



Identification of QTLs for relative root traits associated with phosphorus efficiency in two culture systems in Brassica napus

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1 **Identification of QTLs for relative root traits associated with phosphorus efficiency in two**
2 **culture systems in *Brassica napus***

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33

34 **Abstract** Modifications of root system morphology and architecture are considered important
35 strategies of plant tolerance to phosphorus (P) deficiency. However, the effect of culture system
36 on the responses of root traits to P deficiency is not well documented. In this study, the responses
37 of root traits to P deficiency were recorded in a *Brassica napus* double haploid population
38 consisting of 182 lines derived from a cross between cultivar ‘Tapidor’ and ‘Ningyou 7’ using
39 an ‘agar’ system and a ‘pouch and wick’ system. Under P deficient conditions, more DH lines
40 had greater total root length, primary root length, total lateral root length, mean lateral root
41 length and less lateral root density in the ‘pouch and wick’ system than the ‘agar’ system. Ten
42 and two quantitative trait loci (QTLs) were detected for the relative root traits in the ‘agar’
43 system and the ‘pouch and wick’ system, respectively. The QTL for the same trait in the ‘agar’
44 system did not overlap with that in the ‘pouch and wick’ system. Two QTL clusters identified
45 in the ‘agar’ system were located on chromosome A09 (Cluster1 and Cluster2) and one on C04
46 (Cluster3), respectively. RLRN_A04b, RSDW_A09a and Cluster1 were found to affect the seed
47 yield and/or yield-related traits in two field trials. Overall, this study demonstrated a significant
48 impact of different culture systems on the responses of root traits to P deficiency and on the
49 detection of QTLs for the relative root traits, and identified three major QTLs that could be
50 employed for marker assisted selection of P efficient cultivars.

51

52 **Keywords** Root traits; Quantitative trait loci (QTLs); Phosphorus deficiency; ‘agar’ system;
53 ‘pouch and wick’ system; *Brassica napus*

54 **Abbreviations** DH, double haploid; LP, a low phosphorus supply; LRD, lateral root density;
55 LRL, total lateral root length; LRN, lateral root number; MLRL, mean lateral root length; OP,
56 an optimal phosphorus supply; P, phosphorus; Pi, inorganic phosphate; PRL, primary root
57 length; QTL, quantitative trait loci; RLRD, relative lateral root density; RLRL, relative total
58 lateral root length; RLRN, relative lateral root number; RMLRL, relative mean lateral root
59 length; RPRL, relative primary root length; RRFW, relative root fresh weight; RSDW, relative
60 shoot dry weight; RTDW, relative total dry weight; RTRL, relative total root length; TRL, total
61 root length

62 **Introduction**

63

64 Phosphorus (P) is a component of cellular membranes as phospholipids, and is involved in
65 multiple biological functions, such as energy transfer, photosynthesis, metabolic processes,
66 intracellular signal transduction and gene replication and expression (Hawkesford et al. 2012).
67 However, 50% of agricultural soils in the world are deficient in plant-available P, which leads
68 to growth reduction, developmental delays, and severe crop failures (Lynch 2011; Elser 2012).
69 In response to persistent P deficiency, plants have evolved a wide array of adaptive mechanisms
70 to improve P acquisition efficiency and P utilization efficiency, including increased root/shoot
71 ratio, modifications in root architecture to forage soil horizons for high phytoavailable P,
72 increased number and length of lateral roots and root hairs, the induction of high-affinity
73 inorganic phosphate (Pi) transporters, more exudation of acid phosphatases, organic acids or
74 protons, symbiosis with arbuscular mycorrhizal (AM) fungi and change of metabolic processes
75 (Hermans et al. 2006; Fita et al. 2011; Tian et al. 2012; Veneklaas et al. 2012; Haling et al. 2013;
76 Lambers et al. 2013; White et al. 2013a, 2013b; Lapis-Gaza et al. 2014; López-Arredondo et al.
77 2014; Walder et al. 2015).

78 The alteration of root system architecture is a well-documented phenomenon in response to
79 P starvation (Liao et al. 2004; Zhu et al. 2005a, 2005b; Wang et al. 2010; Bayuelo-Jiménez et
80 al. 2011; Lambers et al. 2011, 2013; Lynch 2011). In the model plant *Arabidopsis*, root system
81 architecture responses to P deficiency have been well characterized (White et al. 2005).
82 Typically, a reduction of the primary root length (Williamson et al. 2001; Linkohr et al. 2002;
83 López-Bucio et al. 2002; Svistoonoff et al. 2007) concomitantly associated with an increase in
84 the number and length of lateral roots (Williamson et al. 2001; Linkohr et al. 2002; Al-Ghazi et
85 al. 2003; López-Bucio et al. 2005; Nacry et al. 2005; Reymond et al. 2006) are observed in P-
86 starved *Arabidopsis*, but this root responses are largely genotype dependent. Compared with a
87 P-rich medium, 37 of 73 *Arabidopsis* ecotypes showed both reduced primary root length and
88 lateral root number at the P-poor medium, and 25% were affected in only one trait while the
89 remaining accessions displayed no response to P availability (Chevalier et al. 2003), suggesting
90 different physiological strategies are exploited to adapt to P deficiency within a species.

91 Additionally, the growth medium has strong effect on the root responses to P deficiency. For
92 example, when grown in P-deficient nutrient solution, most of the tested rice genotypes formed
93 longer root hairs, but many of these rice varieties tended to produce shorter root hairs in an
94 upland field with a low P supply (Nestler and Wissuwa 2016).

95 Two genes have been cloned for the modifications of the root traits response to P starvation
96 by forward genetics (Svistoonoff et al. 2007; Gamuyao et al. 2012). In *Arabidopsis*, *Low*
97 *Phosphate Root1 (LPR1)* encoding a multicopper oxidase (MCO) functionally plays an
98 important role in primary root development in response to P deficiency (Svistoonoff et al. 2007).
99 In rice, *phosphorus-starvation tolerance 1 (PSTOL1)* was identified to regulate the early crown
100 root development and root proliferation at a low P supply (Gamuyao et al. 2012).

101 Traits related to P efficiency in plants are generally divided into single traits and relative traits
102 (Wang et al. 2018). Relative traits are calculated as the quotient of the value of a trait observed
103 when plants are grown at a reduced P supply divided by the value of the trait when plants are
104 grown with optimal P nutrition. These include the P efficiency coefficient (i.e. the ratio of
105 biomass at the seedling stage or grain yield at maturity in plants grown with a low versus an
106 optimal P supply) which has been used to evaluate tolerance to P deficiency in oilseed rape
107 (Duan et al. 2009) and rice (Ni et al. 1998; Ming et al. 2000). Although single traits are more
108 commonly used in quantitative trait loci (QTLs) mapping studies for P efficiency traits and in
109 breeding programs, relative traits indicate the tolerance of a genotype to reduced P availability.
110 Thus the co-located QTLs both for a single trait and for a relative trait should be more useful
111 than the QTLs only for a single trait in the breeding of P efficient cultivars (Wang et al. 2018).

112 Oilseed rape (*Brassica napus* L.) is commonly used to produce cooking oil for human
113 consumption, fodder for animal feeds and renewable feedstock for biodiesel production (Liu et
114 al. 2015). Despite the many QTLs for P-efficiency related traits that have been identified at the
115 seedling stage (Yang et al. 2010, 2011; Shi et al. 2013a; Zhang et al. 2016; Wang et al. 2017)
116 and mature stage (Ding et al. 2012; Shi et al. 2013b), no QTL for P efficiency has been cloned
117 and functionally characterized in *Brassica napus* (*B. napus*) so far. In this study, the genetic
118 variations of the root morphological traits in a double haploid (DH) population of *B. napus*
119 (*Bna*TNDH population) derived from a cross between Tapidor and Ningyou 7 were investigated

120 at a low and an optimal P supply with an ‘agar’ system and a ‘pouch and wick’ system. Ningyou
121 7 was found to have a higher seed yield than that of Tapidor at a low P supply in both pot culture
122 and field trials (Shi et al. 2010, 2013b). The relative root traits were employed to identify QTLs
123 for the plasticity of root traits in response to P deficiency. These will contribute to the
124 understanding of the effect of growth environments on the seedling root traits responding to P
125 deficiency and their QTLs.

126

127 **Materials and methods**

128

129 **Plant materials**

130

131 The *BnaTNDH* mapping population consisted of 182 lines, which was generated through anther
132 culture of the F₁ generation of a cross between *B. napus* cultivar Tapidor and Ningyou 7 (Qiu
133 et al. 2006).

134

135 **High throughput phenotyping and data analysis**

136

137 In the ‘agar’ system, the root traits and biomass traits of the *BnaTNDH* population and the
138 parents had been screened previously at a Pi concentration of 0 mM (a low phosphorus supply,
139 LP) and 0.625 mM (an optimal phosphorus supply, OP), respectively (Shi et al. 2013a). Briefly,
140 surface sterilized seeds were sown into vented polystyrene trays (QTray; 240 × 240 × 20 mm;
141 Molecular Devices, Hampshire, UK) containing 300 mL 0.8% (w/v) agar and a modified basal
142 salt mix (Murashige and Skoog 1962) with either OP added as KH₂PO₄ or LP, with 0.625 mM
143 KCl added to provide K. Seeds were sown 3 cm from the top edge of the tray, with four seeds
144 per line and two lines per tray. Trays were sealed with Nescofilm and placed 10° from vertical
145 in a growth room under a 16-h photoperiod at a constant temperature of 24 °C. Illumination
146 was provided by a bank of 84 100-W cool fluorescent tubes (Philips, Eindhoven, Netherlands),
147 giving a photon flux density between 400 and 700 nm of 80–100 μmol photons m⁻² s⁻¹ at plant
148 height. For each line, 16 seeds were sown across four independent replicates, at both LP and

149 OP. Trays were placed randomly within the growth room. Images of the root systems were
150 captured using a flatbed scanner (Scanjet 3670; Hewlett-Packard, Palo Alto, CA, USA) 12 d
151 after sowing. At harvest, shoot and root fresh weight were determined, respectively. Tissue
152 samples were dried at 80 °C and dry weights (shoot dry weight; root dry weight) determined.
153 Images were loaded into ImageJ (Abràmoff et al. 2004). Primary root length (PRL) and total
154 lateral root length (LRL) were measured. Lateral root numbers (LRN) were counted and used
155 to calculate lateral root density (LRD, LRN/PRL) and mean lateral root length (MLRL,
156 LRL/LRN). Total root length (TRL) was calculated as the sum of PRL and LRL. Raw data were
157 entered into GenStat (15th Edition, VSN International Ltd, Hemel Hempstead, UK). To acquire
158 adjusted line means, the REML (residual maximum likelihood) procedure was performed using
159 the $([P]_{\text{ext}} + \text{Line} + [P]_{\text{ext}} \times \text{Line})$ term as a fixed factor and (Replicate + Replicate/Run +
160 Replicate/Run/Plate + Replicate/Run/Plate/Position) as a random factor.

161 In the ‘pouch and wick’ system, the root traits of the *Bna*TNDH population and the parents
162 had also been investigated previously at a Pi concentration of 0 mM (LP) and 0.25 mM (OP),
163 respectively (Zhang et al. 2016). Briefly, this system comprised growth pouches assembled
164 from blue germination paper (SD7640; Anchor Paper Company, St Paul, MN, USA), re-cut to
165 24 × 30 cm and overlain with black polythene (Cransford Polythene Ltd, Woodbridge, UK).
166 Along their shorter edges, the paper and polythene were clipped together using ‘bulldog’-type
167 fold-back clips to each side of an acrylic bar (Acrylic Online, Hull, UK) giving 2 germination
168 papers per pouch. The growth pouches were suspended above plastic drip trays containing a ¼
169 strength Hoagland’s solution (No. 2 Basal Salt Mixture, Sigma Aldrich, Dorset, UK) with either
170 OP added as KH₂PO₄ or LP, with 0.125 mM K₂SO₄ added for balanced K, supported within
171 lightweight aluminium/polycarbonate frames. A single seed was sown in the middle of the
172 upper edge of each germination paper by pressing the seed into the paper. Each genotype was
173 grown in one experimental run under a 12 h photoperiod with 18/15 °C day/night temperatures
174 and relative humidity of 60–80%, and pouches were randomly allocated to a position within
175 each column of each tank, giving ~24 replicates per run. Photosynthetically Active Radiation
176 (PAR; measured at plant height with a 190 SB quantum sensor; LI-COR Inc., Lincoln, NE,
177 USA) was 207 μmol m⁻² s⁻¹, generated by 400 W white fluorescent lamps (HIT 400w/u/Euro/4K,

178 Venture Lighting, Rickmansworth, UK). Drip trays were replenished with 500 mL of deionized
179 water every 3 d. Fourteen days after sowing, the polythene sheets were removed from all
180 pouches and images were taken using a Digital Single Lens Reflex (DSLR) camera (Canon
181 EOS 1100D, Canon Inc., Tokyo, Japan) with a focal length of 35 mm at a fixed height of 75
182 cm. The root images were cropped by reducing extraneous pixels on bulked images, using
183 XnConvert (Version 1.66, www.xnconvert.com). Cropped images were analysed using
184 RootReader2D (RR2D). PRL, LRL and LRN were automatically calculated by RR2D. LRD
185 was calculated as the ratio of LRN to PRL, and MLRL was calculated as the ratio of LRL to
186 LRN. TRL was calculated as the sum of PRL and LRL. A random term
187 [(Run/Frame/Column/Position/Paper side) + ([P]_{ext} × Line)] except for a fixed factor (Line) was
188 used to estimate line means with the REML procedure.

189 The relative root traits and relative biomass traits of each line were estimated as quotient of
190 the mean value of a trait at LP divided by the mean value of the trait at OP. The plasticity of
191 root traits in response to P deficiency were calculated using the following formula: plasticity =
192 (mean value of a trait at LP – mean value of a trait at OP) / mean value of a trait at OP. The
193 correlation coefficients among these traits were computed using the Pearson's correlation
194 method of SPSS/WIN 18.0 program.

195

196 **QTL mapping**

197

198 A SNP-based high-density *BnaTNDH* genetic map comprising 2041 markers (Zhang et al. 2016)
199 was used for the QTL mapping. The composite interval mapping (CIM) program of
200 WinQTLCart v2.5 (Wang et al. 2011) was used to detect significant QTLs for relative traits of
201 the *BnaTNDH* population. The number of control markers, window size and walking speed
202 were set to 5, 10 cM and 1 cM, respectively. The backward regression algorithm was used to
203 obtain cofactors. Empirical threshold for each trait was computed using the permutation test
204 (1,000 permutations, overall error level 5%) for CIM (Churchill and Doerge 1994). The
205 estimated additive effect and the percentage of phenotypic variation explained by each putative
206 QTL were obtained using the CIM model. The confidence intervals were set as the map interval

207 that corresponded to a 2-LOD decline on either side of the LOD peak.

208 The epistatic QTLs were identified for the relative root traits by the QTL IciMapping v4.1
209 (Meng et al. 2015), which is public and freely available (<http://www.isbreeding.net/software/>).

210 The epistatic QTLs were detected by the ICIM-EPI method using single environment
211 phenotypic values. The *P* values for entering variables (PIN) and removing variables (POUT)
212 were set at 0.0001 and 0.0002, respectively, and the scanning step was 5 cM. The LOD
213 threshold for the epistatic QTL was set as the default manual input value of the software. The
214 proportion of observed phenotypic variance explained by each epistatic QTL and the
215 corresponding additive effects were also estimated.

216

217 **Identification and integration of the QTL clusters**

218

219 A QTL cluster was defined as two or more significant QTLs with overlapping confidence
220 interval. Individual QTLs for relative root traits and relative biomass traits in a QTL cluster
221 were integrated in a meta-analysis using BioMercator v4.2 (Arcade et al. 2004). Meta-analysis
222 computing is based on the position of each input QTL, and on the variance of this position,
223 assessed through confidence interval values. The algorithm developed by Goffinet and Gerber
224 (2000) was employed to conduct the QTL meta-analysis, and the model with lowest Akaike
225 value was selected for QTL integration. The principle of integration is that the confidence
226 interval of integrated QTL should contain the peak position of component QTLs. The integrated
227 QTL for each QTL cluster was mapped to the reference genome (Darmor-*bzh*) according to the
228 physical position of the two flanking markers. The available reference genome of *B. napus*
229 (Chalhoub et al. 2014) and the functional annotation of the *Arabidopsis* genome
230 (<https://www.arabidopsis.org/>) were employed for the prediction of putative candidate genes.

231

232

233

234

235

236 **Results**

237

238 **Difference in root traits of *Bna*TNDH population in respond to Pi starvation between**
239 **‘agar’ system and ‘pouch and wick’ system**

240

241 When compared with OP, more DH lines had greater TRL, PRL, LRL and MLRL in the ‘pouch
242 and wick’ system than the ‘agar’ system at LP (Fig. 1a–d). Accordingly, the mean plasticity of
243 TRL, PRL, LRL, and MLRL of the *Bna*TNDH population in the ‘pouch and wick’ system was
244 larger than that in the ‘agar’ system (Supplementary Fig. 1a–d). Nearly 65.0% of the DH lines
245 had an increase in LRN at LP compared with OP in both the ‘agar’ and ‘pouch and wick’
246 systems, while the mean plasticity of LRN of the *Bna*TNDH population in the ‘pouch and wick’
247 system was 12.6% greater than that in the ‘agar’ system (Fig. 1e; Supplementary Fig. 1e).
248 Nearly 60% of lines that showed increased LRN at LP were the same in both culture systems.
249 In ‘agar’ system, the LRD of 90.0% of the DH lines was increased at LP as compared with OP
250 and the mean plasticity of LRD of the *Bna*TNDH population was 59%. While in the ‘pouch and
251 wick’ system, the LRD of 75.4% of the DH lines was increased at LP and the mean plasticity
252 of LRD of the *Bna*TNDH population was only 14.1% (Fig. 1f; Supplementary Fig. 1f).
253 Similarly, when compared with OP, the TRL, PRL, LRL and MLRL of cultivars Tapidor and
254 Ningyou 7 was increased at LP in the ‘pouch and wick’ system but decreased in the ‘agar’
255 system, while the LRN and LRD of cultivars Tapidor and Ningyou 7 was increased at LP in
256 both the ‘agar’ system and the ‘pouch and wick’ system (Fig. 1).

257

258 **Phenotypic variation and correlation among relative root traits in the ‘agar’ and ‘pouch**
259 **and wick’ systems**

260

261 A wide range of variation was observed in all the relative traits among the *Bna*TNDH lines in
262 both culture systems (Table 1; Fig. 2). Values of the six relative root traits of Tapidor were all
263 higher than that of Ningyou 7 in both culture systems, except relative primary root length
264 (RPRL) in both culture systems and relative mean lateral root length (RMLRL) in the ‘agar’

265 system (Table 1; Fig. 2). The mean of all the relative traits, except for the relative lateral root
266 density (RLRD), of *Bna*TNDH population and both parental lines was higher in the ‘pouch and
267 wick’ system than that in the ‘agar’ system (Table 1). Moreover, larger coefficients of variation
268 (CVs) of these traits, except for RLRD, of *Bna*TNDH population were observed in the ‘pouch
269 and wick’ system compared with that in the ‘agar’ system (Table 1). In both culture systems,
270 the frequency distribution of all the traits showed continuous phenotypic variation, and
271 significant transgressive segregations were observed in the population (Table 1; Fig. 2).

272 Pearson’s correlation coefficients between relative root traits were calculated
273 (Supplementary Table 1). Significant positive correlations between relative total root length
274 (RTRL) and the other five relative root traits of *Bna*TNDH population were observed in both
275 culture systems. Of these, the correlation of RTRL and relative total lateral root length (RLRL)
276 in the ‘pouch and wick’ system ($r = 0.93$; $P < 0.001$) was much larger than that in the ‘agar’
277 system ($r = 0.53$; $P < 0.001$). RPRL and RLRL were significantly correlated in both the ‘agar’ (r
278 $= 0.27$; $P < 0.001$) and ‘pouch and wick’ systems ($r = 0.49$; $P < 0.001$). There was a significant
279 positive correlation between RPRL and relative lateral root number (RLRN) in the ‘pouch and
280 wick’ system ($r = 0.80$; $P < 0.001$), while there was no correlation between them in the ‘agar’
281 system ($r = 0.06$). In the ‘agar’ system, a significant negative correlation was observed between
282 RPRL and RLRD ($r = -0.39$; $P < 0.001$), but no correlation was observed in the ‘pouch and wick’
283 system ($r = 0.06$; $P = \$\$\$\$$). RLRL was significantly correlated with RMLRL and RLRN in both
284 the ‘agar’ and ‘pouch and wick’ systems. There was a significant positive correlation between
285 RLRL and RLRD in the ‘pouch and wick’ system ($r = 0.35$; $P < 0.001$), while the correlation
286 was not significant in the ‘agar’ system ($r = 0.05$). There was no correlation between RMLRL
287 and RLRN in the ‘pouch and wick’ system ($r = 0.01$), but a weak negative correlation was
288 observed in the ‘agar’ system ($r = -0.17$; $P < 0.05$). A strong positive correlation and a moderate
289 positive correlation were observed between RLRN and RLRD in the ‘agar’ system ($r = 0.83$;
290 $P < 0.001$) and the ‘pouch and wick’ system ($r = 0.47$; $P < 0.001$), respectively. Moreover, in the
291 ‘agar’ system, the relative biomass traits were significantly correlated with relative root traits
292 (Supplementary Table 2), such as between relative total dry weight (RTDW) and RTRL ($r =$
293 0.65 ; $P < 0.001$), and between relative shoot dry weight (RSDW) and RPRL ($r = 0.52$; $P < 0.001$).

294 However, no correlation was observed for the same trait between the two culture systems
295 (Supplementary Table 1).

296

297 **QTLs for relative root and biomass traits of *Bna*TNDH mapping population in the ‘agar’**
298 **and ‘pouch and wick’ systems**

299

300 A QTL analysis was performed to identify the genetic factors responsible for the relative root
301 traits in both the ‘agar’ and the ‘pouch and wick’ systems. In the ‘agar’ system, a total of 10
302 significant QTLs were identified for six relative root traits across six of the 19 chromosomes
303 (Supplementary Table 3). Among them, one QTL for RTRL, one for RPRL, one for RLRL, one
304 for RMLRL and one for RLRD were mapped on chromosomes A09, A08, A09, A07 and C04,
305 respectively, accounting for 7.4%–12.8% of the phenotypic variation. Five QTLs for RLRN
306 were mapped on A04, C04 and C08, which jointly explained 46.7% of the phenotypic variation.
307 With the exception of one QTL on A07 for RMLRL (RMLRL_A07), all the QTLs for relative
308 root traits had a negative additive effect (Supplementary Table 3). The alleles from Tapidor
309 increased the values of all the relative root traits except for RMLRL. Moreover, two QTLs for
310 RTDW, two for RSDW, one for relative shoot fresh weight on chromosome A09, and two for
311 relative root fresh weight (RRFW) on both chromosome A09 and C09 were detected,
312 respectively, which explained 7.9%–15.3% of the phenotypic variation (Supplementary Table
313 3). Among these nine QTLs, the alleles of seven QTLs from Tapidor contributed to the increase
314 of relative traits except for two QTLs on chromosome C09 for RRFW (RRFW_C09a,
315 RRFW_C09b) (Supplementary Table 3). In the ‘pouch and wick’ system, one QTL for RPRL
316 and one for RLRL were detected on chromosomes A03 and C04, respectively, and no QTLs
317 were identified for RTRL, RMLRL, RLRN and RLRD with the *Bna*TNDH mapping population
318 (Supplementary Table 3). The QTL for the same trait in the ‘agar’ system did not overlap with
319 that in the ‘pouch and wick’ system, which was consistent with the poor correlation of these
320 traits between the two culture systems among genotypes.

321 Epistatic interaction analysis was conducted with the ICIM approach using phenotypic
322 values from the ‘agar’ and ‘pouch and wick’ systems independently (Supplementary Table 4).

323 In the ‘agar’ system, one epistatic QTL was identified for RTRL, accounting for 3.6% of the
324 phenotypic variation. In the ‘pouch and wick’ system, there was one epistatic QTL controlling
325 RPRL and another one controlling RLRD, which explained 4.9% and 13.2% of the phenotypic
326 variation, respectively. These three epistatic QTLs had a negative effect of additive by additive
327 interaction, indicating that two loci from different parental lines take the positive effects
328 (Supplementary Table 4).

329

330 **QTL clusters identified in the ‘agar’ system**

331

332 Two QTL clusters identified in the ‘agar’ system were located on chromosome A09 (Cluster1
333 and Cluster2) and one was located on C04 (Cluster3) (Fig. 3; Supplementary Table 5). Cluster1
334 contained one QTL for RTRL, one for RTDW, and one for RRFW. Four QTLs controlling
335 RTDW, RSDW, relative shoot fresh weight and RRFW were co-located in Cluster2. In Cluster3,
336 a QTL associated with RLRN was co-located with a QTL for RLRD (Fig. 3; Supplementary
337 Table 5). The average LOD score of the component QTLs in these three QTL clusters ranged
338 from 5.03–5.35, and each cluster accounted for 11.2%–12.2% of the average phenotypic
339 variation (Supplementary Table 5). The confidence intervals of Cluster1, Cluster2 and Cluster3
340 were defined as 129.5–131.3, 135.9–138.1 and 30.9–32.9 cM, respectively, using BioMercator
341 v4.2 by QTL meta-analysis (Supplementary Table 5).

342

343 **Co-located QTLs for the relative root traits and for the single root traits or the seed yield- 344 related traits**

345

346 We have mapped the significant QTLs for the single root traits of the *BnaTNDH* population at
347 LP and OP in the ‘agar’ system (Shi et al. 2013a), in the ‘pouch and wick’ system (Zhang et al.
348 2016), and the seed yield and yield-related traits in the field trials (Shi et al. 2013b). These
349 QTLs had been summarized in Supplementary Table 6 and Supplementary Table 7. In the ‘agar’
350 system, the average number of QTLs detected for each single root trait was 2.7 at LP and 2.3 at
351 OP, while the average number of QTLs detected for each relative root trait was 1.7. In the

352 'pouch and wick' system, the average number of QTLs detected for each single root trait was
353 3.7 at LP and 1.7 at OP, while the average number of QTLs detected for each relative root trait
354 was only 0.3. Therefore, the QTLs for the relative root traits were less than that for the single
355 root traits. In the 'agar' system, two of the ten QTLs for the relative root traits co-located with
356 the QTL for respective single root trait at OP, including RMLRL_A07 and RLRN_C08
357 (Supplementary Fig. 2). In the 'pouch and wick' system, RPRL_A03 (one of the two QTLs for
358 relative root traits) was found to overlap with the QTL for its single root trait at OP
359 (Supplementary Fig. 2).

360 Seven QTLs associated with the relative root trait and/or relative biomass trait were co-
361 located with the QTLs for seed yield and yield-related traits (Table 2). Among these QTLs,
362 RLRN_A04b, RSDW_A09a and Cluster1 were found to affect the seed yield and yield-related
363 traits in two of three field trials (Table 2). RLRN_A04b was co-located with the QTL for seed
364 weight of 1,000 seeds at OP (P₂O₅, 90 Kg ha⁻¹) in Tri.1 (field trial conducted from Sept 2008
365 to May 2009), and at LP (P₂O₅, 9 Kg ha⁻¹) and OP (P₂O₅, 90 Kg ha⁻¹) in Tri.2 (field trial
366 conducted from Sept 2009 to May 2010). RSDW_A09a was co-located with the QTL for seed
367 yield per hectare at LP (P₂O₅, 9 Kg ha⁻¹) and plant height at OP in Tri.1, and for height to the
368 first primary branch at OP in Tri.2. Cluster1 was co-located with the QTL for seed yield per
369 hectare at LP in Tri.1, and for height to the first primary branch, plant height, seed yield per
370 hectare at OP in Tri.2. Additionally, RSDW_A09a, and Cluster1 and Cluster2 were co-located
371 with QTLs SY_LP_A09a (R₂ = 4.5%) and SY_LP_A09b (R₂ = 5.8%) associated with seed yield
372 based on best linear unbiased estimation (BLUE) across three field trials at LP, respectively
373 (Supplementary Table 8). In the genomic regions of RLRN_A04b (17364075–17578367 bp),
374 RSDW_A09a (32624626–32900779 bp) and Cluster1 (32900851–33338388 bp), there were 53,
375 60 and 97 annotated genes, respectively (Supplementary Table 9). In RLRN_A04b and
376 RSDW_A09a, there seemed to be no annotated genes known to be involved in tolerance to P
377 deficiency. In Cluster1, *BnaA09g50010D* is orthologous to *AT1G06160 (ERF59)* in
378 *Arabidopsis*, which encodes a member of the ERF (ethylene response factor) subfamily B-3 of
379 ERF/AP2 transcription factor family.

380

381 **Discussion**

382

383 **Difference in the root plasticity of *B. napus* in response to P deficiency between ‘agar’**
384 **system and ‘pouch and wick’ system**

385

386 Phosphorus plays a critical role in all major developmental processes and reproduction in plants,
387 including seed germination, seedling growth, flower initiation and seed formation (Hawkesford
388 et al. 2012). P deficiency in soil is one of the major limiting factors for crop production
389 throughout the world (Lynch 2007; Veneklaas et al. 2012). Root system architecture traits are
390 vital for soil exploration and nutrient acquisition (Lynch 2007). The remodeling of root
391 morphology and architecture is the most evident change in response to P deficiency, which
392 provides a shallower growth angle of axial roots for obtaining P in the top part of soils (Lynch
393 2007; Liang et al. 2014), the proteoid roots (cluster roots: dense clusters of short side roots)
394 releasing carboxylates for mobilising P to improve soil P availability (Shane and Lambers 2005;
395 Lambers et al. 2011, 2013), and/or an increase in number and length of lateral roots and root
396 hairs for enlarging the root surface area scavenging for P in soils (Jain et al. 2007; Lynch 2011;
397 Haling et al. 2013; Niu et al. 2013). In this study, there were significant differences in the
398 response of root traits of *B. napus* to P starvation between an ‘agar’ system and a ‘pouch and
399 wick’ system (Fig. 1; Supplementary Fig. 1). At LP compared with OP, there was a decrease in
400 TRL of most DH lines and both parental lines in the ‘agar’ system, which was caused by a
401 reduction in both PRL and LRL, while an increased TRL was observed in the ‘pouch and wick’
402 system. A reduced PRL is a widely reported physiological response in P-deficient *Arabidopsis*
403 grown on vertical agar plates (Williamson et al. 2001; Linkohr et al. 2002; Al-Ghazi et al. 2003;
404 López-Bucio et al. 2005; Nacry et al. 2005; Reymond et al. 2006; Ward et al. 2008; Müller et
405 al. 2015; Mora-Macías et al. 2017) and is possibly the result of iron (Fe) toxicity (Ward et al.
406 2008). The reduced PRL of *B. napus* at LP compared to OP in the ‘agar’ system is consistent
407 with these studies on *Arabidopsis*. However, the reduction in LRL of *B. napus* at LP compared
408 to OP in the ‘agar’ system contrasts with an increase in LRL observed in *Arabidopsis* grown on
409 agar plates at LP compared to OP (Williamson et al. 2001; Linkohr et al. 2002; Al-Ghazi et al.

410 2003; López-Bucio et al. 2005; Nacry et al. 2005; Reymond et al. 2006). The P-deficient *B.*
411 *napus* plants grown in the ‘pouch and wick’ system seemed to have greater PRL, which is
412 consistent with studies of plants grown in nutrient solutions (Zhang 2009; Wang et al. 2017).
413 The LRL of *B. napus* grown in the ‘pouch and wick’ system was greater at LP than at OP, which
414 is consistent with studies of *Brassica oleracea* grown on vertical glass plates supported on blue
415 blotter paper (Hammond et al. 2009).

416 Under P-limited conditions, an increased LRN was observed in both culture systems (Fig.
417 1e; Supplementary Fig. 1e). Enhanced lateral root formation at LP has been reported for
418 *Arabidopsis* grown on vertically oriented agar plates (López-Bucio et al. 2002; Sánchez-
419 Calderón et al. 2006; Péreztorres et al. 2008), *Brassica oleracea* grown on vertical glass plates
420 supported on blue blotter paper (Hammond et al. 2009) and oilseed rape grown hydroponically
421 (Zhang et al. 2011). The reduction in MLRL at LP compared to OP in the ‘agar’ system contrasts
422 with an increase in MLRL at LP compared to OP in the ‘pouch and wick’ system (Fig. 1d;
423 Supplementary Fig. 1d). A significant increase in LRD at LP was found in both culture systems
424 (Fig. 1f; Supplementary Fig. 1f). However, the LRD in the ‘agar’ system had a significantly
425 greater plasticity than that in the ‘pouch and wick’ system, mainly because of a large reduction
426 of PRL in the ‘agar’ system (Fig. 1b; Supplementary Fig. 1b).

427 The response of roots to unilateral light is a species-dependent and can include positive
428 phototropism, negative phototropism and no phototropism (Hubert and Funke 1937; Kutschera
429 and Briggs 2012). The roots of *Arabidopsis* display a negative phototropic response
430 (Boccalandro et al. 2008; Kutschera and Briggs 2012). Negative root phototropism prevents
431 light stress in the upper layers of the soil where light penetration is greatest, reduced desiccation
432 phenomena, and enhanced seedling survival under dry and windy conditions by mediating
433 plastic increases in the efficiency of root growth near the soil surface (Galen et al. 2004, 2006;
434 Kutschera and Briggs 2012). In this study, the root system was exposed to light in the ‘agar’
435 system (Shi et al. 2013a), while polythene sheets were employed to cover the root in the ‘pouch
436 and wick’ system (Zhang et al. 2016). Increased PRL and LRL were generally observed in P-
437 deficient *B. napus* plants in the ‘pouch and wick’ system, while the situation in the ‘agar’ system
438 was opposite. Moreover, when oilseed rape seedlings were grown in P-deficient nutrient

439 solution with roots in dark, the TRL and PRL were both enhanced compared with plants in P-
440 replete nutrient solution (Zhang 2009; Zhang et al. 2011; Wang et al. 2017). These observations
441 suggest that shielding roots from light reduced the sensitivity of root system elongation to P
442 deficiency in *B. napus*. In *Arabidopsis*, plants with roots in darkness had longer PRL and more
443 LRN than plants with roots exposed to light conditions under P sufficient conditions (Silva-
444 Navas et al. 2016). P deficiency significantly inhibited the elongation of the primary root of
445 *Arabidopsis* and *B. napus* cultivars when roots were exposed to light, but had no effect when
446 roots grew in darkness (Supplementary Figs. 3–5). Similarly, in *Arabidopsis*, roots grown in
447 darkness showed less sensitivity to nitrogen deficiency and salt stress compared with those
448 exposed to light (Silva-Navas et al. 2015). The increased number of lateral roots under P
449 deficient conditions happened in both the ‘agar’ and ‘pouch and wick’ systems, indicating that
450 the increase in LRN is not light-sensitive.

451 Plants grown in ‘agar’ and ‘pouch and wick’ systems can be used to remove the influence of
452 complex soil environment on root growth. However, in agricultural system, natural soils exhibit
453 considerable spatial and temporal variability in structure and resource availability (Jin et al.
454 2017). Since significant $G \times E$ interaction occur for root system architecture in the field (White
455 et al. 2013b), only a few QTLs for the seed yield and yield-related traits of the *Bna*TNDH
456 population investigated in the field (Shi et al. 2013b) co-located with the QTLs for the root or
457 relative root traits investigated by ‘agar’ and ‘pouch and wick’ systems. Some promising
458 technologies, such as X-ray computed tomography or magnetic resonance imaging have been
459 developed for visualizing plant root systems within their natural soil environment
460 noninvasively (Metzner et al. 2015) and may prove useful in the future for identifying QTL
461 associated with root traits in response to abiotic factors.

462

463 **QTLs for relative root traits of *B. napus***

464

465 Relative root traits were used to evaluate the root plasticity of *B. napus* in response to P
466 deficiency in this study. Considerable transgression of six relative root traits of *B. napus* were
467 observed in both the ‘agar’ and ‘pouch and wick’ culture systems (Table 1; Fig. 2), indicating

468 that both parental lines carry genes with alleles contributing to an increase or a decrease of the
469 relative root traits. The culture system had a significant influence on the relative root traits of
470 two parental lines, and greater differences in all relative root traits except RLRN were observed
471 between the two parental lines in the ‘pouch and wick’ system than in the ‘agar’ system (Table
472 1). Pairs of relative root traits, such as RTRL and RPRL, RTRL and RLRL, RLRL and RMLRL,
473 were significantly correlated across the two culture systems (Supplementary Table 1), which is
474 consistent with the correlation between TRL and PRL, TRL and LRL, LRL and MLRL (Zhang
475 et al. 2016). However, correlations between some pairs of relative traits, e.g. RPRL and RLRN,
476 RPRL and RLRD were not stable across the two culture systems (Supplementary Table 1),
477 suggesting that there are different P deficiency-induced modulations of root system architecture
478 in the two culture systems.

479 There was a difference in the genetic control of the relative root traits between plants grown
480 in the ‘agar’ and ‘pouch and wick’ systems. One QTL for RTRL, one for RPRL, one for RLRL,
481 one for RMLRL, one for RLRD, and five QTLs for RLRN were identified in the ‘agar’ system,
482 while only one QTL for RPRL and one for RLRL were detected in the ‘pouch and wick’ system
483 (Fig. 3; Supplementary Table 3). The QTLs identified for the same trait in the two culture
484 systems were not co-located, which could account for the poor correlations among genotypes
485 for the same traits studied in the two culture systems (Supplementary Table 1). The different
486 genetic control of the relative root traits between plants grown in the ‘agar’ and ‘pouch and
487 wick’ systems indicates that the plasticity of root system architecture responding to P deficiency
488 is largely influenced by environmental factors like the light in this study. Larger coefficients of
489 variation (CVs) of RTRL, RMLRL and RLRN were observed in the ‘pouch and wick’ system
490 compared to the ‘agar’ system (Table 1), but no QTL was discovered for these traits in the
491 ‘pouch and wick’ system. Thus, the trait variation may be not a good indicator for the number
492 of QTLs that could be identified (Ghandilyan et al. 2009).

493 The QTLs for the relative root traits, RPRL_A03, RMLRL_A07 and RLRN_C08, co-located
494 with the QTLs for the respective single root traits at OP (Supplementary Fig. 2), implying that
495 these three QTLs only affected their respective single root trait at OP. The co-located QTLs
496 both for a relative root trait and for a single root trait at LP should be more useful than the QTLs

497 only for a relative root trait in the breeding of P efficient cultivars. In this study, RLRL_C04
498 was discovered to overlap with a significant SNP (Bn-scaff_15712_8-p121295) for LRL at LP
499 identified by genome-wide association studies (GWAS) in the ‘pouch and wick’ system (Wang
500 et al. 2017), which may play an important role in lateral root growth and development in
501 response to P deficiency at seedling stage.

502 Three QTLs for the relative root traits were identified to affect the seed yield and yield-
503 related traits in two of three field trials (Table 2). In the intervals of QTLs RLRN_A04b and
504 RSDW_A09a, no annotated genes were involved in the response of plant to P deficiency, which
505 indicated that there were novel genes in these QTL regions or several annotated genes had novel
506 function associated with P deficiency response. In Cluster1, *BnaA09g50010D* (homologous to
507 *ATIG06160*) was predicted as a promising candidate gene. *ATIG06160* (*ERF59*) is a member
508 of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family
509 (Nakano et al. 2006). *ERF1* and *ERF2* have been demonstrated to regulate the root growth of
510 *Arabidopsis* and rice, respectively (Mao et al. 2016; Xiao et al. 2016). The candidate gene
511 underlying the three target QTLs can be identified by investigating the phenotype of the
512 *Arabidopsis* mutant at LP and OP, or by developing near-isogenic lines to allow further fine
513 mapping of these QTLs and the cloning of potential candidate genes.

514

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522

523 **Compliance with ethical standards**

524

525 **Conflict of interest**

526 The authors declare that they have no competing interests.

527

528 **References**

529

530 Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. *Biophoton Int*
531 11:36–42

532 Al-Ghazi Y, Muller B, Pinloche S, Tranbarger TJ, Nacry P, Rossignol M, Tardieu F, Doumas
533 P (2003) Temporal responses of *Arabidopsis* root architecture to phosphate starvation:
534 evidence for the involvement of auxin signalling. *Plant Cell Environ* 26:1053–1066

535 Arcade A, Labourdette A, Falque M, Mangin B, Chardon F, Charcosset A, Joets J (2004)
536 BioMercator: integrating genetic maps and QTL towards discovery of candidate genes.
537 *Bioinformatics* 20:2324–2326

538 Bayuelo-Jiménez JS, Gallardo-Valdéz M, Pérez-Decelis VA, Magdaleno-Armas L, Ochoa I,
539 Lynch JP (2011) Genotypic variation for root traits of maize (*Zea mays* L.) from the
540 Purhepecha Plateau under contrasting phosphorus availability. *Field Crops Res* 121:350–
541 362

542 Boccalandro HE, De Simone SN, Bergmann-Honsberger A, Schepens I, Fankhauser C, Casal
543 JJ (2008) PHYTOCHROME KINASE SUBSTRATE1 regulates root phototropism and
544 gravitropism. *Plant Physiol* 146:108–115

545 Chalhoub B, Denoëud F, Liu S, Parkin IA, Tang H, Wang X, Chiquet J, Belcram H, Tong
546 C, Samans B, Corrêa M, Da Silva C, Just J, Falentin C, Koh CS, Le Clainche I, Bernard
547 M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud
548 C, Alamery S, Jabbari K, Zhao M, Edger PP, Chelaifa H, Tack D, Lassalle G, Mestiri
549 I, Schnel N, Le Paslier MC, Fan G, Renault V, Bayer PE, Golicz AA, Manoli S, Lee
550 TH, Thi VH, Chalabi S, Hu Q, Fan C, Tollenaere R, Lu Y, Battail C, Shen J, Sidebottom
551 CH, Wang X, Canaguier A, Chauveau A, Bérard A, Deniot G, Guan M, Liu Z, Sun
552 F, Lim YP, Lyons E, Town CD, Bancroft I, Wang X, Meng J, Ma J, Pires JC, King
553 GJ, Brunel D, Delourme R, Renard M, Aury JM, Adams KL, Batley J, Snowdon RJ, Tost
554 J, Edwards D, Zhou Y, Hua W, Sharpe AG, Paterson AH, Guan C, Wincker P (2014)
555 Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome.
556 *Science* 345:950–953

557 Chevalier F, Pata M, Nacry P, Doumas P, Rossignol M (2003) Effects of phosphate availability
558 on the root system architecture: large-scale analysis of the natural variation between
559 *Arabidopsis* accessions. *Plant Cell Environ* 26:1839–1850

560 Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping.
561 *Genetics* 138:963–971

562 Ding G, Zhao Z, Liao Y, Hu Y, Shi L, Long Y, Xu F (2012) Quantitative trait loci for seed

563 yield and yield-related traits, and their responses to reduced phosphorus supply in *Brassica*
564 *napus*. *Ann Bot* 109:747–759

565 Duan HY, Shi L, Ye XS, Wang YH, Xu FS (2009) Identification of phosphorous efficient
566 germplasm in oilseed rape. *J Plant Nutr* 32:1148–1163

567 Elser JJ (2012) Phosphorus: a limiting nutrient for humanity? *Curr Opin Biotechnol* 23:833–
568 838

569 Fita A, Nuez F, Picó B (2011) Diversity in root architecture and response to P deficiency in
570 seedlings of *Cucumis melo* L. *Euphytica* 181:323–339

571 Galen C, Huddle J, Liscum E (2004) An experimental test of the adaptive evolution of
572 phototropism: blue-light receptors controlling phototropism in *Arabidopsis thaliana*.
573 *Evolution* 58:515–523

574 Galen C, Rabenold JJ, Liscum E (2006) Functional ecology of a blue light photoreceptor:
575 effects of phototropin-1 on root growth enhance drought tolerance in *Arabidopsis thaliana*.
576 *New Phytol* 173:91–99

577 Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C, Slamet-Loedin I,
578 Tecson-Mendoza EM, Wissuwa M, Heuer S (2012) The protein kinase Pstol1 from
579 traditional rice confers tolerance of phosphorus deficiency. *Nature* 488:535–539

580 Ghandilyan A, Ilk N, Hanhart C, Mbengue M, Barboza L, Schat H, Koornneef M, El-Lithy
581 M, Vreugdenhil D, Reymond M, Aarts MG (2009) A strong effect of growth medium and
582 organ type on the identification of QTLs for phytate and mineral concentrations in three
583 *Arabidopsis thaliana* RIL populations. *J Exp Bot* 60:1409–1425

584 Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. *Genetics* 155:463–473

585 Haling RE, Brown LK, Bengough AG, Young IM, Hallett PD, White PJ, George TS (2013)
586 Root hairs improve root penetration, root-soil contact, and phosphorus acquisition in soils
587 of different strength. *J Exp Bot* 64:3711–3721

588 Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead
589 A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use
590 efficiency in *Brassica oleracea* and correlates with root architecture traits. *J Exp Bot*
591 60:1953–1968

592 Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Skrumsager Møller I, White P
593 (2012) Chapter 6: Functions of macronutrients. In: Marschner P (ed) *Marschner's Mineral*
594 *Nutrition of Higher Plants*, 3rd edn. Academic Press, London, pp 135–189

595 Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient
596 shortage by biomass allocation? *Trends Plant Sci* 11:610–617

597 Hubert B, Funke GL (1937) The phototropism of terrestrial roots. *Biol Jaarboek* 4:286–315

598 Jain A, Poling MD, Karthikeyan AS, Blakeslee JJ, Peer WA, Titapiwatanakun B, Murphy

599 AS, Raghothama KG (2007) Differential effects of sucrose and auxin on localized
600 phosphate deficiency-induced modulation of different traits of root system architecture in
601 *Arabidopsis*. *Plant Physiol* 144:232–247

602 Jin K, White PJ, Whalley WR, Shen J, Shi L (2017) Shaping an optimal soil by root–soil
603 interaction. *Trends Plant Sci* 22:823–829

604 Kutschera U, Briggs WR (2012) Root phototropism: from dogma to the mechanism of blue
605 light perception. *Planta* 235:443–452

606 Lambers H, Clements JC, Nelson MN (2013) How a phosphorus-acquisition strategy based on
607 carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*,
608 *Fabaceae*). *Am J Bot* 100:263–288

609 Lambers H, Finnegan PM, Laliberté E, Pearse SJ, Ryan MH, Shane MW, Veneklaas EJ (2011)
610 Phosphorus nutrition of proteaceae in severely phosphorus-impooverised soils: are there
611 lessons to be learned for future crops? *Plant Physiol* 156:1058–1066

612 Lapis-Gaza HR, Jost R, Finnegan PM (2014) *Arabidopsis* PHOSPHATE TRANSPORTER1
613 genes *PHT1;8* and *PHT1;9* are involved in root-to-shoot translocation of orthophosphate.
614 *BMC Plant Biol* 14:334

615 Liang C, Wang J, Zhao J, Tian J, Liao H (2014) Control of phosphate homeostasis through gene
616 regulation in crops. *Curr Opin Plant Biol* 21:59–66

617 Liao H, Yan X, Rubio G, Beebe SE, Blair MW, Lynch JP (2004) Genetic mapping of basal root
618 gravitropism and phosphorus acquisition efficiency in common bean. *Funct Plant Biol*
619 31:959–970

620 Linkohr BI, Williamson LC, Fitter AH, Leyser HM (2002) Nitrate and phosphate availability
621 and distribution have different effects on root system architecture of *Arabidopsis*. *Plant J*
622 29:751–760

623 Liu J, Hua W, Hu Z, Yang H, Zhang L, Li R, Deng L, Sun X, Wang X, Wang H (2015) Natural
624 variation in *ARF18* gene simultaneously affects seed weight and silique length in polyploid
625 rapeseed. *Proc Natl Acad Sci* 112:E5123–E5132

626 López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-
627 Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. *Annu*
628 *Rev Plant Biol* 65:95–123

629 López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J,
630 Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in
631 hormone sensitivity in the *Arabidopsis* root system. *Plant Physiol* 129:244–256

632 López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Pérez-Torres A, Rampey RA, Bartel
633 B, Herrera-Estrella L (2005) An auxin transport independent pathway is involved in
634 phosphate stress-induced root architectural alterations in *Arabidopsis*: identification of

635 *BIG* as a mediator of auxin pericycle cell activation. *Plant Physiol* 137:681–691

636 Lynch JP (2007) Roots of the second green revolution. *Aust J Bot* 55:493–512

637 Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools
638 for future crops. *Plant Physiol* 156:1041–1049

639 Mao JL, Miao ZQ, Wang Z, Yu LH, Cai XT, Xiang CB (2016) *Arabidopsis* ERF1 mediates
640 cross-talk between ethylene and auxin biosynthesis during primary root elongation by
641 regulating *ASA1* expression. *PLoS Genet* 12:e1005760

642 Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic
643 linkage map construction and quantitative trait locus mapping in bi-parental populations.
644 *Crop J* 3:265–279

645 Metzner R, Eggert A, van Dusschoten D, Pflugfelder D, Gerth S, Schurr U, Uhlmann
646 N, Jahnke S (2015) Direct comparison of MRI and X-ray CT technologies for 3D imaging
647 of root systems in soil: potential and challenges for root trait quantification. *Plant Methods*
648 11:17

649 Ming F, Zheng X, Mi G, He P, Zhu L, Zhang F (2000) Identification of quantitative trait loci
650 affecting tolerance to low phosphorus in rice (*Oryza Sativa* L.). *Chinese Sci Bull* 45:520–
651 525

652 Mora-Macías J, Ojeda-Rivera JO, Gutiérrez-Alanís D, Yong-Villalobos L, Oropeza-Aburto A,
653 Raya-González J, Jiménez-Domínguez G, Chávez-Calvillo G, Rellán-Álvarez R, Herrera-
654 Estrella L (2017) Malate-dependent Fe accumulation is a critical checkpoint in the root
655 developmental response to low phosphate. *Proc Natl Acad Sci* 114:E3563–E3572

656 Müller J, Toev T, Heisters M, Teller J, Moore KL, Hause G, Dinesh DC, Bürstenbinder K, Abel
657 S (2015) Iron-dependent callose deposition adjusts root meristem maintenance to
658 phosphate availability. *Dev Cell* 33:216–230

659 Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco
660 tissue cultures. *Physiol Plant* 15:473–497

661 Nacry P, Canivenc G, Muller B, Azmi A, Van Onckelen H, Rossignol M, Doumas P (2005) A
662 role for auxin redistribution in the responses of the root system architecture to phosphate
663 starvation in *Arabidopsis*. *Plant Physiol* 138:2061–2074

664 Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene
665 family in *Arabidopsis* and rice. *Plant Physiol* 140:411–432

666 Nestler J, Wissuwa M (2016) Superior root hair formation confers root efficiency in some, but
667 not all, rice genotypes upon P deficiency. *Front Plant Sci* 7:1935

668 Ni JJ, Wu P, Senadhira D, Huang N (1998) Mapping QTLs for phosphorus deficiency tolerance
669 in rice (*Oryza sativa* L.). *Theor Appl Genet* 97:1361–1369

670 Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS (2013) Responses of root architecture

671 development to low phosphorus availability: a review. *Ann Bot* 112:391–408

672 Péreztorres CA, Lópezbucio J, Cruzramírez A, Ibarra-Laclette E, Dharmasiri S, Estelle M,
673 Herrera-Estrella L (2008) Phosphate availability alters lateral root development in
674 *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin
675 receptor. *Plant Cell* 20:3258–3272

676 Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann
677 T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt
678 R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and
679 its use for QTL analysis of seed oil and erucic acid content. *Theor Appl Genet* 114:67–80

680 Reymond M, Svistoonoff S, Loudet O, Nussaume L, Desnos T (2006) Identification of QTL
681 controlling root growth response to phosphate starvation in *Arabidopsis thaliana*. *Plant*
682 *Cell Environ* 29:115–125

683 Sánchez-Calderón L, López-Bucio J, Chacón-López A, Gutiérrez-Ortega A, Hernández-Abreu
684 E, Herrera-Estrella L (2006) Characterization of *low phosphorus insensitive* mutants
685 reveals a crosstalk between low phosphorus-induced determinate root development and
686 the activation of genes involved in the adaptation of *Arabidopsis* to phosphorus deficiency.
687 *Plant Physiol* 140:879–889

688 Shane MW, Lambers H (2005) Cluster roots: a curiosity in context. *Plant Soil* 274:101–125

689 Shi L, Shi T, Broadley MR, White PJ, Long Y, Meng J, Xu F, Hammond JP (2013a) High-
690 throughput root phenotyping screens identify genetic loci associated with root
691 architectural traits in *Brassica napus* under contrasting phosphate availabilities. *Ann Bot*
692 112:381–389

693 Shi T, Li R, Zhao Z, Ding G, Long Y, Meng J, Xu F, Shi L (2013b) QTL for yield traits and
694 their association with functional genes in response to phosphorus deficiency in *Brassica*
695 *napus*. *PLoS One* 8:e54559

696 Shi TX, Wang SS, Shi L, Meng JL, Xu FS (2010) Effects of different nitrogen and phosphorus
697 levels on seed yield and quality parameters of double high and double low *Brassica napus*.
698 *Plant Nutr Fert Sci* 16:959–964

699 Silva-Navas J, Moreno-Risueno MA, Manzano C, Pallero-Baena M, Navarro-Neila S, Téllez-
700 Robledo B, Garcia-Mina JM, Baigorri R, Gallego FJ, del Pozo JC (2015) D-Root: a
701 system for cultivating plants with the roots in darkness or under different light conditions.
702 *Plant J* 84:244–255

703 Silva-Navas J, Moreno-Risueno MA, Manzano C, Téllez-Robledo B, Navarro-Neila
704 S, Carrasco V, Pollmann S, Gallego FJ, del Pozo JC (2016) Flavonols mediate root
705 phototropism and growth through regulation of proliferation-to-differentiation transition.
706 *Plant Cell* 28:1372–1387

707 Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L,
708 Desnos T (2007) Root tip contact with low-phosphate media reprograms plant root
709 architecture. *Nat Genet* 39:792–796

710 Tian J, Wang X, Tong Y, Chen X, Liao H (2012) Bioengineering and management for efficient
711 phosphorus utilization in crops and pastures. *Curr Opin Biotechnol* 23:866–871

712 Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA,
713 Scheible WR, Shane MW, White PJ, Raven JA (2012) Opportunities for improving
714 phosphorus-use efficiency in crop plants. *New Phytol* 195:306–320

715 Walder F, Brulé D, Koegel S, Wiemken A, Boller T, Courty PE (2015) Plant phosphorus
716 acquisition in a common mycorrhizal network: regulation of phosphate transporter genes
717 of the Pht1 family in sorghum and flax. *New Phytol* 205:1632–1645

718 Wang S, Basten CJ, Zeng ZB (2011) Windows QTL Cartographer 2.5. Department of Statistics.
719 North Carolina State University, Raleigh, NC

720 Wang W, Ding GD, White PJ, Wang XH, Jin KM, Xu FS, Shi L (2018) Mapping and cloning
721 of quantitative trait loci for phosphorus efficiency in crops: opportunities and challenges.
722 *Plant Soil*. <https://doi.org/10.1007/s11104-018-3706-6>

723 Wang X, Chen Y, Thomas CL, Ding G, Xu P, Shi D, Grandke F, Jin K, Cai H, Xu F, Yi B,
724 Broadley MR, Shi L (2017) Genetic variants associated with the root system architecture
725 of oilseed rape (*Brassica napus* L.) under contrasting phosphate supply. *DNA Res* 24:407–
726 417

727 Wang X, Yan X, Liao H (2010) Genetic improvement for phosphorus efficiency in soybean: a
728 radical approach. *Ann Bot* 106:215–222

729 Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG (2008) The effect of iron on the
730 primary root elongation of *Arabidopsis* during phosphate deficiency. *Plant Physiol*
731 147:1181–1191

732 White PJ, Broadley MR, Greenwood DJ, Hammond JP (2005) Genetic modifications to
733 improve phosphorus acquisition by roots. *Proceedings* 568. York, UK: International
734 Fertiliser Society

735 White PJ, George TS, Dupuy LX, Karley AJ, Valentine TA, Wiesel L, Wishart J (2013a) Root
736 traits for infertile soils. *Front Plant Sci* 4:193

737 White PJ, George TS, Gregory PJ, Bengough AG, Hallett PD, McKenzie BM (2013b) Matching
738 roots to their environment. *Ann Bot* 112:207–222

739 Williamson LC, Ribrioux SP, Fitter AH, Leyser HM (2001) Phosphate availability regulates
740 root system architecture in *Arabidopsis*. *Plant Physiol* 126:875–882

741 Xiao G, Qin H, Zhou J, Quan R, Lu X, Huang R, Zhang H (2016) OsERF2 controls rice root
742 growth and hormone responses through tuning expression of key genes involved in

743 hormone signaling and sucrose metabolism. *Plant Mol Biol* 90:293–302

744 Yang M, Ding G, Shi L, Feng J, Xu F, Meng J (2010) Quantitative trait loci for root morphology
745 in response to low phosphorus stress in *Brassica napus*. *Theor Appl Genet* 121:181–193

746 Yang M, Ding G, Shi L, Xu F, Meng J (2011) Detection of QTL for phosphorus efficiency at
747 vegetative stage in *Brassica napus*. *Plant Soil* 339:97–111

748 Zhang H, Huang Y, Ye X, Xu F (2011) Genotypic variation in phosphorus acquisition from
749 sparingly soluble P sources is related to root morphology and root exudates in *Brassica*
750 *napus*. *Sci China Life Sci* 54:1134–1142

751 Zhang HW (2009) Study on physiological mechanisms of phosphorus efficiency in *Brassica*
752 *napus*. Dissertation, Huazhong Agricultural University

753 Zhang Y, Thomas CL, Xiang J, Long Y, Wang X, Zou J, Luo Z, Ding G, Cai H, Graham NS,
754 Hammond JP, King GJ, White PJ, Xu F, Broadley MR, Shi L, Meng J (2016) QTL meta-
755 analysis of root traits in *Brassica napus* under contrasting phosphorus supply in two
756 growth systems. *Sci Rep* 6:33113

757 Zhu J, Kaeppler SM, Lynch JP (2005a) Mapping of QTLs for lateral root branching and length
758 in maize (*Zea mays* L.) under differential phosphorus supply. *Theor Appl Genet* 111:688–
759 695

760 Zhu J, Kaeppler SM, Lynch JP (2005b) Topsoil foraging and phosphorus acquisition efficiency
761 in maize (*Zea mays*). *Funct Plant Biol* 32:749–762

Table 1 Means, ranges and coefficients of variation (CVs) of the relative root traits in the parental lines and the *BnaTNDH* mapping population in the ‘agar’ system and the ‘pouch and wick’ system

Trait	Culture system	Parental lines		<i>BnaTNDH</i> lines		
		Tapidor	Ningyou 7	Mean	Range	CV%
RTRL	‘agar’ system	0.89	0.88	0.80	0.41–1.83	22.8
	‘pouch and wick’ system	2.20	1.76	1.41	0.31–5.45	55.8
RPRL	‘agar’ system	0.82	0.85	0.76	0.46–1.43	19.9
	‘pouch and wick’ system	1.21	1.49	1.07	0.53–2.69	33.1
RLRL	‘agar’ system	1.00	0.89	0.91	0.23–5.00	54.0
	‘pouch and wick’ system	2.94	1.86	1.60	0.24–7.68	70.8
RMLRL	‘agar’ system	0.80	0.90	0.80	0.32–1.75	34.0
	‘pouch and wick’ system	1.68	1.03	1.17	0.45–3.42	41.3
RLRN	‘agar’ system	1.25	1.00	1.17	0.21–4.47	37.2
	‘pouch and wick’ system	1.87	1.72	1.30	0.40–5.90	49.3
RLRD	‘agar’ system	1.45	1.18	1.59	0.20–4.96	39.0
	‘pouch and wick’ system	1.63	1.09	1.14	0.68–1.77	19.3

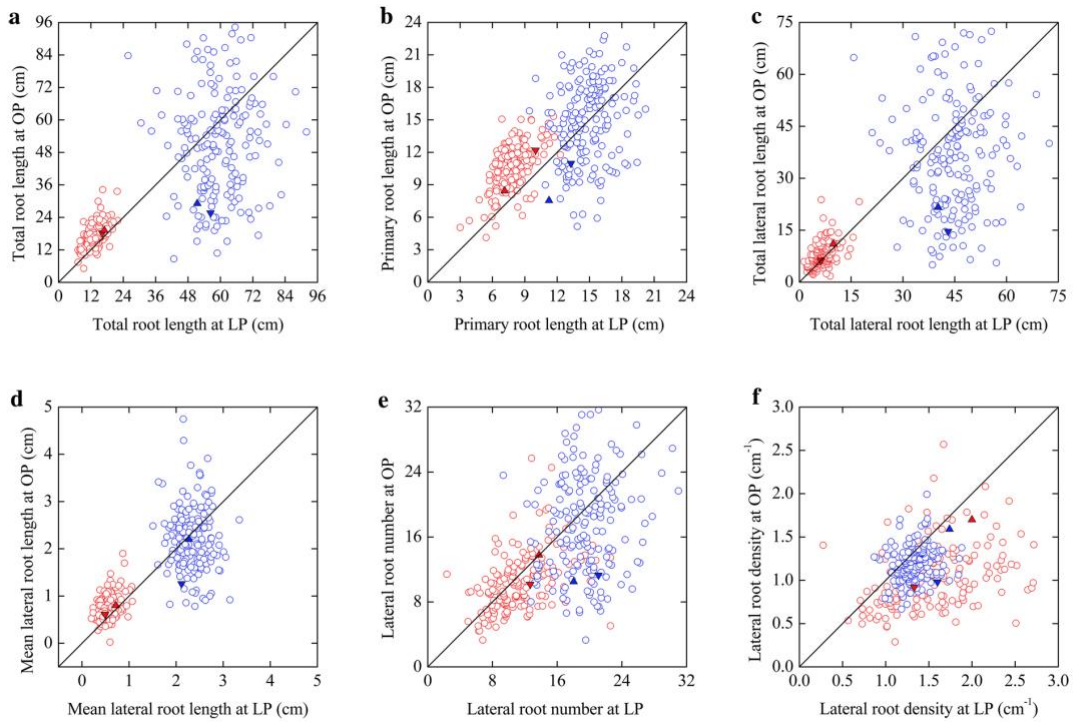
762 RTRL, relative total root length; RPRL, relative primary root length; RLRL, relative total lateral root length; RMLRL, relative mean lateral root length; RLRN,
763 relative lateral root number; RLRD, relative lateral root density

Table 2 Co-located QTLs for the relative traits in the ‘agar’ system and the ‘pouch and wick’ system with the seed yield and yield-related traits in the field trials

z	QTLs for the relative traits			QTLs for the seed yield and yield-related traits in the field trials	
	Culture system	QTL name	Confidence interval (cM)	QTL name	Confidence interval (cM)
A04	‘agar’ system	RLRN_A04b	10.0–12.3	SW_OP1_A04a	9.9–14.8
				SW_LP2_A04b	9.0–14.8
				SW_OP2_A04b	7.4–15.4
A09		RLRL_A09	52.5–59.4	BN_OP3_A09b	45.8–52.6
		RSDW_A09a	124.4–129.4	SY_LP1_A09a	124.3–129.4
				PH_OP1_A09a	124.5–129.4
				FBH_OP2_A09a	128.8–134.3
		Cluster1	129.5–131.3	SY_LP1_A09b	130.9–135.4
				FBH_OP2_A09a	128.8–134.3
				PH_OP2_A09a	129.4–132.3
				SY_OP2_A09	130.4–135.4
		Cluster2	135.9–138.1	FBH_OP3_A09	137.3–139.4
C09		RRFW_C09b	61.4–64.2	RBH_OP3_C09a	61.4–64.3
A03	‘pouch and wick’ system	RPRL_A03	3.1–23.6	SW_LP1_A03a	20.4–27.8
				BN_OP1_A03	0–8.9

The QTLs for the seed yield and yield-related traits in the three field trials were denominated as "trait+P treatment+trial number+chromosome+the serial letter". Height to the first primary branch (cm; FBH), plant height (cm; PH), relative first primary branch height (the ratio of FBH to PH; RBH), number of primary branches per plant (N; BN), seed weight of 1,000 seeds (g per 1000 seeds; SW), seed yield per hectare (kg·ha⁻¹; SY). LP, a low phosphorus supply. OP, an optimal phosphorus supply

765 **Figures**

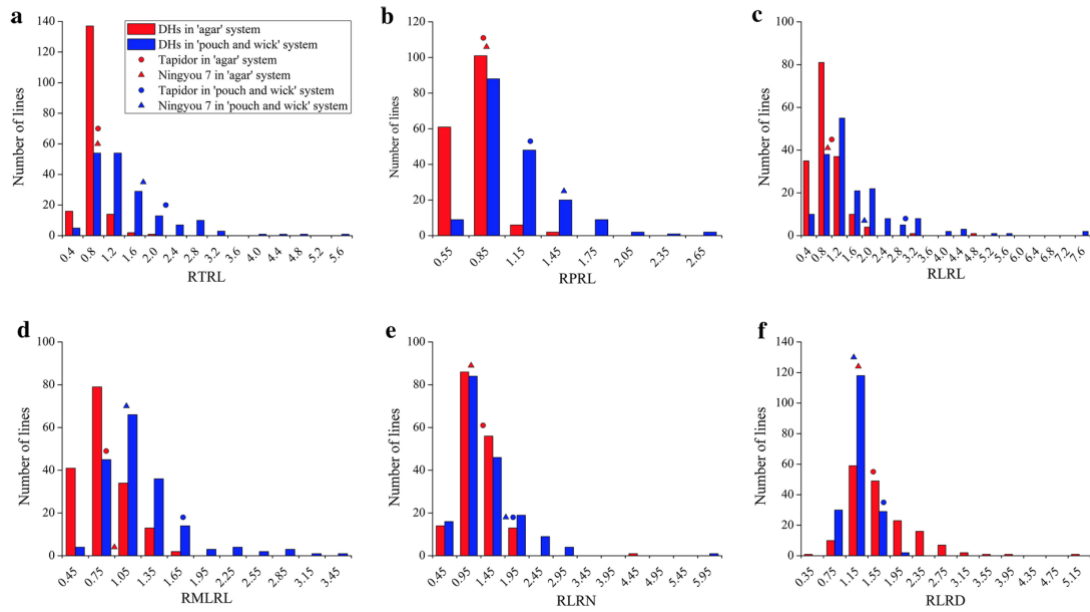


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768 **Fig. 1** Variation in total root length (a), primary root length (b), total lateral root length (c),
 769 mean lateral root length (d), lateral root number (e), lateral root density (f) of the *BnaTNDH*
 770 mapping population in the ‘agar’ and ‘pouch and wick’ systems. The open red circle and open
 771 blue circle represent the DH lines in the ‘agar’ system and the ‘pouch and wick’ system,
 772 respectively. The solid red downtriangle and solid red uptriangle represent Tapidor and Ningyou
 773 7 in the ‘agar’ system, respectively. The solid blue downtriangle and solid blue uptriangle
 774 represent Tapidor and Ningyou 7 in the ‘pouch and wick’ system, respectively. The continuous
 775 line represents the 1 : 1 line

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778

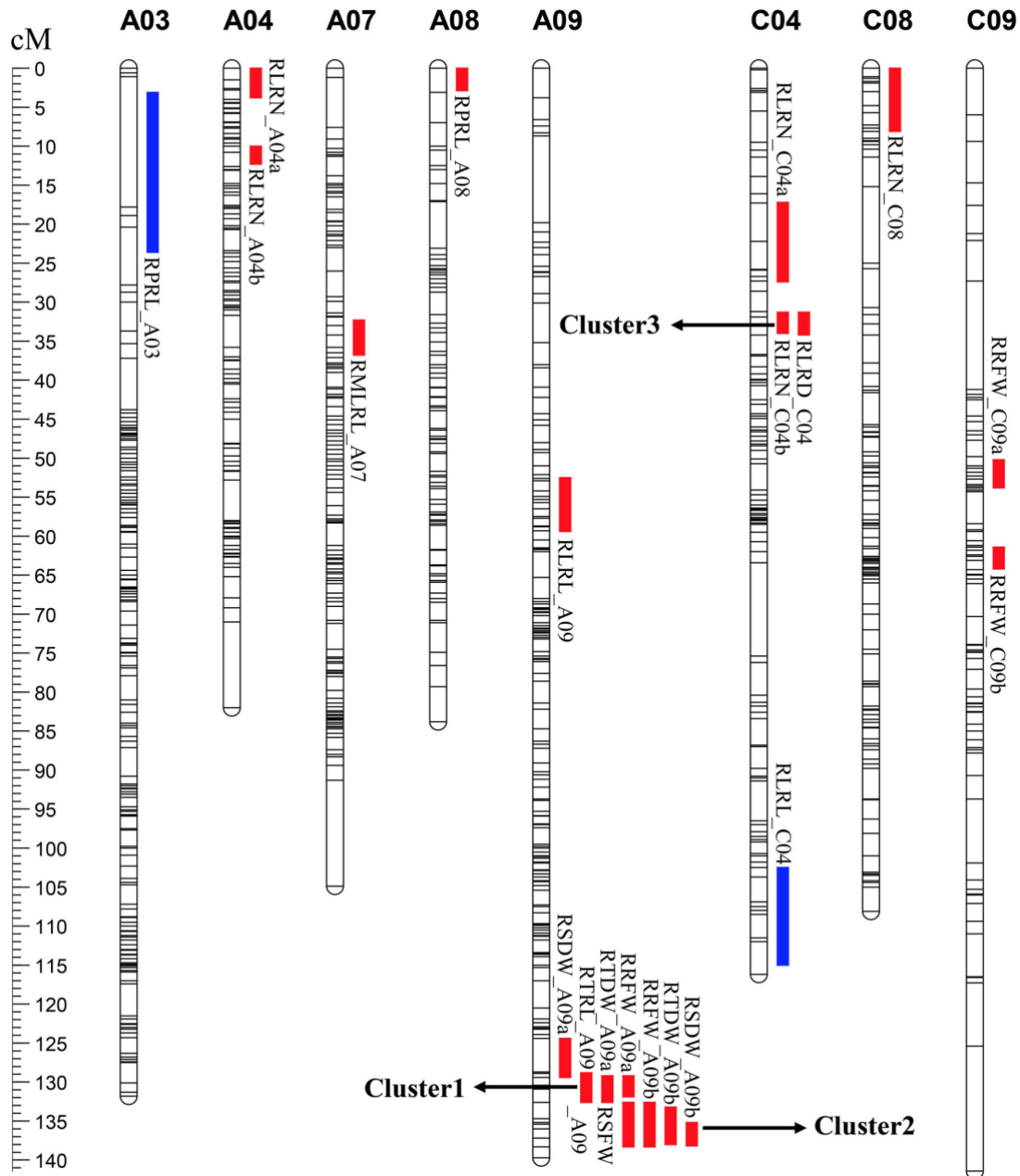
779

780 **Fig. 2** Frequency distribution of relative root traits of the *BnaTNDH* mapping population in the
 781 'agar' (red bar) and 'pouch and wick' (blue bar) systems. RTRL (a), relative total root length;
 782 RPRL (b), relative primary root length; RLRL (c), relative total lateral root length; RMLRL (d),
 783 relative mean lateral root length; RLRN (e), relative lateral root number; RLRD (f), relative
 784 lateral root density. The solid red circle and solid red uptriangle represent Tapidor and Ningyou
 785 7 in the 'agar' system, respectively. The solid blue circle and solid blue uptriangle represent
 786 Tapidor and Ningyou 7 in the 'pouch and wick' system, respectively

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792 **Fig. 3** Locations of QTLs for relative root traits and relative biomass traits in the *BnaTNDH*
 793 mapping population in the 'agar' and 'pouch and wick' systems. QTLs are indicated on the right
 794 side of each chromosome. The red and blue bars denote the QTLs identified in the 'agar' system
 795 and the 'pouch and wick' system, respectively. The QTL confidence intervals are set as the map
 796 interval corresponding to a 2-LOD decline on either side of the LOD peak. RTRL, relative total
 797 root length; RPRL, relative primary root length; RLRL, relative total lateral root length;
 798 RMLRL, relative mean lateral root length; RLRN, relative lateral root number; RLRD, relative
 799 lateral root density; RTDW, relative total dry weight; RSDW, relative shoot dry weight; RSWF,
 800 relative shoot fresh weight; RRFW, relative root fresh weight

801 **Supplementary Figure Legends**

802 **Supplementary Fig. 1** The plasticity of root traits of the *Bna*TNDH mapping population in
803 response to phosphorus deficiency in the ‘agar’ and ‘pouch and wick’ systems. **a**, total root
804 length (TRL); **b**, primary root length (PRL); **c**, total lateral root length (LRL); **d**, mean lateral
805 root length (MLRL); **e**, lateral root number (LRN); **f**, lateral root density (LRD). Boxes
806 represent the mid two quartiles with the median and mean drawn. Whiskers are the 95%
807 confidence limits plus extremes

808

809 **Supplementary Fig. 2** Location of QTLs for TRL (total root length), PRL (primary root length),
810 LRL (total lateral root length), MLRL (mean lateral root length), LRN (lateral root length),
811 LRD (lateral root density) and its relative traits. The red bar above the chromosome denotes the
812 QTL identified at a low P supply. The green bar below the chromosome denotes the QTL
813 identified at an optimal P supply. The purple bar inside the chromosome denotes the QTL for
814 relative root trait. The red star indicates that the QTL for a root trait is co-located with the QTL
815 for its relative trait

816

817 **Supplementary Fig. 3** The illumination of roots altered the response of root architecture to
818 phosphate deprivation in *Arabidopsis thaliana*. Col-0 seedlings were grown at a low (-P, 0 mM)
819 and an optimal P supply (+P, 0.625 mM) with the root exposed to light (LGR, light-grown roots)
820 or in darkness (DGR, dark grown roots) in an illuminated culture room with 16 h photoperiod
821 of approximately 300–320 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature at 18–24 °C and a relative humidity of 65–
822 80 % for 21 days. Scale bar = 2 cm

823

824 **Supplementary Fig. 4** The illumination of roots altered the response of root architecture to
825 phosphate deprivation in *Brassica napus*. Tapidor and Ningyou 7 seedlings were grown at a
826 low (-P, 0 mM) and an optimal P supply (+P, 0.625 mM) with the root exposed to light (LGR,
827 light-grown roots) or in darkness (DGR, dark grown roots) in an illuminated culture room with
828 16 h photoperiod of approximately 300–320 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature at 18–24 °C and a relative
829 humidity of 65–80 % for 9 days. Scale bar = 3 cm

830

831 **Supplementary Fig. 5** Total root length (**a**), primary root length (**b**), total lateral root length
832 (**c**), mean lateral root length (**d**), lateral root number (**e**), lateral root density (**f**) of Tapidor and
833 Ningyou 7 seedlings grown at a low (-P, 0 mM) and an optimal P supply (+P, 0.625 mM) with
834 the root exposed to light (LGR, light-grown roots) or in darkness (DGR, dark grown roots).
835 Data are shown as mean \pm SD ($n = 3-6$). Asterisks indicate statistically significant differences
836 between -P and +P (*, $P < 0.05$; **, $P < 0.01$) according to Student’s *t*-test.