA01g21470D*) were identified in the candidate region. On
4 chromosome A03, the significant SNP chrA03_20898013 was also detected by GLM
5 and MLM model simultaneously, with $P = 4.97E-07$ and $R^2 = 9.21\%$ (Supplementary
6 Data Table S5-S6). Two genes encoding heat shock transcription factor A7A
7 (*BnaA03g41540D* and *BnaA03g41550D*) were identified in the upstream region from
8 chrA03_20898013 and their homolog in *Arabidopsis* responds to abiotic stresses (Lin
9 *et al.*, 2018). In addition, a phosphatidic acid phosphatase family protein
10 (*BnaA03g41050D*) and a pyruvate orthophosphate dikinase (*BnaA03g41960D*) were
11 identified in the down-stream region of chrA03_20898013. On chromosome C07, a
12 total of 62 genes were detected underlying the candidate region around the SNP
13 ‘chrC07_521008’ ($P = 5.95E-07$). Among these candidate genes, an EXP1 gene
14 (*BnaC07g00140D*), which is involved in unidimensional cell growth and plant-type cell
15 wall loosening, and may be involved in BN determination at LP (Supplementary Data
16 Table S8).

17

18 *Comparison of the significant SNPs with QTLs for P efficiency*

19 Based on the Darmor-*bzh* reference genome, we analyzed the co-localization of
20 the significant SNPs detected in our study and the QTLs detected by previous study (y
21 (Shi *et al.*, 2013). Thirty-three significant SNPs detected by GWAS co-localized with
22 the intervals of the QTLs for the same traits in a previous linkage analyses of the

1 *BnaTNDH* population (Shi *et al.*, 2013), including four SNPs on A02, seven SNPs on
 2 A09, seven SNPs on C06 and fifteen SNPs on C07 (Supplementary Data Table S9).
 3 Among them, four co-located SNPs on A02 chromosome associated with PH_LP (Fig.
 4 3A-3C), including the P-tolerant T allele of chrA02_23692807, C allele of
 5 chrA02_23713660, A allele of chrA02_23899688 and T allele of chrA02_23912345
 6 (Fig. 3D). A total of 152 candidate genes were within the QTL (PH_LP1_A02a; Shi *et*
 7 *al.*, 2013) confidence interval on A02 (Fig. 3A, Supplementary Data Table S10).
 8 Among the four co-localized SNPs, the lead SNP chrA02_23899688 ($P = 1.85E-07$)
 9 located in the CDS region of *BnaA02g33340D* (Supplementary Data Table 3). Since
 10 *BnaA02g33340D* was identified by association with PH_LP, we examined the
 11 association of sequence variation in *BnaA02g33340D* with the PH_LP of the
 12 association panel. Five SNPs in *BnaA02g33340D* were detected to be significantly
 13 associated with the PH_LP (Fig. 3E). Further analyses showed that G allele of
 14 chrA02_23899623, G allele of chrA02_23899654, T allele of chrA02_23899669, C
 15 allele of chrA02_23899686 and A allele of chrA02_23899688 were P-tolerant alleles
 16 (Fig. 3F). There were two major haplotypes associated with PH and Hap1 (GGTCA)
 17 had higher PH at low P than Hap2 (ATATC) with a P-value of 0.0010, suggesting that
 18 the variation in *BnaA02g33340D* was associated with PH_LP (Fig. 3G). Further
 19 analysis indicated that chrA02_23899623 (G/A), chrA02_23899654 (G/T) and
 20 chrA02_23899669 (T/A) were located in the intron region, and the SNP
 21 'chrA02_23899686 (C/T)' was located in the exon region and was a synonymous
 22 mutation, which were unlikely to affect the function of the *BnaA02g33340D* protein

1 (Table 3). The SNP ‘chrA02_23899688 (A/C)’ was located in the exon region of the
2 gene *BnaA02g33340D* and resulted in amino acid changes from histidine to proline and
3 may be causative in the difference of PH_LP (Table 3). These observations
4 demonstrated that *BnaA02g33340D* is most likely to be the candidate gene for the locus
5 represented by lead SNP chrA02_23899688.

6

7 **DISCUSSION**

8 *Significant differences in the PH and BN in the association panel of B. napus at low P*
9 *supply*

10 In this study, the soil available P concentration in three fields ranged from 11.56 to
11 16.95 mg/kg, which are between P deficient and slightly P deficient (Zou et al. 2009.).
12 Some typical P deficient symptoms were observed in most lines in the field, such as
13 greater anthocyanin visibility in old leaves, restricted shoot growth at the seedling stage
14 (Supplementary Data Fig. S1 A-C), and reduced PH and BN at the mature stage
15 (Supplementary Data Fig. S1 D-F). PH and BN are essential components of plant
16 canopy architecture in *B. napus*, which are closely correlated with its yield (Zheng *et*
17 *al.*, 2020). Compared to SP, PH and BN of ‘Eyou Changjia’ (a P-efficient variety)
18 decreased by 11% and 25.4%, respectively; and PH and BN of B104-2’ (a P-inefficient
19 variety) decreased by 23%, and 46.9%, respectively (Ding *et al.*, 2012). In addition, the
20 PH and BN of ‘Eyou Changjia’ were higher than those of ‘B104-2’ under LP in two
21 field Trials (Ding *et al.*, 2012). Cultivars ‘Tapidor’ and ‘Ningyou 7’ are parental
22 cultivars of the *BnaTNDH* population and PH and BN under LP were also less than

1 those under SP (Shi *et al.*, 2013). Compared to the control (no P application), the PH of
2 *B. napus* increased by 11.2%, 16.7% and 19.4%, respectively; and the BN of *B. napus*
3 increased by 25.5%, 36.2% and 40.4% in the treatments of P application rate of 103.0,
4 206.0 and 309.0 kg/hm² (Lu *et al.*, 2005).

5 Extensive phenotypic variations for PH and BN are observed in several natural
6 populations of *B. napus* at SP (Li *et al.*, 2016; Sun *et al.*, 2016; Luo *et al.*, 2017; Zheng
7 *et al.*, 2017). For example, the PH varied from 48.3 to 228.3 cm among 496 *B. napus*
8 accessions, with an average ranging from 133.5 cm to 187.3 cm across six environments
9 (Sun *et al.*, 2016); and varied from 86.2 cm to 206.0 cm among 333 *B. napus* accessions
10 across 2 years, with 1.6 to 2.4 fold variations across the two years (Zheng *et al.*, 2017).

11 In this study, we measured PH and BN in a panel of 403 *B. napus* accessions across two
12 years under both LP and SP conditions. At SP, the PH varied from 84.0 cm to 235.0 cm
13 and BN varied from 1 to 15 per plant (Table 2; Supplementary Data Table S1 and Fig.
14 S2). Compared with the association panel with 496 *B. napus* accessions (Sun *et al.*
15 2016), the natural population in this study has more extensive genetic variation at SP.
16 At LP conditions, the PH varied from 57.5 cm to 179.2 cm and BN varied from 0 to 11
17 per plant (Table 2; Supplementary Data Table S1 and Fig. S2). These data show that P
18 deficiency significantly reduced the PH and BN of *B. napus* compared to those grown
19 under SP in our study and others. In addition, extensive phenotypic variations for PH
20 and BN at LP would be useful for the screening the P efficient cultivars that have greater
21 potential to yield higher under LP.

22

1 *Candidate genes associated with significant SNPs for PH and BN of B. napus under LP*

2 GWAS has been successfully applied to the genetic dissection of low-P tolerance in
3 different crops, such as in maize (Xu *et al.* 2018; Luo *et al.* 2019), rice (Wissuwa *et al.*
4 2015), wheat (Liu *et al.* 2015), soybean (Zhang *et al.* 2014) and oilseed rape (Wang *et al.*
5 *et al.* 2017). However, up to now, there is no report on the GWAS of *B. napus* PH and BN
6 under LP, major determinants of seed yield and yield in *B. napus*. A total of 2127
7 significant SNPs were detected for PH and BN and thirty-three SNPs were identified
8 co-localized with previously-identified QTLs (Supplementary Data Table S5, S6, S9).
9 Closer examination of allelic frequency of the four co-located SNPs on chromosome
10 A02 among this association panel indicated that the inbred lines carrying a minor allele
11 have lower PH than inbred lines carrying major allele under LP (Fig. 3D). Interestingly,
12 the PH of the accessions carrying the minor allele and major allele did not meet
13 significant difference at a sufficient P supply (Supplementary Data Fig. S8). The four
14 co-localized SNPs decrease the PH at low P and might affect the seed yield.
15 ChrA10_8216711 for PH_LP and PHr in both tria2_R2 and tria2_BLUP was located
16 in the genetic region of a gene with unknown function, *BnaA10g09290D*.
17 ChrC08_27999846 for PHr was located in the exon of *BnaC08g26640D*. We performed
18 candidate gene association analysis and haplotype analysis for *BnaA10g09290D* and
19 *BnaC08g26640D*, and high P efficiency haplotypes of “*BnaA10g09290Hap1*” and
20 “*BnaC08g26640Hap1*” were identified, respectively (Fig. 1G, Fig. 1L, Fig. 2G).

21 In addition, some genes reported to be associated with P uptake and homeostasis were
22 also detected in our association analysis (Supplementary Data Table S11). The

1 *PHOSPHATE1 (PHO1)*, a gene which encodes a membrane protein consisting of SPX,
 2 plays an important role in loading P into xylem in roots (Hamburger *et al.* 2002; Pacak
 3 *et al.* 2016; Che *et al.* 2020). The peak SNP ‘chrA07_6641098’ on A07 was identified
 4 to be located within 280 kb of *B. napus BnaA07.PHO1* (Supplementary Data Table S11).
 5 Many plant proteins with SPX domains are involved in Pi signaling (Wang *et al.* 2004;
 6 Duan *et al.* 2008). In this study, *BnaC03g65110D*, a gene with SPX
 7 (SYG1/Pho81/XPR1) domain-containing protein, was located within the interval of the
 8 SNP ‘chrC03_54249584’ for the trait of PHr (Supplementary Data Table S11).
 9 *BnaC05g27910D* was a homologous gene of *BnaC03g65110D*, also containing SPX
 10 (SYG1/Pho81/XPR1) domain, and was located within the interval of the leading SNP
 11 ‘chrC05_25541489’ for the trait of PHr (Supplementary Data Table S11). In
 12 *Arabidopsis*, *AtUBP14* is involved in the response of root systems to P deficiency (Lee
 13 *et al.*, 2008). In this study, *BnaA08g08470D (BnaA08.UBP20)*, a gene which contains
 14 ubiquitin-specific protease activity, was located within the interval of the SNP
 15 ‘chrA08_8488725’ and linked with the trait for PHr (Supplementary Data Table S11).
 16 PHT1 family encode plant Pi transporters to transport P into plants (Huang *et al.*, 2019).
 17 Recently, *BnaPht1;4* was found to participate in the uptake and transportation of P, and
 18 promotes seed germination and seedling growth of *B. napus* by regulating the
 19 biosynthesis of ABA and GA (Huang *et al.*, 2019). In this study, we identified the
 20 *BnaA04g22280D (BnaA04.PHT1;4)* by SNP ‘chrA04_16843321’ for BN_LP, which
 21 was likely to affect the growth and development of BN under LP. (Supplementary Data
 22 Table S11).

1 In addition, we identified BnaA09g16430D (BnaA09.Pht1;6) by SNP
2 chrA09_9605643 for BN_LP, which is located 170 kb downstream of the SNP
3 chrA09_9605643 (Supplementary Data Table S11). Purple acid phosphatases (PAPs)
4 are a family of binuclear metalloenzymes (Olczak *et al.*, 2003). Overexpression of
5 OsPAP10c (purple acid phosphatase 10c) enhances the utilization of phytate-P, root
6 growth and yield at LP in rice (Deng *et al.*, 2020). BnaA03g41050D (BnaA03.PAP2)
7 was located 310 kb downstream of the SNP chrA03_20898013 and linked with the
8 trait for BN_LP (Supplementary Data Table S11). These candidate genes might play
9 important roles in the development of PH and BN in *B. napus* at a deficient P supply.

10

11 *Co-location of the significant SNPs for root system architecture traits and PH and BN*
12 *of B. napus at a deficient P supply*

13 Previously, 285 SNPs were detected associated with root related traits of *B. napus* at
14 low P supply and a P efficient haplotype '*BnA03Hap*' on A03 chromosome was
15 identified (Wang *et al.*, 2017). Fifty-three significant SNPs in this study were adjacent
16 to previously published significant SNPs associated with root system architecture at
17 low P supply, which may control both root system and plant height or branch numbers
18 at low P (Supplementary Data Table S12). For example, SNP chrA08_10449680 was
19 associated with PHr in this study and chrA08_10481443 was associated with Primary
20 Root Length under LP (PRL_LP) in Wang et al. (2017), respectively. *BnaA08g11400D*
21 was detected by chrA08_10449680 and linked with the trait for PHr and located 106 kb
22 upstream of chrA08_10449680 (Supplementary Data Table S12). *BnaA08g11400D*

1 was homologous to *Arabidopsis* root hair defective 6-like 2 (*RSL2*). *RSL2* encodes a
2 basic Helix- Loop-Helix transcription factors that promotes root hair initiation, growth,
3 and elongation (Han *et al.*, 2017; Yi *et al.*, 2010; Mangano *et al.*, 2018). In addition,
4 mutants lacking *RSL2* have disrupted root hair formation under LP (Lan *et al.*, 2012;
5 Bhosale *et al.*, 2018). *BnaA08g11400D* may effect PH by regulating root hair
6 development at low P or have pleiotropic effects on shoot growth and development in
7 *B. napus*.

8

9 CONCLUSIONS

10 Taken together, the PH and BN of a panel of 403 *B. napus* accessions collected
11 worldwide were investigated under LP and SP, with 2127 significant SNPs associated
12 with these traits identified by GWAS. Thirty-three SNPs co-localized with previous
13 QTLs, including four under LP and 22 under SP. In addition, candidate gene association
14 and haplotype analysis of *BnaA02g33340D*, *BnaA10g09290D* and *BnaC08g26640D*
15 revealed several P tolerant haplotypes. These significant SNP loci, favorable alleles and
16 haplotypes, and the accessions carrying these desired alleles and haplotypes will be
17 useful for breeding low P tolerance *B. napus* cultivars.

18

19 FIGURE LEGEDGS

20 **Figure 1. The co-localized locus and haplotypes on chromosome A10 associated**
21 **with PH_LP and PHr of *B. napus*.** (A) Manhattan plot of co-localized locus for
22 PH_LP and PHr in Trial 2_R2 and Trial 2_BLUP. (B) Significant SNPs associated with
23 PH_LP and PHr on chromosome A10. The big red dots represent the significant SNPs.

1 (C) Candidate gene association analysis of *BnaA10g09290D* with PH_LP. Association
2 of the three alleles in chrA10_8216680 (D), chrA10_8216711 (E) and chrA10_8216756
3 (F) with PH_LP, respectively. (G) Two haplotypes of *BnaA10g09290D*. (H) Candidate
4 gene association analysis of *BnaA10g09290D* with PHr. Association of the three alleles
5 in chrA10_8216680 (I), chrA10_8216711 (J) and chrA10_8216756 (K) with PHr,
6 respectively. (L) Two haplotypes of *BnaA10g09290D*. The number of inbred lines
7 harboring the corresponding allele are shown in the bracket at the bottom. PH, plant
8 height; LP, low phosphorus supply; SP, sufficient phosphorus supply; PHr, ratio of
9 PH_LP to PH_SP. R2, replication 2; BLUP, best linear unbiased prediction.

10 **Figure 2. The co-localized locus and haplotypes on chromosome C08 associated**
11 **with PHr of *B. napus*.** (A) Manhattan plot of co-localized locus for PHr in Trial 1_R2,
12 Trial 1_R3, Trial 1_BLUP and Trial 2_BLUP. (B) Significant SNP associated with PHr
13 on chromosome C08. The big red dots represent the significant SNPs. (C) Candidate
14 gene association analysis of *BnaC08g26640D* with PHr. Association of the three alleles
15 in chrC08_27999709 (D), chrC08_27999778 (E) and chrC08_27999846 (F) with PHr,
16 respectively. (G) Two haplotypes of *BnaC08g26640D*. The number of inbred lines
17 harboring the corresponding allele are shown in the bracket at the bottom. PH, plant
18 height; LP, low phosphorus supply; SP, sufficient phosphorus supply; PHr, ratio of
19 PH_LP to PH_SP; R2, replication 2; R3, replication 3; BLUP, best linear unbiased
20 prediction.

21 **Figure 3. Co-localized locus on chromosome A02 for plant height of *B. napus* at LP.**

22 (A) QTL detected for plant height in the *BnaTNDH* population linkage analysis (Shi et

1 al., 2013). (B) Manhattan plot of PH_LP (Trial 2_R1). (C) Peak SNP for PH_LP (Trial
2 2_R1). (D) Association of the co-localized locus with plant height at LP, respectively.
3 (E) Candidate gene association analysis of *BnaA02g33340D*. (F) Association of the five
4 alleles in chrA02_23899623, chrA02_23899654, chrA02_23899669,
5 chrA02_23899686 and chrA02_23899688 with plant height at LP, respectively. (G)
6 Two haplotypes of *BnaA02g33340D*. The number of inbred lines harboring the
7 corresponding allele are shown in the bracket at the bottom. PH, plant height; LP, low
8 phosphorus supply; R1, replication 1.

1 Table 1 Soil physical and chemical properties in the field trials

Trials (Year)	Replications	pH(1: 1 H ₂ O)	Organic matter (g/kg)	Total N concentr ation (g/kg)	Available P concentrati on (mg/kg)	Total P concentrati on (g/kg)	Total K concentrati on (g/kg)
Trial 1	1	5.61	7.37	0.91	11.56	0.57	13.2
(2018-	2	5.69	8.04	1.12	11.71	0.53	12.1
2019	3	5.65	8.33	1.30	12.19	0.46	14.6
Trial 2	1	5.70	8.42	1.19	12.51	0.63	11.8
(2019-	2	5.58	7.88	1.33	16.95	0.56	10.5
2020)	3	5.56	7.03	1.06	14.10	0.62	12.7

2

3

1 Table 2. Mean, maximum (max), minimum (min), range, heritability and coefficient of variation
2 (CV, %) of the plant height (PH), branch number (BN), PHr (ratio of PH_LP to PH_SP) and BNr
3 (ratio of BN_LP to BN_SP) in an association panel of *B. napus* in Trial 1 and Trial 2, at low
4 phosphorus (LP) and sufficient phosphorus (SP) supplies.

Trait	Year	Replications	Mean	Min	Max	Range	CV	h^2 (%)
PH_LP (cm)	Trial 1	1	121.89	73.75	165.50	91.75	14.80%	63.26
		2	132.02	74.95	179.25	104.30	14.31%	
		3	132.15	79.00	179.00	100.00	14.31%	
	Trial 2	1	128.66	57.50	176.00	118.50	18.23%	
		2	126.93	60.50	170.50	110.00	16.42%	
BN_LP (No./ plant)	Trial 1	1	2.9	0.0	8.0	8.0	53.76%	61.01
		2	4.2	0.0	9.5	9.5	45.80%	
		3	4.3	0.0	10.5	10.5	46.65%	
	Trial 2	1	4.4	0.0	10.0	10.0	45.98%	
		2	4.0	0.0	11.0	11.0	51.15%	
PH_SP (cm)	Trial 1	1	137.43	90.50	194.00	103.50	12.72%	88.51
		2	151.11	84.00	207.50	123.50	12.25%	
		3	159.85	97.00	214.00	117.00	11.02%	
	Trial 2	1	170.31	97.00	216.00	119.00	12.82%	
		2	160.16	107.00	204.00	97.00	11.67%	
		3	171.78	90.00	235.00	145.00	11.76%	
BN_SP (No./ plant)	Trial 1	1	4.2	1.0	9.5	8.5	39.48%	70.12
		2	5.9	1.5	13.0	11.5	31.31%	
		3	6.7	3.0	15.0	12.0	28.75%	
	Trial 2	1	7.2	1.0	15.0	14.0	26.85%	
		2	6.7	3.0	14.0	11.0	26.69%	
		3	7.0	2.5	15.5	13.0	26.77%	
PHr	Trial 1	1	0.84	0.45	1.00	0.54	12.22%	69.64
		2	0.83	0.52	1.00	0.47	12.93%	
		3	0.81	0.50	1.00	0.50	13.98%	
	Trial 2	1	0.75	0.40	1.00	0.60	16.17%	
		2	0.73	0.40	0.97	0.57	15.93%	
BNr	Trial 1	1	0.61	0.10	1.00	0.90	40.97%	61.33
		2	0.64	0.10	1.00	0.90	37.03%	
		3	0.63	0.13	1.00	0.88	34.79%	
	Trial 2	1	0.59	0.11	1.00	0.89	35.81%	
		2	0.59	0.11	1.00	0.89	37.74%	

5
6
7

1 Table 3 List of synonymous and non-synonymous SNP variants identified in the candidate genes of
2 *BnaA02g33090D*, *BnaA10g09290D* and *BnaC08g26640D*

Gene	SNP	Major allele	Minor allele	SNP location	SNP types	Amino acid changes
<i>BnaA02g33090D</i>	chrA02_23899623	G	A	Intron	-	-
	chrA02_23899654	G	T	Intron	-	-
	chrA02_23899669	T	A	Intron	-	-
	chrA02_23899686	C	T	Exon	Synonymous	-
	chrA02_23899688	A	C	Exon	Non-synonymous	histidine to proline
<i>BnaA10g09290D</i>	chrA10_8216680	T	A	Exon	Non-synonymous	isoleucine to asparagine
	chrA10_8216711	C	T	Intron	-	-
	chrA10_8216756	A	T	Exon	Synonymous	-
<i>BnaC08g26640D</i>	chrC08_27999709	A	T	Intron	-	-
	chrC08_27999778	A	T	Exon	Non-synonymous	isoleucine to asparagine
	chrC08_27999846	T	C	Exon	Synonymous	-

3 Note: SNP, Single-nucleotide polymorphism.

1 **SUPPLEMENTARY DATA**

2 **Figure S1: Shoot and root growth of an association panel of *B. napus* in the field**

3 **at LP and SP.** (A) an association panel of *B. napus* at the seedling stage (left, SP; right,
4 LP); (B) L452 at the seedling stage (left, SP; right, LP); (C) P deficient symptoms of *B.*
5 *napus* at the seedling stage; (D) P deficient symptoms of *B. napus* at the flowering stage;
6 (E) P deficient symptoms of *B. napus* at the silique stage. (F) Difference in the plant
7 architecture in the selected lines in the association panel of *B. napus* at LP at maturity
8 stage; (G) L528 (left, LP; right, SP); (H) L768 (left, LP; right, SP); (I) L236 (left, LP;
9 right, SP). LP, low phosphorus supply; SP, sufficient phosphorus supply.

10 **Figure S2: Frequency distribution of plant height and branch number of an**

11 **association panel of *B. napus* in Trial 1 and Trial 2 at low and sufficient phosphorus**

12 **supplies.** (A) Plant height in Trial 1. (B) Plant height in Trial 2. (C) Branch number in

13 Trial 1. (D) Branch number in Trial 2. PH, Plant height; BN, Branch number; PHr, ratio

14 of PH_LP to PH_SP; BNr, ratio of BN_LP to BN_SP; LP, low phosphorus supply; SP,

15 sufficient phosphorus supply.

16 **Figure S3: Correlation coefficients of PH and BN of *B. napus* between Trial 1 and**

17 **Trial 2 at LP and SP.** (A) PH. (B) BN. PH, plant height; BN, branch number; LP, low

18 phosphorus supply; SP, sufficient phosphorus supply.

19 **Figure S4: The LD decay of an association panel of *B. napus***

20 **Figure S5: Population structure of an association panel of *B. napus* with K from 2**

21 **to 8.** (A) The x axis represented the different accessions, the y axis quantified cluster

22 membership, and each accession shown as a vertical line partitioned into K colored

1 components represented inferred membership in K genetic clusters. (B) The K value
2 estimated for population structure analysis. There was a minimum K-value when K =
3 5. CV value, cross validation value.

4 **Figure S6: The kinship of an association panel of 403 *B. napus* accessions.** (A) A
5 heatmap of the kinship value. (B) The distribution of pairwise relative kinship

6 **Figure S7: Genome-wide association study of PH and BN in an association panel**
7 **of *B. napus* at low and sufficient phosphorus supplies.** (A) Manhattan and QQ plot

8 of the GLM model (Q) for PH in Trial 1. (B) Manhattan and QQ plot of the MLM model
9 (Q+K) for PH in Trial 1. (C) Manhattan and QQ plot of the GLM model (Q) for PH in

10 Trial 2. (D) Manhattan and QQ plot of the MLM (Q+K) model for PH in Trial 2. (E)

11 Manhattan and QQ plot of the GLM model (Q) for BN in Trial 1. (F) Manhattan and

12 QQ plot of the MLM model (Q+K) for BN in Trial 1. (G) Manhattan and QQ plot of

13 the GLM model (Q) for BN in Trial 2. (H) Manhattan and QQ plot of the MLM model

14 (Q+K) for BN in Trial 2. PH, Plant height; BN, Branch number; PHr, ratio of PH_LP

15 to PH_SP; BNr, ratio of BN_LP to BN_SP; LP, low phosphorus supply; SP, sufficient

16 phosphorus supply.

17 **Figure S8: GO enrichment analysis of candidate genes.** (A) GO enrichment analysis

18 of candidate genes within 300 kb up and down the lead SNP of chrA10__8216711 on

19 A10 chromosome for PH_LP. (B) GO enrichment analysis of candidate genes within

20 300 kb up and down the lead SNP of chrC08__27999846 on C08 chromosome for PHr.

21 (C) GO enrichment analysis of candidate genes within 300 kb up and down the lead

22 SNP of chrA01__13846343 on A01 chromosome for BN_LP. (D) GO enrichment

1 analysis of candidate genes within 300 kb up and down the lead SNP of
 2 chrA03__20898013 on A03 chromosome for BN_LP. (E) GO enrichment analysis of
 3 candidate genes within 300 kb up and down the lead SNP of chrC07__521008 on C07
 4 chromosome for BN_LP. (F) GO enrichment analysis of candidate genes located in the
 5 QTL linkage disequilibrium intervals on chromosome A02 for PH_LP. Blue, green, and
 6 red bars indicate molecular function, cellular component and biological process,
 7 respectively. PH, Plant height; BN, Branch number; PHr, ratio of PH_LP to PH_SP; LP,
 8 low phosphorus supply; SP, sufficient phosphorus supply.

9 **Figure S9: Association of alleles for PH_SP.** (A) chrA02_23692807, (B)
 10 chrA02_23713660, (C) chrA02_23899688, (D) chrA02_23912345, (E)
 11 chrA02_23899623, (F) chrA02_23899654, (G) chrA02_23899669, (H)
 12 chrA02_23899686. The number of inbred lines harboring the corresponding allele are
 13 shown in the bracket at the bottom. SP, a sufficient phosphorus supply.

14

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LITERATURE CITED

- Barrett JC, Fry B, Maller J, et al. 2005.** Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**: 263-265.
- Bhosale R, Giri J, Pandey BK, et al. 2018.** A mechanistic framework for auxin dependent *Arabidopsis* root hair elongation to low external phosphate. *Nature Communications* **9**: 1409.
- Bradbury PJ, Zhang Z, Kroon DE, et al. 2007.** TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**: 2633-2635.
- Chen B, Xu K, Li J, et al. 2014.** Evaluation of yield and agronomic traits and their genetic variation in 488 global collections of *Brassica napus* L. *Genetic Resources and Crop Evolution* **61**: 979-999.
- Che J, Yamaji N, Miyaji T, et al. 2020.** Node-localized transporters of phosphorus essential for seed development in rice. *Plant cell physiology* **61**: 1387-1398.
- Chen ZH, Jenkins I, Nimmo HG, et al. 2008.** Identification of an F-box protein that negatively regulates Pi starvation responses. *Plant cell physiology* **49**: 1902-1906.
- Crenrn JM, Srrupsow GM. 1978.** Influence of irrigation and sebding rates on yield and yield components of *Brassica napus*. *Canadian Journal of Plant Science* **58**: 731-737.
- Dai X, Wang Y, Zhang WH, et al. 2016.** OsWRKY74, a WRKY transcription factor, modulates tolerance to phosphate starvation in rice. *Journal of experimental botany* **67**: 947-960.

1 **Deng S, Lu L, Li JL, et al. 2020.** *Purple acid phosphatase 10c* encodes a major acid
2 phosphatase that regulates plant growth under phosphate-deficient conditions in
3 rice. *Journal of experimental botany* **71**: 4321-4332.

4 **Ding GD, Zhao ZK, Liao Y, et al. 2012.** Quantitative trait loci for seed yield and yield-
5 related traits, and their responses to reduced phosphorus supply in *Brassica*
6 *napus*. *Annals of Botany* **109**: 747-759.

7 **Duan K, Yi KK, Dang L, et al. 2008.** Characterization of a sub-family of *Arabidopsis*
8 genes with the SPX domain reveals their diverse functions in plant tolerance to
9 phosphorus starvation. *The Plant Journal* **54**: 965-975.

10 **Han HM, Wang HF, Han Y, et al. 2017.** Altered expression of the TaRSL2 gene
11 contributed to variation in root hair length during allopolyploid wheat evolution.
12 *Planta* **246**: 1019-1028.

13 **Han K, Lee HY, Ro NY, et al. 2018.** QTL mapping and GWAS reveal candidate genes
14 controlling capsaicinoid content in *Capsicum*. *Plant biotechnology journal* **16**:
15 1548-1558.

16 **Hamburger D, Rezzonico E, MacDonald CP, et al. 2002.** Identification and
17 characterization of the *Arabidopsis* PHO1 gene involved in phosphate loading
18 to the xylem. *Plant Cell* **14**: 889-902.

19 **He QJ, Lu H, Guo HX, et al. 2020.** OsbHLH6 interacts with OsSPX4 and regulates
20 the phosphate starvation response in rice. *The Plant Journal* **105**: 649-667.

21 **He YJ, Wu DM, Wei DY, et al. 2017.** GWAS, QTL mapping and gene expression
22 analyses in *Brassica napus* reveal genetic control of branching morphogenesis.

1 *Scientific Reports* **7**: 15971.

2 **Huang KL, Wang H, Wei YL, et al. 2019.** The high-affinity transporter *BnPHT1;4* is
3 involved in phosphorus acquisition and mobilization for facilitating seed
4 germination and early seedling growth of *Brassica napus*. *BMC Plant*
5 *Biology* **19**: 156.

6 **Holford ICR. 1997.** Soil phosphorus: its measurement and its uptake by plants.
7 *Australian Journal of Soil Research* **35**: 227 -39.

8 **Korte A, Farlow A. 2013.** The advantages and limitations of trait analysis with GWAS:
9 a review. *Plant Methods* **9**: 29.

10 **Jain A, Vasconcelos MJ, Raghothama K, et al. 2007.** Molecular mechanisms of plant
11 adaptation to phosphate deficiency. *Plant breeding reviews* **29**: 359 -419.

12 **Kumar SA, Muhlroth A, Jouhet J, et al. 2020.** The Myb-like transcription factor
13 phosphorus starvation response (PtPSR) controls conditional P acquisition and
14 remodelling in marine microalgae. *New phytologist* **225**: 2380-2395.

15 **Kochian LV. 2012.** Plant nutrition: Rooting for more phosphorus. *Nature* **488**: 7412,
16 466-467.

17 **Lan P, Li W, Wen TN, et al. 2012.** Quantitative phosphoproteome profiling of iron-
18 deficient Arabidopsis roots. *Plant physiology* **159**: 403-417.

19 **Laperche A, Aigu Y, Jubault M, et al. 2017.** Clubroot resistance QTL are modulated
20 by nitrogen input in *Brassica napus*. *Theor Appl Genet* **130**: 669-684.

21 **Lee YS, Huang KX, Florante AQ, et al. 2008.** Molecular basis of cyclin-CDK-CKI
22 regulation by reversible binding of an inositol pyrophosphate. *Nature Chemical*

1 *Biology* **4**: 25 -32.

2 **Li S, Zhu Y, Varshney RK, et al. 2020.** A systematic dissection of the mechanisms
3 underlying the natural variation of silique number in rapeseed (*Brassica napus*
4 L.) germplasm. *Plant biotechnology journal* **18**: 568-580.

5 **Li TG, Ma XF, Li NY, et al. 2017.** Genome-wide association study discovered
6 candidate genes of Verticillium wilt resistance in upland cotton (*Gossypium*
7 *hirsutum* L.). *Plant biotechnology journal* **15**: 1520-1532.

8 **Li C, Liu XY, Ruan H, et al. 2019.** *GmWRKY45* Enhances Tolerance to Phosphate
9 Starvation and Salt Stress, and Changes Fertility in Transgenic *Arabidopsis*.
10 *Frontiers in Plant Science* **10**: 1714.

11 **Li F, Chen B, Xu K, et al. 2016.** A genome-wide association study of plant height and
12 primary branch number in rapeseed (*Brassica napus*). *Plant science* **242**: 169-
13 177.

14 **Lu JW, Chen F, Zhang ZQ, et al. 2005.** Effect of phosphor application rate on rapeseed
15 yield, nutrient absorption and profit. Chinese journal of oil crop sciences 27 (in
16 Chinese with English abstract).

17 **Luo X, Ding Y, Zhang LZ, et al. 2017.** Genomic prediction of genotypic effects with
18 epistasis and environment interactions for yield-related traits of rapeseed
19 (*Brassica napus* L.). *Front Genet* **8**: 15.

20 **Lin KF, Tsai MY, Lu CA, et al. 2018.** The roles of *Arabidopsis* HSFA2, HSFA4a, and
21 HSFA7a in the heat shock response and cytosolic protein response. *Botanical*
22 *studies* **59**: 15.

1 **Liu Y, Wang L, Deng M, et al. 2015.** Genome-wide association study of phosphorus-
2 deficiency-tolerance traits in *Aegilops tauschii*. *Theoretical and Applied*
3 *Genetics* **128**: 2203-2212.

4 **Luo B, Ma P, Nie Z, et al. 2019.** Metabolite profiling and genome-wide association
5 studies reveal response mechanisms of phosphorus deficiency in maize seedling.
6 *The Plant Journal* **97**: 947-969.

7 **Mangano S, Denita-Juarez SP, Marzol E, et al. 2018.** High auxin and high phosphate
8 impact on RSL2 expression and ROS-homeostasis linked to root hair growth in
9 *Arabidopsis thaliana*. *Frontiers in Plant Science* **9**: 1164.

10 **Murakami H, Kakutani N, Kuroyanagi Y, et al 2020.** MYB-like transcription factor
11 NoPSR1 is crucial for membrane lipid remodeling under phosphate starvation
12 in the oleaginous microalga *Nannochloropsis oceanica*. *Febs Letters* **594**: 3384-
13 3394.

14 **Nordborg M, Weigel D. 2008.** Next-generation genetics in plants. *Nature* **456**: 720-
15 723.

16 **Olczak M, Morawiecka B, Watorek W. 2003.** Plant purple acid phosphatases-genes,
17 structures and biological function. *Acta Biochimica Polonica* **50**: 1245-1256.

18 **Pacak A, Barciszewska PM, Swida BA, et al. 2016.** Heat stress affects Pi-related
19 genes expression and inorganic phosphate deposition/accumulation in barley.
20 *Frontiers in Plant Science* **7**: 926.

21 **Pu Z, Pei Y, Yang J, et al. 2018.** A QTL located on chromosome 3D enhances the
22 selenium concentration of wheat grain by improving phytoavailability and root

1 structure. *Plant and Soil* **425**: 287-296.

2 **Qiu D, Morgan C, Shi J, et al .2006.** A comparative linkage map of oilseed rape and
3 its use for QTL analysis of seed oil and erucic acid content. *Theoretical and*
4 *Applied Genetics* **114**: 67-80.

5 **Rose TJ, Wissuwa M. 2012.** Rethinking internal phosphorus utilization efficiency: a
6 new approach is needed to improve PUE in grain crops. *In Advances in*
7 *Agronomy Volume* **116**: 185-217.

8 **Shi T, Li R, Zhao Z, et al. 2013.** QTL for yield traits and their association with
9 functional genes in response to phosphorus deficiency in *Brassica napus*. *Plos*
10 *One* **8**: e54559.

11 **Si LZ, Chen JY, Huang XH, et al .2016.** *OsSPL13* controls grain size in cultivated
12 rice. *Nature genetics* **48**: 447-456.

13 **Su JY, Xiao YM, Li M, et al. 2006.** Mapping QTLs for phosphorus-deficiency
14 tolerance at wheat seedling stage. *Plant and Soil* **281**: 25-36.

15 **Sun CM, Wang BQ, Yan L, et al. 2016.** Genome-wide association study provides
16 insight into the genetic control of plant height in rapeseed (*Brassica napus* L.).
17 *Frontiers in Plant Science* **7**:1102.

18 **Tang S, Zhao H, Lu SP, et al. 2020.** Genome- and transcriptome-wide association
19 studies provide insights into the genetic basis of natural variation of seed oil
20 content in *Brassica napus*. *Molecular plant* **1**: 470-484.

21 **Vance CP, Uhde-Stone C, Allan DL. 2003.** Phosphorus acquisition and use: critical
22 adaptations by plants for securing a nonrenewable resource. *New phytologist*

1 157: 423-447.

2 **Wang Xh, Chen Yl, Thomas CL, et al. 2017.** Genetic variants associated with the root
3 system architecture of oilseed rape (*Brassica napus* L.) under contrasting
4 phosphate supply. *DNA Research* **24**: 407-417.

5 **Wang Y, Ribot C, Rezzonico E, et al. 2004.** Structure and expression profile of the
6 *Arabidopsis* PHO1 gene family indicates a broad role in inorganic phosphate
7 homeostasis. *Plant Physiology* **135**: 400-411.

8 **Wissuwa M, Kondo K, Fukuda T, et al. 2015.** Unmasking novel loci for internal
9 phosphorus utilization efficiency in rice germplasm through genome-wide
10 association analysis. *Plos One* **10**: e0124215.

11 **Wissuwa M, Wegner J, Ae N, Yano M .2002.** Substitution mapping of Pup1: a major
12 QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil.
13 *Theoretical and Applied Genetics* **105**: 890-897.

14 **Wu P, Xu J. 2010.** Does OsPHR2, central Pi-signaling regulator, regulate some
15 unknown factors crucial for plant growth? *Plant Signal Behavior* **5**: 712-714.

16 **Xiao YJ, Liu HJ, Wu LJ, et al. 2017.** Genome-wide association studies in maize:
17 Praise and Stargaze. *Molecular plant* **10**: 359-374.

18 **Xu C, Zhang Hw, Sun JH, et al. 2018.** Genome-wide association study dissects yield
19 components associated with low-phosphorus stress tolerance in maize.
20 *Theoretical and Applied Genetics* **131**, 1699-1714.

21 **Xu LP, Hu KN, Zhang ZQ, et al. 2016.** Genome-wide association study reveals the
22 genetic architecture of flowering time in rapeseed (*Brassica napus* L.). *DNA*

1 *Research* **23**, 43-52.

2 **Yang M, Ding GD, Shi L, et al. 2010a.** Quantitative trait loci for root morphology in
3 response to low phosphorus stress in *Brassica napus*. *Theoretical and Applied*
4 *Genetics* **121**: 181-193.

5 **Yang SY, Huang TK, Kuo HF, et al. 2017.** Role of vacuoles in phosphorus storage
6 and remobilization. *Journal of experimental botany* **68**: 3045-3055.

7 **Yang M, Ding GD, Shi L, et al. 2010b.** Detection of QTL for phosphorus efficiency at
8 vegetative stage in *Brassica napus*. *Plant and Soil* **339**: 97-111.

9 **Yi K, Menand B, Bell E, et al 2010.** A basic helix-loop-helix transcription factor
10 controls cell growth and size in root hairs. *Nature genetics* **42**: 264-267.

11 **Zhang C, Dong SS, Xu JY, et al. 2019.** PopLDdecay: a fast and effective tool for
12 linkage disequilibrium decay analysis based on variant call format files.
13 *Bioinformatics* **35**: 1786-1788.

14 **Zhang D, Song H, Cheng H, et al. 2014.** The acid phosphatase-encoding gene
15 *GmACPI* contributes to soybean tolerance to low-phosphorus stress. *Plos*
16 *genetics* **10**: e1004061.

17 **Zhang Y, Thomas CL, Xiang JX, et al. 2016.** QTL meta-analysis of root traits in
18 *Brassica napus* under contrasting phosphorus supply in two growth systems.
19 *Scientific Reports* **6**: 33113.

20 **Zheng M, Peng C, Liu H, et al. 2017.** Genome-wide association study reveals
21 candidate genes for control of plant height, branch initiation height and branch
22 number in rapeseed (*Brassica napus* L.). *Frontiers in Plant Science* **8**: 1246.

1 **Zheng M, Zhang L, Tang M, et al. 2020.** Knockout of two *BnaMAX1* homologs by
2 CRISPR/Cas9-targeted mutagenesis improves plant architecture and increases
3 yield in rapeseed (*Brassica napus* L.). *Plant biotechnology journal* **18**: 644-654.

4 **Zhang Y, Wan J, He L, et al. 2019.** Genome-wide association analysis of plant height
5 using the maize F1 Population. *Plants (Basel)* **8**: 432.

6 **Zou J, Lu JW, Chen F, et al. 2009.** Study on abundance and deficiency indices of soil
7 available P, K and B for winter rapeseed in Yangtze River Valley based on ASI
8 method. *Scientia Agricultura Sinica*. **42**: 2028–2033. (in Chinese with English
9 abstract)

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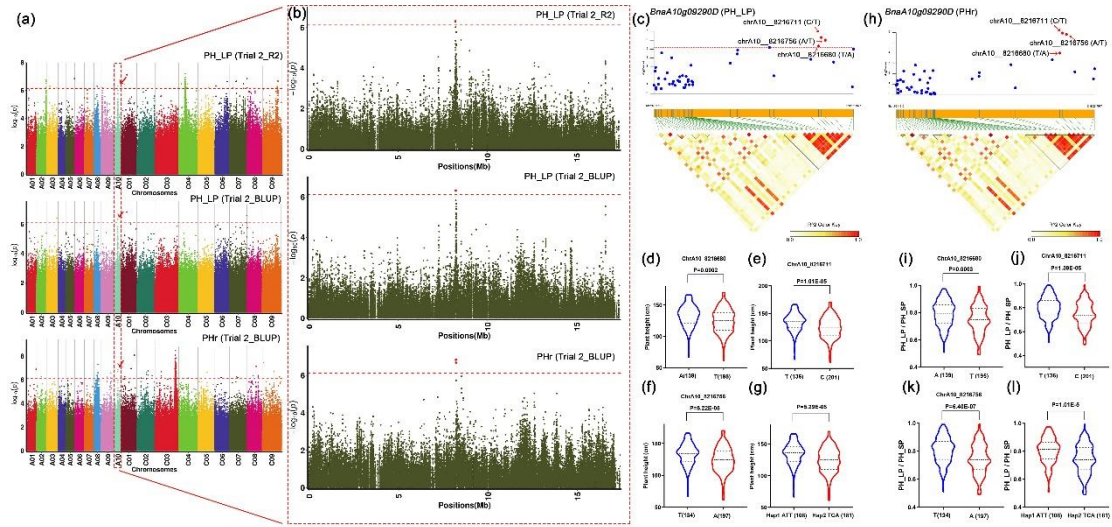


Fig. 1. The co-localized locus and haplotypes on chromosome A10 associated with PH_LP and PHr of *B. napus*. (A) Manhattan plot of co-localized locus for PH_LP and PHr in Trial 2_R2 and Trial 2_BLUP. (B) Significant SNPs associated with PH_LP and PHr on chromosome A10. The big red dots represent the significant SNPs. (C) Candidate gene association analysis of *BnaA10g09290D* with PH_LP. Association of the three alleles in chrA10_8216680 (D), chrA10_8216711 (E) and chrA10_8216756 (F) with PH_LP, respectively. (G) Two haplotypes of *BnaA10g09290D*. (H) Candidate gene association analysis of *BnaA10g09290D* with PHr. Association of the three alleles in chrA10_8216680 (I), chrA10_8216711 (J) and chrA10_8216756 (K) with PHr, respectively. (L) Two haplotypes of *BnaA10g09290D*. The number of inbred lines harboring the corresponding allele are shown in the bracket at the bottom. PH, plant height; LP, low phosphorus supply; SP, sufficient phosphorus supply; PHr, ratio of PH_LP to PH_SP. R2, replication 2; BLUP, best linear unbiased prediction.

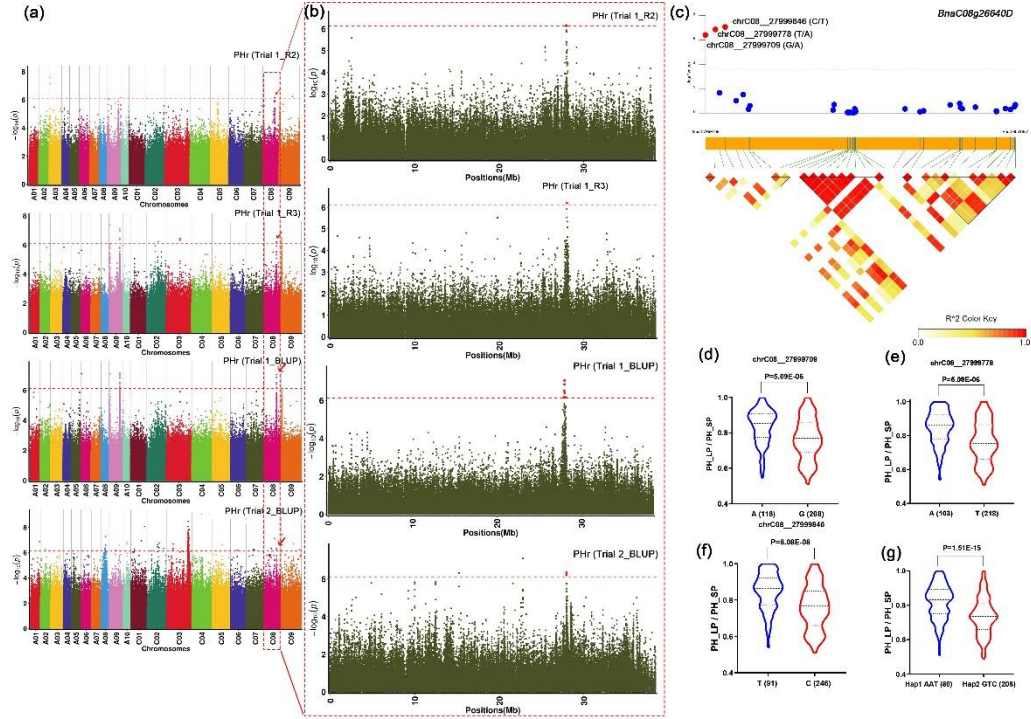


Fig. 2. The co-localized locus and haplotypes on chromosome C08 associated with PHr of *B. napus*. (A) Manhattan plot of co-localized locus for PHr in Trial 1_R2, Trial 1_R3, Trial 1_BLUP and Trial 2_BLUP. (B) Significant SNP associated with PHr on chromosome C08. The big red dots represent the significant SNPs. (C) Candidate gene association analysis of *BnaC08g26640D* with PHr. Association of the three alleles in chrC08_27999709 (D), chrC08_27999778 (E) and chrC08_27999846 (F) with PHr, respectively. (G) Two haplotypes of *BnaC08g26640D*. The number of inbred lines harboring the corresponding allele are shown in the bracket at the bottom. PH, plant height; LP, low phosphorus supply; SP, sufficient phosphorus supply; PHr, ratio of PH_LP to PH_SP; R2, replication 2; R3, replication 3; BLUP, best linear unbiased prediction.

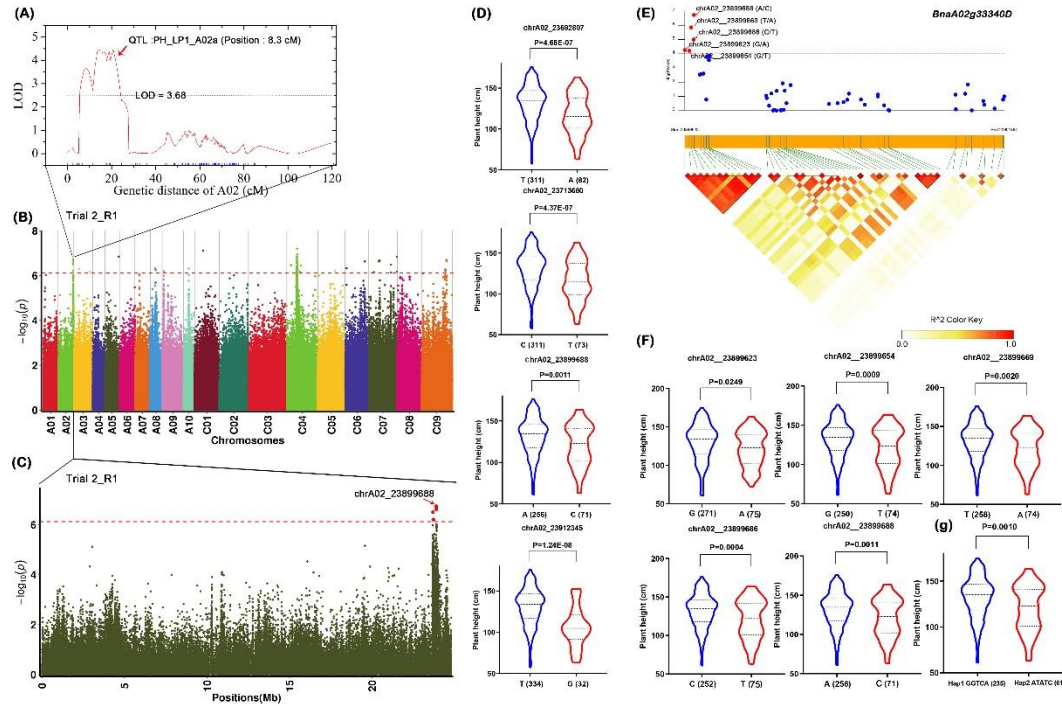


Fig. 3 Co-localized locus on chromosome A02 for plant height of *B. napus* at LP. (A) QTL detected for plant height in the *BnaTNDH* population linkage analysis (Shi *et al.*, 2013). (B) Manhattan plot of PH_LP (Trial 2_R1). (C) Peak SNP for PH_LP (Trial 2_R1). (D) Association of the co-localized locus with plant height at LP, respectively. (E) Candidate gene association analysis of *BnaA02g33340D*. (F) Association of the five alleles in chrA02_23899623, chrA02_23899654, chrA02_23899669, chrA02_23899686 and chrA02_23899688 with plant height at LP, respectively. (G) Two haplotypes of *BnaA02g33340D*. The number of inbred lines harboring the corresponding allele are shown in the bracket at the bottom. PH, plant height; LP, low phosphorus supply; R1, replication 1.