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Article

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Local and systemic responses conferring adaptation of *Brassica napus* roots to low phosphorus conditions

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Running Head: Root plasticity of *B. napus* with heterogenous Pi availability

Summary: This work reveals the mechanistic basis of locally and systemically regulated responses of *B. napus* root architecture to heterogenous Pi distribution under in vitro conditions.

Abstract

Due to the non-uniform distribution of inorganic phosphate (Pi) in the soil, plants modify their root architecture to improve acquisition of this nutrient. In this study, a split-root system was employed to assess the nature of local and systemic signals that modulate root architecture of *Brassica napus* grown with non-uniform Pi availability. Lateral root (LR) growth was regulated systemically by non-uniform Pi distribution, by increasing the density of the second-order LR (2°LR) in compartments with luxury Pi supply but decreasing the 2°LR density in compartments with low Pi availability. Transcriptomic profiling identified groups of genes regulated, both locally and systemically, by Pi starvation. The number of systemically induced genes was greater than the number that was locally induced and included genes related to abscisic acid (ABA) and jasmonic acid (JA) signalling pathways, reactive oxygen species (ROS) metabolism, sucrose, and starch metabolism. Physiological studies confirmed the involvement of ABA, JA, sugars, and ROS in the systemic Pi starvation response. The data reported reveal the mechanistic basis of local and systemic responses of *B. napus* to Pi starvation and provide new insights into the molecular and physiological basis of root plasticity.

Key words: *Brassica napus*, heterogeneous Pi availability, local regulation, systemic regulation, abscisic acid, jasmonic acid, sugar, ROS, phosphate, phosphorus

Introduction

Phosphorus (P) is one of the most critical macronutrients for plant growth and development (Hawkesford *et al.*, 2012). Although the total content of P in the soil can be high, in many cases the availability of inorganic phosphate (Pi), the main form of P that can be taken up by plants, is limited. Pi can precipitate with calcium, magnesium, aluminium and iron, and the high sorption capacity of Pi to soil particles results in a very low availability and heterogeneous distribution in soil (Obersteiner *et al.*, 2013; Zhang *et al.*, 2013; Lynch and Wojciechowski, 2015; Jin *et al.*, 2017).

To counter these constraints, plants have evolved various adaptive strategies to detect Pi distribution in their environment and adapt their morphology and physiology to variations in Pi concentration (Williamson *et al.*, 2001; Lynch, 2011). Root system architecture (RSA) is highly plastic in response to the heterogeneous distribution of Pi, with plants varying both the length and density of their primary (PR) and lateral (LR) roots and root hairs (Péret *et al.*, 2014; Bouain *et al.*, 2016; Gutiérrez-Alanís *et al.*, 2018). For example, shallow root systems have more LRs distributed in the topsoil for better acquisition of the poorly mobile Pi (Jin *et al.*, 2017; van der Bom *et al.*, 2020). This plasticity in RSA in response to localised Pi availability is highly species-specific. For example, in wheat and chickpea root proliferation was significantly increased in the Pi-enriched zone, whereas RSA in maize and faba bean were not responsive to local Pi availability (Li *et al.*, 2014). In *Arabidopsis*, localised Pi availability resulted in a significant increase in LR length in the Pi-enriched zone, whereas LR density was not affected (Linkohr *et al.*, 2002) or even decreased.

Modulation of RSA in response to Pi starvation is driven by two partially independent signalling pathways: local (confined to roots) and systemic (involving long-distance root-to-shoot and shoot-to-root communication) (Chien *et al.*, 2018; Ham *et al.*, 2018; Oldroyd and Leyser, 2020). Local

79 responses are modulated by the external Pi availability in the growth medium,
80 while systemic responses depend on the internal Pi concentrations in the plant
81 (Svistoonoff *et al.*, 2007; Lin *et al.*, 2014). The root cap is positioned at the very
82 end of root tip and is responsible for sensing Pi availability (Svistoonoff *et al.*,
83 2007; Ticconi *et al.*, 2009; Ravelo-Ortega *et al.*, 2022). Manipulating local Pi
84 availability through split-root experiments mimics the heterogeneous Pi
85 distribution in soil and allows changes in RSA and local and systemic
86 responses to Pi starvation to be determined (Franco-Zorrilla, 2005; Thibaud *et*
87 *al.*, 2010). At the same time, genome-wide transcriptome analysis has been
88 successfully used to elucidate molecular mechanisms underlying complex
89 adaptations of crops to Pi deficiency using the RNA-seq technique (Wang *et al.*,
90 2016; Xue *et al.*, 2018; Wang *et al.*, 2019). Combined, these two techniques
91 provide an excellent tool to understand the mechanistic basis of modulation of
92 RSA and the molecular nature of the local and systemic signals involved.

93 LOW PHOSPHATE ROOT1 and 2 (LPR1 and LPR2) proteins play a critical
94 role in sensing local Pi availability in *Arabidopsis*, since LPR1 is expressed in
95 the root cap (Svistoonoff *et al.*, 2007). PHOSPHATE DEFICIENCY
96 RESPONSE 2 (PDR2) and LPR1 may function together in mediating
97 responses of the root meristem to external Pi availability (Ticconi *et al.*, 2009;
98 Ruiz-Herrera *et al.* 2015). Local low Pi sensing enhances auxin responses and
99 involves Mitogen-Activated Protein Kinase 6 (MPK6) signalling within the root
100 tip, particularly the root cap via SOMBRERO (Pérez-Torres *et al.*, 2008;
101 López-Bucio *et al.*, 2019; Ravelo-Ortega *et al.*, 2022). In addition, the
102 SENSITIVE TO PROTON RHIZOTOXICITY1 - MEDIATOR16 -
103 ALUMINUM-ACTIVATED MALATE TRANSPORT1 (STOP1 - MED16 - ALMT1)
104 signalling module is involved in root system remodelling in response to low Pi
105 availability (Raya-González *et al.*, 2021; Ruiz-Herrera *et al.*, 2021). Most of the
106 current knowledge comes from *Arabidopsis* plants and, in the light of the
107 species-specificity of RSA responses to Pi starvation, direct translation of

108 some findings to other species is debatable.

109 Oilseed rape (*Brassica napus* L.) is one of the most important oil crops
110 cultivated throughout the world and is extremely sensitive to Pi deficiency
111 (Chen *et al.*, 2015). No study has investigated the nature of the local and
112 systemic responses to Pi starvation in this species. In this work, we took
113 advantage of the availability of high-quality genomic sequences of *B. napus*
114 (Sun *et al.*, 2017; Song *et al.*, 2020) and utilised a split-root system to
115 investigate local and systemic regulation of RSA in response to homogeneous
116 and heterogeneous Pi availability in this species, both at transcriptional and
117 functional levels.

118

119 **Materials and methods**

120 ***Split-root experiments***

121 ‘ZhongShuang11 (ZS11)’, a semi-winter *B. napus* cultivar used in this work, is
122 the most popular cultivar grown in the middle and downstream regions of the
123 Yangtze River basin. Seeds of ‘ZS11’ were kindly provided by the Oil Crops
124 Research Institute, Chinese Academy of Agriculture Science. Seeds were
125 surface sterilized in 1.0 % (v/v) NaClO for 20 min, rinsed five times in sterile
126 distilled water, and then sown in sterile Petri dishes (13 × 13 × 1 cm) containing
127 60 mL Murashige–Skoog (MS) salt with 1.0 % (w/v) agar (Sigma-Aldrich, St.
128 Louis, MO, catalogue no. A1296) and 625 μM KH₂PO₄ (+P). After 3 days,
129 uniform *B. napus* seedlings were selected. The primary root tip was removed
130 mechanically to induce the formation of lateral roots and allow the
131 development of a split-root system. After another 3 d, seedlings with two lateral
132 roots of the same length were transferred to the bigger (25 × 25 × 2 cm) sterile
133 Petri dish (growth chamber) containing 110 mL MS with 1.0 % (w/v) agar. A thin
134 plastic sheet was inserted to separate the chamber into two compartments,
135 with contrasting Pi availability: (1) compartment containing 625 μM KH₂PO₄

136 (abbreviated as +P), and (2) 0 μM KH_2PO_4 (abbreviated as -P). In the latter
137 case, KH_2PO_4 in MS media was replaced with KCl. The pH was adjusted to 5.6
138 in both compartments. One lateral root (LR) of the first order (1°) was placed
139 into the +P compartment (abbreviated hereafter as R+), and one was placed
140 into the -P compartment (R-). For controls, chambers with uniform P
141 distribution between compartments - either with 625 μM P supply in both
142 compartments (abbreviated as R++) or without P (abbreviated as R--) were
143 used. Each Petri dish contained two plants. Plants were grown in a controlled
144 environment chamber with a photoperiod of 16 h of light and 8 h of darkness at
145 22~24 °C. The light intensity was 300-320 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (photon flux density)
146 and the relative humidity was 60-75%. Plants were photographed, and then
147 shoots (S++, S+- and S--) and roots (R++, R+, R- and R--) were harvested
148 after 9 d of treatment.

149 To check the effect of JA, ABA and sugars on plant responses to Pi
150 availability in a split-root experiment, 1 μM JA, 10 μM DIECA
151 (diethyldithiocarbamic acid, a JA biosynthesis inhibitor), 5 μM ABA, 3 μM FLD
152 (fluridone, an ABA biosynthesis inhibitor), and 1% sucrose were added
153 separately to the -P compartment, and the seedlings were sampled after 9 d.
154 JA and ABA were dissolved in ethanol, and DIECA, FLD and sucrose were
155 dissolved in pure water. Accordingly, the mock controls for the JA and ABA
156 treated experiment contained ethanol, and those for the DIECA, FLD and
157 sucrose treatments were pure water.

158

159 ***Root morphology and tissue Pi content assay***

160 Seedlings grown on the plates were photographed with a digital camera
161 (NIKON D750). The length of 1°LR and 2°LR , 2°LR number and total 2°LR
162 length were measured using ImageJ software. The number of 2°LRs (including
163 LR and LR primordial; VII and VIII stages) of the seedlings was counted
164 under a stereomicroscope (Olympus SZ61) (Péret *et al.* 2009).

165 The tissue Pi concentration was measured using the method described by
166 Wang *et al.* (2012), with some modification. Briefly, 50 mg of fresh tissue was
167 homogenized with 50 μ L of 5 M H_2SO_4 and 950 μ L H_2O . The homogenate was
168 centrifuged at 10000 g for 10 min at 4 °C. The supernatant was collected and
169 diluted to an appropriate concentration. The diluted supernatant was mixed
170 with a malachite green reagent in 3:1 ratio and analysed after 30 min. The
171 absorption values for the solution at 650 nm were determined using a
172 Multifunctional Enzyme Marker (TECAN infinite M200).

173

174 ***Determination of H_2O_2 , O_2^- , POD, SOD, soluble sugars, and sucrose***

175 For analyses of H_2O_2 , and O_2^- content, peroxidase (POD) and superoxide
176 dismutase (SOD) activities, and soluble sugars and sucrose content, 0.1 g
177 fresh weight root samples were homogenized in 2 mL cold extraction buffer
178 (0.1 M phosphate buffer, pH 7.0). After centrifugation for 10 min at 8000 rpm,
179 the supernatants were used for measurements of the parameters using
180 appropriate assay kits (COMINBO, Suzhou, China (www.cominbio.com))
181 according to the manufacturer's instructions as described previously (Anwar *et al.*
182 *et al.*, 2018; Chen *et al.*, 2019). H_2O_2 , O_2^- , soluble sugars, sucrose, POD and
183 SOD were expressed on a fresh weight basis.

184

185 ***Histochemical detection of H_2O_2 , O_2^- and callose***

186 H_2O_2 was detected in roots using the DAB (3,3-diaminobenzidine,
187 Sigma-Aldrich) staining method as described previously (Vanacker *et al.*,
188 2000). The staining solution contained 1 mg/mL DAB in a 10 mM sodium
189 phosphate buffer (pH 7.0) with Tween 20 (0.05% v/v). Roots were incubated in
190 the staining solution at room temperature for 1 h. The root tips of 1stLR were
191 then visualized and imaged with a stereomicroscope (Olympus SZ61)
192 equipped with a digital camera (Olympus DP73) after rinsing five times in
193 medium solution. Generation of O_2^- in roots was detected by dihydroethidium

194 (DHE, Invitrogen) staining according to the method described by Yamamoto *et*
195 *al.* (2002). Roots were incubated with liquid MS medium for 2 h, then loaded
196 with 10 μ M DHE for 20 min. After being washed five times with the MS solution,
197 the root tips of 1°LR were imaged under a confocal microscope (Olympus FV
198 1000; excitation 543 nm, emission 600–675 nm for DHE). For callose staining,
199 roots were treated for 1.5 h with 0.1 % (w/v) aniline blue (AppliChem) in 100
200 mM Na-phosphate buffer (pH 7.2) according to the method described by Müller
201 *et al.* (2015). The relative staining/fluorescence intensities of R++ were set as
202 100%, and the fluorescence intensities of other roots were calculated as the
203 percentage of that for R++ as per He *et al.* (2012). Data are presented as the
204 mean value of at least 20 roots.

205

206 ***Trypan blue staining***

207 Roots of seedlings were incubated in 0.4% trypan blue staining solution at
208 room temperature for 3 min, and then transferred to PBS (phosphate buffer
209 saline) for washing, kept in distilled water and observed under a
210 stereomicroscope (Olympus SZ61).

211

212 ***Quantification of callose content in roots***

213 1,3- β -D-glucan (callose) content of roots was quantified using the method
214 described by Santos *et al.* (2005). Briefly, 0.2 g fresh weight root samples were
215 placed in micro centrifuge tubes containing 95 % ethanol for at least 1 h. The
216 alcohol was subsequently decanted and 200 μ L of 1 M NaOH was added into
217 the tubes. The samples were ground and then placed in a water bath at 80 °C
218 for 15 min to solubilize callose and then centrifuged at 15000 g for 4 min. The
219 supernatant (400 μ L) was incubated with 800 μ L 0.1 % aniline blue, 420 μ L 1
220 M HCl, and 1180 μ L glycine-NaOH buffer (pH 9.5) for 20 min at 50°C and then
221 for 30 min at the room temperature. Callose content was estimated using a
222 Multifunctional Enzyme Marker (TECAN infinite M200) with excitation at 398

223 nm and emission at 495 nm. Pachyman (Calbiochem, LaJolla, CA, USA) was
224 used as an external standard and callose content was expressed as mg
225 Pachyman equivalent (PE) per g root fresh weight.

226

227 ***Determination of MDA content***

228 The MDA content was measured according to the modified thiobarbituric acid
229 (TBA) method described by Wang *et al.* (2009). Approximately 0.1 g fresh
230 weight root samples were homogenized in 750 μ L of 5 % tri chloroacetic acid
231 (TCA) and centrifuged at 3000 rpm for 10 min. The supernatant was mixed
232 with 200 μ L of 5 % TCA containing 0.67 % TBA. The mixture was heated to
233 100 °C for 30 min and cooled on ice. After centrifugation at 12000 rpm for
234 5 min, the absorbance of the supernatant at 532 nm was recorded.
235 Non-specific absorbance at 600 nm was measured and subtracted from the
236 readings recorded at 532 nm. Concentration of malonaldehyde (MDA) was
237 calculated using its extinction co-efficient, 155 mM⁻¹ cm⁻¹.

238

239 ***Extraction and analysis of endogenous plant hormones***

240 Extraction and analysis of endogenous plant hormones were conducted
241 according to the method described by Liu *et al.* (2012). The supernatant was
242 gathered and injected into UFLC-ESI-MS/MS (ultrafast liquid
243 chromatography-electrospray ionization/tandem-mass spectrometry system.
244 Five biological replicates were analysed for each treatment. The standard of
245 JA was purchased from Sigma-Aldrich (St. Louis, MO, USA), and the
246 standards for ABA and JA-Ile were purchased from OlChemIm (OlChemIm,
247 Olomouc, Czech Republic). The internal standards were ²H₆ABA (Olchemin)
248 for ABA, 10-dihydro-JA (DHJA; Olchemin) for JA and JA-Ile. All these
249 standards and internal standards were kindly provided by Dr. Hongbo Liu from
250 the National Key Laboratory of Crop Genetic Improvement, Huazhong
251 Agricultural University.

252

253 ***RNA-seq and analysis***

254 Four biological replicates from each treatment were used for transcriptome
255 analyses. Each biological replicate was a composite sample, which had 20
256 roots from independent plates. Total RNA was extracted using a RNAiso Plus
257 kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The
258 integrity of the RNA was checked by electrophoresis on a 1% agarose gel and
259 spectrophotometrically with a NanoDropTM2000 UV-vis Spectrophotometer
260 (Thermo Scientific, Waltham, MA). The transcriptome sequencing was
261 performed by Novogene (Beijing, China). The library construction was carried
262 out according to the Illumina standard instructions and was sequenced on the
263 Illumina HiSeq 2000 platform. To obtain high-quality clean reads, the adaptor
264 reads, unknown nucleotides, low-quality reads were removed from the raw
265 reads. The clean reads were then aligned to the ZS11 genome (Sun *et al.*,
266 2017; Song *et al.*, 2020) using Bowtie2 and HISAT2 software. The Fragments
267 Per Kilobase of transcript per Million mapped reads (FPKM) method was used
268 to calculate the expression levels of genes. Differential expression of the
269 genes between treatments was then analysed using the DESeq R package
270 (<http://www.bioconductor.org/packages/release/bioc/html/DESeq.html>). Genes
271 with fold change $|\log_2FC| > 1$ and $P < 0.05$ were deemed to be significantly
272 differentially expressed genes (DEGs). To determine the biological significance
273 of the DEGs, transcripts in all samples were searched by BLASTN with an $E <$
274 10^{-5} against the TAIR database (<http://www.Arabidopsis.org/Blast/index.jsp>).
275 Then, the unigenes (AGI identifiers) were used to annotate these DEGs.
276 Annotated genes were attributed functions using the GO database
277 (<http://geneontology.org/>) using the Blast2Go program and to biological
278 pathways using the KEGG database (<http://www.genome.jp/kegg>). GO terms
279 and biological pathways with a $P < 0.05$ were deemed to be significantly
280 enriched in DEGs.

281

282 ***Real-time quantitative reverse transcription PCR (RT-qPCR) analysis***

283 To assay the relative expression levels, RT-qPCR analysis was performed with
284 the total RNA extracted as described above. The total RNA was used as
285 templates (1 µg each) for the first-strand cDNA synthesis with a HiFiScript
286 cDNA Synthesis Kit (CWBIO, Beijing, China) according to the manufacturer's
287 instructions. RT-qPCR analysis with gene-specific primers (Table S2) was
288 conducted on a CFX96™ Real-time PCR Detection System (Bio-Rad,
289 Hercules, CA, USA) using Hieff qPCR SYBR Green Master Mix (Yeasten,
290 Shanghai, China) based on the manufacturer's protocol. *Tubulin* and *EF1α*
291 were used as internal control gene to normalize samples, and relative gene
292 expression levels were measured using the $2^{-\Delta\Delta C_T}$ method. Three biological
293 replicates were used for each sample.

294 In order to check the early transcriptional responses of *B. napus* in split-root
295 treatments, the expression levels of some representative genes (ABA, JA,
296 sugar and ROS related genes) were examined by RT-qPCR.

297

298 ***Statistical analysis***

299 Statistical analysis of the data was conducted using one-way analysis of
300 variance (ANOVA) or t-test in SPSS (IBM, New York, NY) and Microsoft Office
301 Excel, assuming $P < 0.05$ as a significance threshold.

302

303 **Results**

304 ***Heterogeneous availability of Pi significantly alters the RSA of B. napus*** 305 ***seedlings***

306 The shoot fresh weight of the plants grown with a heterogenous Pi supply (S+-)
307 was similar to that of the plants with homogenous Pi availability (S++), but both
308 were significantly larger than that of Pi-starved plants (S--) (Figure 1b-c). Root

309 fresh weights followed the series $R^+ > R^{++} > R^{--} > R^-$ (Figure 1b, c).
310 Furthermore, the Pi concentration in S^{+-} was 25% lower than that in S^{++} , but
311 100% higher than that in S^{--} (Figure 1d). The Pi concentration in R^{--} was only
312 25% of that in R^{++} , whereas the Pi concentration in the R^+ was similar to that
313 of R^{++} . The Pi concentration in R^- was higher than that in R^{--} , but lower than
314 that in R^+ (Figure 1d).

315 The plants grown with a homogenous low Pi supply had shorter 1°LR, but
316 more 2°LR than those grown with a homogenous high Pi supply (Figure 1b,
317 e-g). Root morphology of the plants grown with heterogeneous Pi availability
318 was significantly different to the roots of the plants grown with homogenous Pi
319 availability (Figure 1b). The elongation rate of 1°LR of R^+ was similar to R^{++} ,
320 but that of R^- was significantly greater than R^{--} (Figure 1e, f). Also, the
321 development of 2°LR (Figure 1g, h) and their length (Figure 1i, j) were greatest
322 in R^+ and least in R^- . Collectively, these data suggest that LR growth of *B.*
323 *napus* seedlings is mainly regulated by systemic signalling.

324

325 ***Transcriptomic analysis of genes locally or systemically regulated by Pi*** 326 ***starvation***

327 In order to understand the molecular mechanisms regulating the morphological
328 and physiological responses of *B. napus* seedlings to heterogeneous Pi
329 availability, RNA-seq analysis was performed, and pairwise comparisons of
330 gene expression levels among treatments conducted. Following quality checks
331 and exclusion of null reads, a total of 108.7 Gb (with a GC content of 46.31 and
332 a Q30 of 89.48%) of paired-end clean reads were generated across 16 root
333 samples (Table S1). These clean reads were mapped to the *B. napus* ZS11
334 reference genome (Sun *et al.*, 2017; Song *et al.*, 2020).

335 Transcriptomic differences in the R^{++} vs. R^{--} comparison were the largest
336 among all the pairwise comparisons with a total of 4793 differentially
337 expressed genes (DEGs), 2935 being up-regulated and 1858 down-regulated

338 (Figure 2a). The comparison with the smallest difference in DEGs was R- vs.
339 R+, with only 306 DEGs identified, 150 being up-regulated and 156
340 down-regulated. In addition, a total of 4080 and 1653 DEGs were identified in
341 R-- vs. R- and R++ vs. R+, respectively (Figure 2a). The transcriptome data
342 were validated by quantitative reverse transcription-PCR (RT-qPCR). The
343 expression patterns of the 14 randomly selected genes assayed by RT-qPCR
344 were largely in agreement with those assayed by RNA-seq, as reflected by a
345 high correlation coefficient ($R^2 = 0.88$) between the two methods (Figure S1).

346 The DEGs were divided into two categories according to the relative
347 expression levels of genes between R++ and R--. The genes whose
348 expression levels in R-- were significantly higher and lower than that in R++
349 were defined as Pi-starvation-induced genes and Pi-starvation-repressed
350 genes, respectively. These DEGs were then classified according to their
351 expression levels in R+ or R-: (i) locally regulated genes were designated as
352 transcripts with a similar expression level between R++ and R+ or R-- and R-
353 (Figure 2b, c), whereas (ii) transcripts with significantly different expression
354 levels in R+ and R- versus their respective controls (R++ and R--) were termed
355 systemically regulated genes (Figure 2 d-g). We identified 894 locally induced
356 and 971 locally repressed genes by Pi starvation, respectively. These genes
357 were only regulated by the Pi levels in the adjacent medium and the
358 transcription levels of the up-regulated or down-regulated genes between R+
359 and R++, and between R- and R-- were similar (Figure 2b, c). The systemically
360 regulated genes were divided into four groups based on the transcription levels
361 of R+ and R-, reflecting a hierarchical change in the response to Pi starvation.
362 Two groups are based on the systemic repression of genes in R- or R+ when
363 compared with R-- or R++ but where the transcription level displayed no
364 significant difference between R++ and R+ or R-- and R-, respectively (Figure
365 2d and f). A total of 1778 and 328 genes were identified in these two groups,
366 they were either systemically induced or repressed by Pi starvation (Figure 2d,

f). Among these two groups, the induced genes in R- (Figure 2d) and the repressed genes in R+ (Figure 2f) were modulated systemically, and their transcriptional levels were lower than that in R-- and R++, respectively. In the other two groups, the transcription level of genes in R+ (Figure 2e) and R- (Figure 2g) were both modulated systemically and they were between the transcription level of R++ and R--. The number of genes in these two groups were 52 and 51, respectively. Taken together, the number of systemically regulated genes was more than that of locally regulated genes, and almost half (45%) the Pi starvation-regulated genes were systemically induced, indicating systemically induced genes may play vital roles in response to Pi starvation.

The P1BS element is central to the responses of plants to Pi starvation (Sobkowiak *et al.*, 2012). The proportion of genes containing a putative P1BS element in their promoter in different groups of genes was investigated (Figure 2h). Compared to locally regulated genes and systemically repressed genes, systemically induced genes had a higher proportion of genes containing P1BS in their promoter region (Figure 2h). This supports the notion that systemically induced genes are central to the Pi-deficiency signal transduction pathway.

384

385 ***Functions of genes regulated locally and systemically by Pi starvation***

Genes locally induced and repressed by Pi starvation were both associated with hormone-related responses, including biosynthesis, transport and response to ethylene (induced: *ERF*, *EDF*, *EFE*), jasmonic acid (induced: *JAZ10*), auxin (induced: *IAA7*, *IAA29*, *PIN*; repressed: SAUR-like auxin-responsive protein family, *IAA16*), abscisic acid (induced: *ZF2*; repressed: BURP domain-containing protein, *PP2C5*), gibberellin (repressed: gibberellin-regulated family protein, gibberellin-oxidase) and involved in the homeostasis of metals such as iron, zinc, copper and potassium, etc. (induced: *FER3*, *YSL2*, *ZIP*, *CCH*, etc.; repressed: *VIT*, *COPT2*, *AKT3*, etc.). In addition, a large number of genes encoded transcription factors (*WRKY*, *NAC*, *MYB*,

396 *bHLH*, etc.). A Pi transporter (*PHT3;1*) and two phosphate transporter traffic
397 facilitator1 (*PHF1*) genes were locally induced and transporters for other
398 substances (*MFS*, *AAP*, *MATE*, *NIP*, etc.) were locally induced or repressed. In
399 addition, 14 locally-induced genes were associated with stress-related
400 responses, which encoded cytochrome P450, and disease resistance and
401 response to stress proteins, 25 locally-repressed genes were implicated in cell
402 wall synthesis (expansin, *CEL3*, *CSLA7*), cell activity and growth (*LRR*,
403 *LRR-RK*, etc.), lateral root primordium (lateral root primordium (LRP)
404 protein-related) and root hair growth (*RSL4*), and Pi recycling (HAD
405 superfamily, subfamily IIIB acid phosphatase and *PAP15*) (Figure 3a and Table
406 1).

407 Genes systemically-induced or repressed by Pi starvation were also
408 associated with hormone-related responses, including biosynthesis, transport
409 and response to auxin (induced and repressed: auxin-responsive family
410 protein, etc.), jasmonic acid (induced: *JAZ*, *HCHIB*; repressed: *SQE3*),
411 abscisic acid (induced: *NCED3*, *HAI2*, *RCAR1*, *PP2C5*, *PLP4*, *PDR*; repressed:
412 *ALDH3I1*, *AAO2*), gibberellin (induced: gibberellin-regulated family protein),
413 ethylene (induced: *ERF*) and salicylic acid (repressed:
414 UDP-glucosyltransferase 75B1). In addition, 24 systemically induced genes
415 involved in Pi recovery, including *PHT1;3*, *PHT1;4*, *PHT1;5*, *PHT1;8*, *PHT1;9*,
416 *PHT4;2* and *PHF1*, and two systemically repressed genes encoded low-affinity
417 Pi transporters *PHT2;1*. In addition to these Pi transporters, systemically
418 regulated genes were also involved in regulating the homeostasis of other
419 nutrients. For example, transporters of sulphur (*LSU2*, *SULTR3*, *AST91*),
420 nitrogen (*NRT1.7*, *NAXT1*, nitrate transmembrane transporters), potassium
421 (*KUP9*, *KAT1*, *KAT2*), zinc (*ZIP4*, *ZIP5*), copper (*COPT1*), and iron (*VIT*) were
422 systemically induced, and those of iron (*FRO2*, *FD3*, *IRT1*), nitrogen (*NRT1.1*,
423 *NRT1.7*, *NIA1*, *TIP2;3*, *AMT1;5*), calcium (*CAX1*, *CAX7*), boron (*NIP6;1*) and
424 sulfur (*SULTR1;2*) were systemically repressed. Similarly, a large number of

transcription factors were systemically induced (120) and repressed (20) by Pi starvation, such as *WRKY*, *NAC*, *MYB*, *bHLH*, *bZIP*, *WOX*, etc. In addition, many genes related to Pi recycling, such as acid phosphates (*PAP*) and enzymes involved in phospholipid remobilization, galacto- or sulfo- lipid synthesis and nucleases (*SQD1*, *SQD2*, *MGDC*, *PLC*, *NPC4*, *PS2* and *BFN1*) were induced. Notably, we found that 7 SPX genes (*SPX1*, *SPX2* and *SPX3*) and one *PHO1;H1* gene associated with Pi signalling and sensing were also induced. Several (28) genes related to metal binding, such as zinc binding (13; *STH*, *BCA*, etc.) and iron binding (15; 2OG and Fe(II)-dependent oxygenase superfamily protein, cytochrome P450, etc.), were systemically repressed by Pi starvation (Figure 3b and Table S3).

GO annotation and KEGG pathway analysis of the DEGs systemically-induced by Pi-starvation

The expression of 96% of 1830 systemically- induced genes were strongly repressed in R- compared to R-- (Figure 4a, b). These genes may play significant roles in the systemic response to Pi starvation. Among the top 20 significantly enriched biological process, many systemically-induced DEGs were enriched in five GO terms associated with hormones, including “response to hormone”, “response to abscisic acid”, “response to jasmonic acid”, “jasmonic acid mediated signalling pathway”, “abscisic acid-activated signalling pathway” and four GO terms related to redox status regulation, including “response to oxygen-containing compound”, “oxidation-reduction process”, “response to oxidative stress” and “regulation of reactive oxygen species metabolic process” (Figure 4c). KEGG enrichment analysis of systemically- induced DEGs showed that the greater number of enriched genes were observed in five pathways, “phenylpropanoid biosynthesis”, “glycerolipid metabolism”, “cutin, suberin and wax biosynthesis”, “starch and sucrose metabolism”, and “plant hormone signal transduction” (Figure 4d).

454

455 ***ABA and JA signalling pathways are involved in Pi starvation responses***

456 Six hormone-related GO terms associated with ABA and JA signalling
457 pathways were significantly enriched in genes induced systemically by Pi
458 starvation (Figure 4c). The expression patterns of 41 genes in the
459 ABA-activated signalling pathway GO term and 43 genes in the JA-mediated
460 signalling pathway GO term were analysed in detail (Figure 5a; Table S4). A
461 total of 18 genes were present in both signalling pathways, such as
462 *jasmonate-zim-domain protein (JAZ)*, *myb-domain protein (MYB)* and
463 *calcineurin B-like protein (CBL)*, implying crosstalk between the ABA and JA
464 pathways in the systemic regulation of Pi starvation responses. Twenty-three
465 genes associated with the ABA-activated signalling pathway GO term showed
466 higher expression in R-- than in R++, R+ and R- (Figure 5a and Table S4). In
467 addition, the expression of *nine-cis-epoxycarotenoid dioxygenase 3 (NCED3*,
468 *BnA01g0036580.1*) that codes a key rate-limiting enzyme in ABA biosynthesis
469 (Zhang *et al.*, 2009; Sun *et al.*, 2012) was highest in R-- roots (Table S4), and
470 so was their ABA concentration (Figure 5b, c).

471 The expression of 25 genes associated with the JA-mediated signalling
472 pathway was highest in R-- plants (Figure 5a; Table S4). JA and its biologically
473 active metabolite JA-isoleucine (JA-Ile) are lipid-derived compounds that are
474 synthesized from α -linolenic acid by one of seven branches of the
475 lipoxygenase (LOX) pathways (Feussner and Wasternack, 2002; Fonseca *et al.*
476 *et al.*, 2009). Lipoxygenases (LOXs) catalyse the oxygenation of fatty acids to
477 their hydroperoxyl derivatives, which are required for JA biosynthesis (Schaller,
478 2001). In our study, the transcript levels of two LOXs were highest in R-- plants
479 (Table S4); this was reflected in tissue JA and JA-Ile concentrations (Figure
480 5d-g) indicating that Pi starvation may trigger changes in root JA levels, which
481 act as part of the systemic signalling mechanism.

482 Functional assays demonstrated that shoot growth of plants grown in the

split-root system with heterogeneous Pi availability was inhibited by exogenous JA but promoted by a JA biosynthesis inhibitor (Figure 6a, b), and that R+ and R- roots responded differently to JA application (Figure 6a, c). When R- was treated with exogenous JA, 1°LR elongation of R+ and R- were both inhibited (Figure 6d), and 2°LR number and total 2°LR length of R- were significantly decreased. However, 2°LR density, 2°LR average length and total 2°LR length of R+ roots were significantly increased (Figure 6f-h). Opposite effects were reported for roots treated with a JA biosynthesis inhibitor (Figure 6d-h). Taken together, these data indicate a functional role of JA in the systemic Pi starvation response (PSR).

ABA significantly inhibited R- growth (Figure 7a-c) and 2°LR growth (Figure 7d-h), but increased 2°LR density of R+ (Figure 7a, f). Reduction of ABA concentration by its biosynthesis inhibitor FLD significantly increased shoot fresh weight (Figure 7a-b), promoted 1°LR elongation of R-, but decreased 2°LR number, 2°LR density, average 2°LR length and total 2°LR length of R+ compared to untreated plants (Figure 7d-h). These data indicate that ABA enhances systemic PSR by inhibiting growth of R- and promoting 2°LR density of R+.

501

Genes for sugar metabolism are involved in systemic regulation of Pi starvation responses

The pathway of starch and sucrose metabolism was enriched in DEGs systemically induced or repressed by Pi starvation, implying that this pathway may be critical for systemic regulation of PSR. Seventeen DEGs in the 'Starch and sucrose metabolism' pathway were analysed in detail. Seven DEGs were associated with sucrose synthesis, and seven other DEGs were associated with fructose or glucose synthesis (Figure 8a). The expression of these genes was highest in R-- roots (Figure 8a), as were the concentrations of soluble sugars and sucrose (Figure 8b-e). These results suggest that the synthesis of

sugars in the shoot and their transport to the root were regulated systemically by Pi starvation, and that sugars might be a key component of the systemic Pi-starvation regulation of RSA.

Sucrose can act as a systemic signal, being transported from the shoot to root (Hammond and White, 2011; Ham *et al.*, 2018). In order to confirm the role of sucrose in regulating RSA under heterogenous Pi-starvation conditions, R- was treated with sucrose. Compared to untreated plants, sucrose did not increase shoot fresh weight, but significantly increased fresh weight of R+ (Figure 9a-c). In the roots of R-, sucrose significantly inhibited 1°LR elongation and 2°LR number of R- (Figure 9d, e), but had no effect on 2°LR density, 2°LR average length and total 2°LR length (Figure 9f-h). In roots of R+, sucrose significantly increased 2°LR average length and total 2°LR length of R+ (Figure 9g, h), but did not alter 1°LR length, 2°LR number or 2°LR density (Figure 9d-f). These findings indicate that sucrose enhances systemic PSR by inhibiting 1°LR growth and 2°LR number of R- and increasing the average length of 2°LR and total 2°LR length of R+ roots.

Oxidative stress-related genes are involved in protecting the plant from Pi-starvation stress

In the biological process GO categories, four terms in antioxidant processes and one term in callose deposition were enriched in DEGs (Figure 4a), suggesting that ROS and callose accumulation may be involved in protecting the plant from stresses associated with Pi-starvation. The transcript levels of 28 genes enriched in the response to oxidative stress GO term were highest in R-- plants (Figure 10a) including those encoding peroxidase superfamily proteins (POD) and three genes encoding copper/zinc superoxide dismutases (Cu/Zn-SOD) responsible for ROS scavenging (Choudhury *et al.*, 2016). The activities of POD and SOD were greatest in R-- plants (Figure 10d, e). ROS induces callose deposition in the cell wall of the root tip, which plays an

important role in root development (Dunand *et al.*, 2006; Benitez-Alfonso *et al.*, 2011). The concentrations of two major ROS, hydrogen peroxide (H₂O₂) and superoxide radical (O²⁻), and callose deposition, in plant roots were observed by DAB, DHE and aniline blue staining, respectively. The histochemical staining with DAB and DHE showed the strongest signals in the root tips of R-- plants (Figure 10b, c), as did the fluorescence intensity of aniline blue. Consistent with these findings was *in situ* accumulation of ROS (H₂O₂ and O₂⁻) and callose in root tips (Figure 10f-h). ROS-induced lipid peroxidation assessed by malondialdehyde (MDA) content showed markedly higher MDA content in R-- compared with R++, R+ and R- (Figure 10i). In general, the accumulation of ROS and ROS-induced callose deposition and lipid peroxidation were all more pronounced in R-- than in R++, R+ and R- (Figure 10a~i).

Discussion

Changes in RSA in response to localised Pi availability

Phosphate availability in the soil often shows a heterogeneous distribution because of its low mobility. To overcome low Pi availability, plants have evolved a wide array of mechanisms aimed at modifying RSA to increase root proliferation in Pi-enriched patches (Sun *et al.*, 2018; Wang *et al.*, 2019). Our previous studies showed that 625 µM phosphate in the agar medium is an optimum P supply (Shi *et al.*, 2013), as SDW and RDW were less at both low/no P conditions as well as at 1250 µM phosphate than at 625 µM phosphate. Thus, in the current study, 625 µM phosphate was used as the control (+P, optimum supply).

In this study, heterogeneous Pi availability (+P/-P) did not affect shoot growth (shoot fresh weight) (Figure 1b-d). At the same time, heterogeneous Pi availability increased root fresh weight of R+ but decreased that of R- (Figure

1c), illustrating the preferential partitioning of biomass to the place with greater Pi availability. Pi concentration in R+ was higher than that in R-, and the density and total length of 2°LR in R+ were also greater than those in R- (Figure 1d, h, j), which allows plants to compensate for restricted Pi acquisition by other parts of the root system. Compared with R++ and R--, Pi distribution in R+ and R- also suggesting relatively high mobility of Pi from R+ to R- via the shoot. Previous studies have also shown that greater root proliferation contributed to Pi uptake capacity in Pi-rich patches and, thereby, maintained biomass production (Shen *et al.*, 2005; Funakoshi *et al.*, 2015; Wang *et al.*, 2019). These data suggest a key strategy to maintain high yields with low fertilizer input by local or banded application of phosphorus fertilizers.

Pi deficiency inhibits primary root elongation and increases lateral root length and density (López-Bucio *et al.*, 2002; Sánchez-Calderón *et al.*, 2005; Richardson and Simpson, 2011; Trachsel *et al.*, 2011; Ruiz-Herrera *et al.*, 2015). In this study, the 1°LR length was inhibited while 2°LR length and density were increased in -P/-P medium (Figure 1b, e-j), implying that 1°LR may function similarly to the primary root after the primary root is removed. 1°LR growth was mainly determined by the Pi concentration in the growth medium whether plants were grown with a heterogeneous P supply or at homogeneous Pi supply (with or without Pi) (Figure 1 b-c, e-f). In addition, this pattern also matched the Pi concentration in roots (Figure 1 d), indicating that 1°LR elongation might be modulated not only by the external Pi concentration in the growth medium, but by the intracellular Pi concentration in roots. 1°LR length of R- was longer than that of R-- (Figure 1b, f), which differed from that observed in *Arabidopsis* (Thibaud *et al.*, 2010). Heterogeneous Pi availability markedly decreased 2°LR density of R- and promoted 2°LR initiation and elongation of R+ (Figure 1g-j), which was indicative of systemic P-demand signals from R- and systemic P-supply signals from R+. To our knowledge, no study reported that 2°LR number, density, average length and total length of R-

598 decreased as compared with R-- in *Arabidopsis* (Thibaud *et al.*, 2010; Oldroyd
599 and Leyser, 2020) and other crops (Wang *et al.*, 2019). RSA responses of *B.*
600 *napus* and *Arabidopsis* to heterogeneous Pi availability are not completely
601 consistent.

602 The seed of *B. napus* is very small, and the seed P reserves probably lasted
603 only 5-6 days in the experiments reported here, judged by the fact that
604 cotyledons became yellow and purple after that time in plants lacking a P
605 supply. In this study, seedlings of *B. napus* were first grown with sufficient Pi for
606 6 d, and then transferred to the split-root system with different Pi availabilities
607 for 9 d (Figure 1a). Both 1°LR and 2°LR growth showed significant differences
608 among treatments after 9 d of treatment (Figure 1e-j). It is, therefore, likely that
609 it was the Pi in the medium, rather than the P in the seed, that affected the
610 responses of *B. napus* to Pi availability in our experiments.

611

612 ***Locally and systemically regulated transcriptional responses to Pi*** 613 ***starvation***

614 Transcriptional changes of gene expression play pivotal roles in the
615 modulation of physiological and biological processes (Zhu, 2016). The
616 pairwise comparison of R++ vs. R-- had the greatest number of DEGs and the
617 R+ vs. R- pairwise comparison had the least number of DEGs, suggesting that
618 gene expression in roots with heterogeneous Pi availability was responding
619 mainly to Pi starvation (Figure 2a). These DEGs were divided into different
620 groups according to a previous study of *Arabidopsis*; but it was recognised that
621 some of systemically induced and repressed genes might also be controlled by
622 local Pi availability (Figure 2d and 2f). This implies the co-regulation of genes
623 by local and systemic signals in response to Pi starvation. Changes in root
624 morphology with heterogeneous Pi availability were mainly regulated
625 systemically (Figure 1b-j). This was consistent with the observation that more
626 genes were regulated systemically than were regulated locally (Figure 2b-g).

627 Furthermore, 1°LR growth was consistent with the expression of systemically-
628 induced genes by Pi starvation (Figure 1f; Figure 2d).

629 Consistent with previous observations (Thibaud *et al.*, 2010), genes
630 associated with Pi homeostasis (Pi recovery, Pi recycling and Pi sensing) were
631 generally systemically induced by Pi starvation (Figure 3b), while genes
632 associated with metal binding were systemically repressed (Figure 3b),
633 implying different strategies for dealing with Pi and metal availability (e.g.,
634 systemic vs local response). In *Arabidopsis*, many genes related to hormonal
635 metabolism were induced locally by Pi starvation (Thibaud *et al.*, 2010).
636 However, our findings revealed that genes associated with hormone-related
637 responses were regulated both locally and systemically by Pi starvation in *B.*
638 *napus* (Figure 3). This is consistent with earlier reports that hormones are
639 implicated in both local and systemic responses to Pi starvation, and that Pi
640 availability can alter hormone biosynthesis, transport and sensitivity (Rubio *et*
641 *al.*, 2009; Chiou *et al.*, 2011; Ham *et al.*, 2018).

642 Pi-starvation-induced genes often contain the P1BS sequence in their
643 promoters (Bustos *et al.*, 2010). In *Arabidopsis*, systemically-induced genes
644 were enriched in the P1BS sequence compared to the entire genome (Thibaud
645 *et al.*, 2010). In our study, 83% of the genes induced systemically by Pi
646 starvation contained the P1BS binding site (Figure 2d and h), and 99% of
647 these genes were specifically, systemically-induced DEGs (Figure 4a). This
648 indicates that BnPHR1 is a major component of the Pi signal transduction
649 pathway regulating systemically-induced genes in the Pi starvation response
650 of *B. napus*.

651 A large number of genes modulated by Pi starvation were regulated locally in
652 *Arabidopsis thaliana* (Thibaud *et al.*, 2010) after 2 days of growth in a split-root
653 system. Our study on *B. napus* involved older plants, and longer (9 d) Pi
654 starvation; and most DEGs were regulated systemically (Figure 2b-g). The
655 transcriptional responses of root to Pi starvation between *B. napus* and

656 *Arabidopsis* are different. A plausible explanation for the contrasting
657 observations of Thibaud *et al.* (2010) and the present paper could be that
658 transcriptional responses to short-term Pi starvation are primarily mediated by
659 external Pi availability, while the response to long-term Pi starvation is mainly
660 mediated by intercellular Pi concentrations. However, the expression patterns
661 of genes related to hormone (JA and ABA) signalling, sugar metabolism and
662 ROS following short-term (2 d) exposure to heterogeneous Pi availability were
663 similar to those following 9 d exposure of *B. napus* to heterogeneous Pi supply
664 (Figure S2).

665

666 ***JA and ABA are involved in systemic responses to Pi starvation***

667 Hormones are important components of Pi signalling regulatory networks (Ha
668 and Tran, 2014; Puga *et al.*, 2017). Hormone-related genes in *Arabidopsis*
669 were only locally regulated by Pi starvation (Thibaud *et al.*, 2010), while
670 hormone-related genes in *B. napus* were both locally regulated and
671 systematically regulated by Pi starvation (Figure 3). A large number of DEGs
672 induced systemically by Pi starvation were significantly enriched in the GO
673 terms of the ABA and JA-mediated signalling pathways (Figure 4c and 5a),
674 suggesting that ABA and JA are both involved in systemic responses of *B.*
675 *napus* to Pi starvation (Figure 4d).

676 Previous studies have indicated that JA induction and Pi starvation share
677 some common phenotypes, including growth reduction and anthocyanin
678 accumulation, implying a potential role of JA in PSR (Shan *et al.*, 2009; Yang *et*
679 *al.*, 2012). It was also suggested that JA may play an important role in the
680 inhibition of PR growth triggered by Pi-starvation (Chacón-López *et al.*, 2011).
681 In our study, the genes associated with JA signalling were systemically
682 induced by Pi starvation (Figure 5a; Table S4). Among them, JAZs are key
683 components in the JA signal transduction pathway and are rapidly induced in
684 response to Pi deficiency (Mosblech *et al.*, 2011; Khan *et al.*, 2016). The

transcript levels of two *BnLOXs* (*BnA03g0128810.1* and *BnC02g0496310.1*), encoding an important enzyme in the JA and JA-Ile biosynthetic pathway (Schaller, 2001), were significantly higher in R-- roots than in R++, R+ and R- roots (Figure 5a and Table S4), and the JA and JA-Ile concentrations in shoots and roots of plants in the -P/-P treatment were significantly higher than those in the -P/+P and +P/+P treatments (Figure 5d-g). Therefore, compared to S-- and R--, less accumulation of JA and JA-Ile in S+- and R- may be a result of higher Pi concentration in S+- and R- (Khan *et al.* 2016).

Addition of JA to -P medium appeared to enhance the Pi-starvation signal from R- in the split-root system that inhibited 1°LR and 2°LR growth of R- and increased 2°LR density, 2°LR average length and total 2°LR length of R+ (Figure 6a, d-h). In contrast, inhibition of JA biosynthesis appeared to reduce the Pi-starvation signal from R- (Figure 6d-h). Taken together, these findings provide strong evidence for the role of JA signalling in systemic regulation of RSA in plants grown with heterogeneous Pi availability.

The transcriptomic analysis of *Arabidopsis* subjected to different hormone treatments and Pi starvation showed that among these hormones, ABA displayed the most interaction with Pi starvation (Woo *et al.*, 2012). We also found that the genes associated with ABA signalling, including *PYL10*, *RCAR1*, *PP2C*, *NCED3*, were systemically induced by Pi starvation (Figure 5a and Table S4). *PYL10* and *RCAR1* (ABA receptors) and their downstream *PP2Cs* are key components in ABA signal transduction (Ma *et al.*, 2009; Hao *et al.*, 2011). Furthermore, the expression of *NCED3* (a key ABA biosynthetic enzyme) was correlated with ABA concentrations in roots (Figure S1 and Figure 5c). Previous studies have similarly reported that greater expression of *NCED3* increases ABA biosynthesis (Takahashi *et al.*, 2018). R-- accumulated more ABA than R++ (Figure 5c), and the inhibition of ABA biosynthesis in R- might be explained by greater Pi concentrations in R- than R-- roots (Figure 1d) or a systemic signal from R+.

714 Exogenous ABA apparently enhanced the Pi-starvation signal from R- roots
715 and systemic PSR by inhibiting 1°LR elongation and 2°LR growth of R- and
716 promoting 2°LR density and root hair growth of R+ (Figure 7a, d-f). Inhibition of
717 ABA biosynthesis significantly decreased the Pi-starvation signal from R- roots
718 and attenuated the systemic PSR by increasing 1°LR length of R- and
719 decreasing 2°LR growth and root hair growth of R+ (Figure 7a, d-f). This
720 indicates that ABA may be also an important signal involved in the long-term
721 systemic responses to Pi starvation.

722

723 ***Sugar metabolism in the systemic responses to Pi starvation***

724 Sucrose and starch metabolism have been reported previously to be involved
725 in PSR, and sucrose has been proposed as an important systemic signal that
726 participates in the regulation of RSA in plants lacking Pi (Hermans *et al.*, 2006;
727 Jain *et al.*, 2007; Hammond and White, 2008; Müller *et al.*, 2007; Hammond
728 and White, 2011; Chiou *et al.*, 2011; Pant *et al.*, 2015). The addition of sucrose
729 to Pi-starved plants enhances PSR gene expression and modifies root growth
730 (Liu *et al.*, 2005; Lei *et al.*, 2011). In this study, many genes related to sugar
731 metabolism were systemically induced by Pi starvation (Figure 8a). These
732 genes included *SPS*, *SPP*, *FRUCT* and *BGLU* that encode enzymes involved
733 in sucrose, glucose and fructose metabolism (Fernández *et al.*, 2004; Chen *et al.*,
734 2005; Haigler *et al.*, 2007; Zhao *et al.*, 2013). The expression of these
735 genes was significantly higher in R-- than in R++, R+ and R- roots (Figure 8a).
736 Also, soluble sugars and sucrose concentrations were significantly higher in
737 S-- than in S++ and S+, and in R-- than in R++, R+ and R- (Figure 8b-e). Pi
738 starvation promotes sugar accumulation in the shoot and also its translocation
739 from the shoot (source) to the root (sink), which suggests that it might act as a
740 systemic Pi signal reporting shoot P-demand to the root and promoting root
741 growth (Ciereszko *et al.*, 2005; Dasgupta *et al.*, 2014). In our study, sugar
742 concentrations in R- were significantly less than that in R--, but similar to those

743 in R+, suggesting that the P-demand of S+- from R- is lower than that of S--
744 from R-- because of the former received a systemic signal of Pi availability
745 from R+ (Figure 1d and Figure 8c, e). Plants treated with exogenous sucrose
746 showed greater Pi starvation symptoms and more P-demand in R- than R+ in
747 the split-root (P+/P-) system, and this enhanced systemic PSR by decreasing
748 1°LR length and 2°LR number of R- and increasing 2°LR average length and
749 total 2°LR length of R+ (Figure 9d-h). Therefore, sugar metabolism is involved
750 in long-term systemic responses to Pi starvation and could potentially be
751 interacting with JA and ABA signalling pathways.

752

753 ***ROS production in Pi-starvation stress***

754 ROS are usually deemed to be toxic and excessive accumulation of ROS
755 leads to inhibition of plant growth and development (Choudhury *et al.*, 2016).
756 ROS concentrations in roots are increased by Pi starvation (Shin *et al.*, 2005).
757 POD and SOD are two key antioxidant enzymes that play a vital role in
758 elimination of H₂O₂ and ·O₂⁻ respectively (Choudhury *et al.*, 2013; Gong *et al.*,
759 2020). Our study indicated that the expression of *POD* and *SOD* genes were
760 systemically induced by Pi starvation (Figure 8a). In agreement with the
761 transcript levels of the genes, POD and SOD activities were also much higher
762 in R-- than in the R++, R+ and R- (Figure 10d, e), suggesting that R-- needs
763 more ROS-scavenging enzymes to detoxify excessive ROS than R++, R+ and
764 R-. This suggestion is supported by the higher accumulation of both H₂O₂
765 and ·O₂⁻ in R-- roots than R++, R+ and R- roots (Figure 10b-c, g-h). These
766 findings agree with previous reports that Pi-starvation increases ROS
767 production and activity of ROS-scavenging enzymes (Shin *et al.*, 2005; Zhang
768 *et al.*, 2020). In addition, there was no significant difference in trypan blue
769 staining among R++, R+ and R-, but staining of R-- was deeper than R++, R+
770 and R-, which indicated that ROS accumulation was caused by Pi-starvation
771 and not by apoptosis (Figure S3). In agreement with the accumulation of ROS

772 in different roots, MDA and callose concentrations in R-- were also higher than
 773 those in R++, R+ and R- (Figure 10b-c, f-i). ROS have been reported to
 774 accumulate in PR meristem and inhibit PR growth in local responses to Pi
 775 starvation (López-Bucio *et al.*, 2002). However, Pi starvation induced ROS
 776 accumulation in the elongation zone of young LR and promoted LR growth,
 777 while in meristem of older LR they inhibited LR growth (Tyburski *et al.*, 2009).
 778 ROS may also act as secondary signals participating in systemic responses to
 779 Pi starvation (Chiou *et al.*, 2011). In our study, ROS accumulation in R- was not
 780 significantly different to R+ and R++, but 1°LR length of R- was shorter than R+
 781 and R++ (Figure 1f and Figure 10b-c, g-h). This might be attributed to the fact
 782 that 1°LR elongation is mainly regulated by external Pi, which resulted in a
 783 slower 1°LR elongation rate of R- than R+ and R++ immediately after transfer
 784 to the treatments (Figure 1e); however, 1°LR elongation rate of R- began to
 785 increase after 4 d (Figure 1e), which could be associated with the decrease in
 786 ROS accumulation in R- at this time. Finally, ROS accumulation in R- was
 787 similar to that in R++ and R+ after 9 d of treatment, but 1°LR length of R- was
 788 shorter than that of R++ and R+ (Figure 1f and Figure 10b-c, g-h).

789

790 **Conclusions**

791 In this study we analysed the changes of RSA to homogeneous and
 792 heterogeneous Pi availability in *B. napus* and found that 2°LR growth was
 793 regulated mainly systemically by Pi starvation. Systemic P-demand (-P)
 794 signalling promotes 2°LR growth of R+ (in blue) and systemic P-supply (+P)
 795 signalling inhibit 2°LR growth of R- (in orange) in split-root plants (Figure 11). A
 796 global transcriptome analysis identified local and systemic regulation of genes
 797 by Pi starvation. Hormones (ABA and JA) and sugars were involved in the
 798 systemic response of RSA to Pi starvation, and ROS were involved in
 799 protecting roots from Pi-starvation (Figure 11). These results provide new
 800 insights to long-term Pi starvation responses by offering new evidence of ABA

801 and JA signalling pathway being involved in the systemic regulation of Pi
802 starvation-induced changes in root system architecture and the mechanistic
803 basis of plant adaptation to low and heterogeneous Pi availability.

804

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812

813 **Author contributions**

814 Y.L. and L.S. designed research; Y.L., X.Y., H.L., W.W., C.W., G.D., F.X., S.W.,
815 H.C. performed research; Y.L., P.W. and J.H. analyzed data; Y.L., L.S., M.Y, S.
816 S., J.H. and P.W. wrote the paper.

817

818 **Conflicts of interest**

819 The authors declare no conflicts of interest.

820

821 **Data Availability**

822 All data supporting the findings of this study are available within the paper and
823 within its supplementary materials published online.

824

825 **Supplementary data**

826 Supplementary data are available at *JXB* online.

827 **Table S1.** Quality of sequencing data

828 **Table S2.** Primer sequences used in the RT-qPCR experiment

829 **Table S3** Functions and differential expression of genes regulated systemically
830 by Pi-starvation in *Brassica napus*

831 **Table S4.** Differentially expressed genes (DEGs) enriching GO terms of
832 ABA-activated and JA-mediated signalling pathways shown in Figure 5a

833 **Figure S1.** Expression of selected differentially expressed genes (DEGs) in
834 the roots of *B. napus* seedlings 9 DAT to the split-root systems illustrated in
835 Figure 1.

836 **Figure S2.** Expression of ten differentially expressed genes (DEGs) related to
837 hormone (JA and ABA) metabolism, sugar metabolism and oxidative stress in
838 the roots of *B. napus* seedlings 2 DAT to the split-root systems.

839 **Figure S3.** Cell activity of roots grown in the split-root systems illustrated in
840 Figure 1.

References

- Anwar A, Bai L, Miao L, Liu Y, Li S, Yu X, Li Y.** 2018. 24-epibrassinolide ameliorates endogenous hormone levels to enhance low-temperature stress tolerance in cucumber seedlings. *International Journal of Molecular Sciences* **19**, 2497.
- Battal P, Turker M, Tileklioğlu B.** 2003. Effects of different mineral nutrients on abscisic acid in maize (*Zea mays*). *Annales Botanici Fennici* **40**, 301–308.
- Benitez-Alfonso Y, Jackson D, Maule A.** 2011. Redox regulation of intercellular transport. *Protoplasma* **248**, 131–140.
- Bouain N, Dumas P, Rouached H.** 2016. Recent advances in understanding the molecular mechanisms regulating the root system response to phosphate deficiency in *Arabidopsis*. *Current Genomics* **17**, 308–314.
- Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, Pérez-Pérez J, Solano R, Leyva A, Paz-Ares J.** 2010. A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*. *Plos Genetics* **6**, e1001102.
- Chacón-López A, Ibarra-Laclette E, Sánchez-Calderón L, Gutiérrez-Alanís D, Herrera-Estrella L.** 2011. Global expression pattern comparison between *low phosphorus insensitive 4* and WT *Arabidopsis* reveals an important role of reactive oxygen species and jasmonic acid in the root tip response to phosphate starvation. *Plant Signaling and Behavior* **6**, 382–392.
- Chen S, Ding G, Wang Z, Cai H, Xu F.** 2015. Proteomic and comparative genomic analysis reveals adaptability of *Brassica napus* to phosphorus-deficient stress. *Journal of Proteomics* **117**, 106–119.
- Chen S, Hajirezaei M, Peisker M, Tschiersch H, Sonnewald U, Börnke F.** 2005. Decreased sucrose-6-phosphate phosphatase level in transgenic tobacco inhibits photosynthesis, alters carbohydrate partitioning, and reduces growth. *Planta* **221**, 479–492.
- Chen ZY, Wang YT, Pan XB, Xi ZM.** 2019. Amelioration of cold-induced oxidative stress by exogenous 24-epibrassinolide treatment in grapevine seedlings: Toward regulating the ascorbate–glutathione cycle. *Scientia Horticulturae* **244**, 379–387.
- Chien PS, Chiang CP, Leong SJ, Chiou TJ.** 2018. Sensing and signaling of

phosphate starvation: from local to long distance. *Plant and Cell Physiology* **59**, 1714–1722.

Chiou TJ, Lin SI. 2011. Signaling network in sensing phosphate availability in plants. *Annual Review of Plant Biology* **62**, 185–206.

Choudhury S, Panda P, Sahoo L, Panda SK. 2013. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signaling and Behavior* **8**, e23681.

Choudhury FK, Rivero RM, Blumwald E, Mittler R. 2016. Reactive oxygen species, abiotic stress and stress combination. *Plant Journal* **90**, 856–867.

Ciereszko I, Johansson H, Kleczkowski LA. 2005. Interactive effects of phosphate deficiency, sucrose and light/dark conditions on gene expression of UDP-glucose pyrophosphorylase in *Arabidopsis*. *Journal of Plant Physiology* **162**, 343–353.

Dasgupta K, Khadilkar AS, Sulpice R, Pant B, Scheible WR, Fisahn J, Stitt M, Ayre BG. 2014. Expression of sucrose transporter cDNAs specifically in companion cells enhances phloem loading and long-distance transport of sucrose but leads to an inhibition of growth and the perception of a phosphate limitation. *Plant Physiology* **165**, 715–731.

Dunand C, Crèvecoeur M, Penel C. 2006. Distribution of superoxide and hydrogen peroxide in *Arabidopsis* root and their influence on root development: possible interaction with peroxidases. *New Phytologist* **174**, 332–341.

Fernández RC, Maresma BG, Juárez A, Martínez J. 2004. Production of fructooligosaccharides by β -fructofuranosidase from *Aspergillus* sp 27H. *Journal of Chemical Technology and Biotechnology* **79**, 268–272.

Feussner I, Wasternack C. 2002. The lipoxygenase pathway. *Annual Review of Plant Biology* **53**, 275–297.

Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C, Solano R. 2009. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology* **5**, 344–350.

Franco-Zorrilla M, Martí AC, Leyva A, Paz-Ares J. 2005. Interaction between phosphate-starvation, sugar, and cytokinin signaling in *Arabidopsis* and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiology* **138**, 847–857.

Funakoshi Y, Daimon H, Matsumura A. 2015. Formation of densely

branched lateral roots in *Sesbania cannabina* triggered by patchily distributed phosphorus in andosolic soils. *Plant Root* **9**, 24–33.

Gong Z, Xiong L, Shi H, Yang S, Zhu JK. 2020. Plant abiotic stress response and nutrient use efficiency. *Science China Life Sciences* **63**, 635–674.

Gutiérrez-Alanís D, Ojeda-Rivera JO, Yong-Villalobos L, Cárdenas-Torres L, Herrera-Estrella L. 2018. Adaptation to phosphate scarcity: tips from *Arabidopsis* roots. *Trends in Plant Science* **23**, 721–730.

Ha S, Tran LS. 2014. Understanding plant responses to phosphorus starvation for improvement of plant tolerance to phosphorus deficiency by biotechnological approaches. *Critical Reviews in Biotechnology* **34**, 16–30.

Haigler CH, Singh B, Zhang D, et al. 2007. Transgenic cotton over-producing spinach sucrose phosphate synthase showed enhanced leaf sucrose synthesis and improved fiber quality under controlled environmental conditions. *Plant Molecular Biology* **63**, 815–832.

Ham BK, Chen J, Yan Y, Lucas WJ. 2018. Insights into plant phosphate sensing and signaling. *Current Opinion in Biotechnology* **49**, 1–9.

Hammond JP, White PJ. 2008. Sucrose transport in the phloem: Integrating root responses to phosphorus starvation. *Journal of Experimental Botany* **59**, 93–109.

Hammond JP, White PJ. 2011. Sugar signaling in root responses to low phosphorus availability. *Plant Physiology* **156**, 1033–1040.

Hao Q, Yin P, Li W, Wang L, Yan C, Lin Z, Wu JZ, Wang J, Yan SF, Yan N. 2011. The molecular basis of ABA-independent inhibition of PP2Cs by a subclass of PYL proteins. *Molecular Cell* **42**, 662–672.

Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P. 2012. Marschner's Mineral Nutrition of Higher Plants. || Functions of Macronutrients. 135-189.

He J, Duan Y, Hua D, Fan G, Wang L, Liu Y, Chen Z, Han L, Qu LJ, Gong Z. 2012. DEXH box RNA helicase-mediated mitochondrial reactive oxygen species production in *Arabidopsis* mediates crosstalk between abscisic acid and auxin signaling. *Plant Cell* **24**, 1815–1833.

Hermans C, Hammond JP, White PJ, Verbruggen N. 2006. How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science* **11**, 610–617.

- Jain A, Poling MD, Karthikeyan AS, Blakeslee JJ, Peer WA, Titapiwatanakun B, Murphy AS, Raghothama KG.** 2007. Differential effects of sucrose and auxin on localized phosphate deficiency-induced modulation of different traits of root system architecture in *Arabidopsis*. *Plant Physiology* **144**, 232–247.
- Jin K, White PJ, Whalley WR, Shen J, Shi L.** 2017. Shaping an optimal soil by root–soil interaction. *Trends in Plant Science* **22**, 823–829.
- Khan GA, Vogiatzaki E, Glauser G, Poirier Y.** 2016. Phosphate deficiency induces the jasmonate pathway and enhances resistance to insect herbivory. *Plant Physiology* **171**, 632–644.
- Lei M, Liu Y, Zhang B, Zhao Y, Wang X, Zhou Y, Raghothama KG, Liu D.** 2011. Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in *Arabidopsis*. *Plant Physiology* **156**, 1116–1130.
- Li H, Ma Q, Li H, Zhang F, Rengel Z, Shen J.** 2014. Root morphological responses to localized nutrient supply differ among crop species with contrasting root traits. *Plant and Soil* **376**, 151–163.
- Lin WY, Huang TK, Leong SJ, Chiou TJ.** 2014. Long-distance call from phosphate: systemic regulation of phosphate starvation responses. *Journal of Experimental Botany* **65**, 1817–1827.
- Linkohr BI, Williamson LC, Fitter AH, Leyser HMO.** 2002. Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*. *The Plant Journal* **29**, 751–760.
- Liu H, Li X, Xiao J, Wang S.** 2012. A convenient method for simultaneous quantification of multiple phytohormones and metabolites: application in study of rice-bacterium interaction. *Plant Methods* **8**, 2.
- Liu J, Samac DA, Bucciarelli B, Allan DL, Vance CP.** 2005. Signaling of phosphorus deficiency-induced gene expression in white lupin requires sugar and phloem transport. *Plant Journal* **41**, 257–268.
- López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L.** 2002. Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. *Plant Physiology* **129**, 244–256.
- López-Bucio JS, Salmerón-Barrera GJ, Ravelo-Ortega G, Raya-González**

- J, León P, de la Cruz HR, Campos-García J, López - Bucio J, Guevara-García ÁA.** 2019. Mitogen-activated protein kinase 6 integrates phosphate and iron responses for indeterminate root growth in *Arabidopsis thaliana*. *Planta* **250**, 1177-1189.
- Lynch JP.** 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiology* **156**, 1041–1049.
- Lynch JP, Wojciechowski T.** 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. *Journal of Experimental Botany* **66**, 2199–2210.
- Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E.** 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**, 1064–1068.
- Mosblech A, Thurow C, Gatz C, Feussner I, Heilmann I.** 2011. Jasmonic acid perception by COI1 involves inositol polyphosphates in *Arabidopsis thaliana*. *Plant Journal* **65**, 949–957.
- Müller R, Morant M, Jarmer H, Nilsson L, Nielsen TH.** 2007. Genome-wide analysis of the *Arabidopsis* leaf transcriptome reveals interaction of phosphate and sugar metabolism. *Plant Physiology* **143**, 156–171.
- Müller J, Toev T, Heisters M, Teller J, Moore KL, Hause G, Dinesh DC, Bürstenbinder K, Abel S.** 2015. Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. *Developmental Cell* **33**, 216–230.
- Obersteiner M, Peñuelas J, Ciais P, Van Der Velde M, Janssens IA.** 2013. The phosphorus trilemma. *Nature Geoscience* **6**, 897–898.
- Oldroyd GED, Leyser O.** 2020. A plant's diet, surviving in a variable nutrient environment. *Science* **368**, eaba0196.
- Pant BD, Pant P, Erban A, Huhman D, Kopka J, Scheible WR.** 2015. Identification of primary and secondary metabolites with phosphorus status-dependent abundance in *Arabidopsis*, and of the transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation. *Plant Cell and Environment* **38**, 172–187.
- Péret B, Desnos T, Jost R, Kanno S, Berkowitz O, Nussaume L.** 2014. Root architecture responses: in search of phosphate. *Plant Physiology* **166**, 1713–1723.

Pérez-Torres CA, López-Bucio J, Cruz-Ramírez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L. 2008. Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 Auxin Receptor. *Plant Cell* **20**, 3258-3272.

Péret B, De Rybel B, Casimiro I, Benková E, Swarup R, Laplace L, Beeckman T, Bennett MJ. 2009. *Arabidopsis* lateral root development: an emerging story. *Trends in Plant Science* **14**, 399-408.

Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J. 2017. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Current Opinion in Plant Biology* **39**, 40–49.

Ravelo-Ortega G, Pelagio-Flores R, López-Bucio J, Campos-García J, de la Cruz RH, López-Bucio JS. 2022. Early sensing of phosphate deprivation triggers the formation of extra root cap cell layers via SOMBRERO through a process antagonized by auxin signaling. *Plant Molecular Biology* **108**,77-91.

Raya-González J, Ojeda-Rivera JO, Mora-Macias J, Oropeza-Aburto A, Ruiz-Herrera LF, López-Bucio J, Herrera-Estrella L. 2021. MEDIATOR16 orchestrates local and systemic responses to phosphate scarcity in *Arabidopsis* roots. *New Phytologist* **229**, 1278-1288.

Richardson AE, Simpson RJ. 2011. Soil microorganisms mediating phosphorus availability. *Plant physiology* **156**, 989–996.

Rubio V, Bustos R, Irigoyen ML, Cardona-López X, Rojas-Triana M, Paz-Ares J. 2009. Plant hormones and nutrient signaling. *Plant Molecular Biology* **69**, 361–373.

Ruiz-Herrera LF, Shane MW , López-Bucio J. 2015. Nutritional regulation of root development. *Wiley Interdisciplinary Reviews Developmental Biology* **4**, 431–443.

Sánchez-Calderón L, López-Bucio J, Chacón-López A, Cruz-Ramírez A, Nieto-Jacobo F, Dubrovsky JG, Herrera-Estrella L. 2005. Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. *Plant and Cell Physiology* **46**, 174–184.

Santos TMM dos, Ślaski JJ, Carvalho MÂAP de, Lor G ory JT, Maria R. Clemente Vieira. 2005. Evaluating the Madeiran wheat germplasm for aluminum resistance using aluminium-induced callose formation in root apices as a marker. *Acta Physiologiae Plantarum* **27**, 297–302.

- Schaller F.** 2001. Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. *Journal of Experimental Botany* **52**, 11–23.
- Shan X, Zhang Y, Peng W, Wang Z, Xie D.** 2009. Molecular mechanism for jasmonate-induction of anthocyanin accumulation in *Arabidopsis*. *Journal of Experimental Botany* **60**, 3849–3860.
- Shen J, Li H, Neumann G, Zhang F.** 2005. Nutrient uptake, cluster root formation and exudation of protons and citrate in *Lupinus albus* as affected by localized supply of phosphorus in a split-root system. *Plant Science* **168**, 837–845.
- Shi L, Shi TX, Broadley MR, White PJ, Long Y, Meng JL, Xu FS, Hammond JP.** 2013. High-throughput root phenotyping screens identify genetic loci associated with root architectural traits in *Brassica napus* under contrasting phosphate availabilities. *Annals of Botany* **112**, 381–389.
- Shin R, Berg RH, Schachtman DP.** 2005. Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. *Plant and Cell Physiology* **46**, 1350–1357.
- Sobkowiak L, Bielewicz D, Malecka EM, Jakobsen I, Albrechtsen M, Szweykowska-Kulinska Z, Pacak A.** 2012. The role of the P1BS element containing promoter-driven genes in Pi transport and homeostasis in plants. *Frontiers in Plant Science* **3**, 58.
- Song JM, Guan Z, Hu J, et al.** 2020. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nature Plants* **6**, 34–45.
- Sun F, Fan G, Hu Q, et al.** 2017. The high-quality genome of *Brassica napus* cultivar ‘ZS11’ reveals the introgression history in semi-winter morphotype. *Plant Journal* **92**, 452–468.
- Sun B, Gao Y, Lynch JP.** 2018. Large crown root number improves topsoil foraging and phosphorus acquisition. *Plant Physiology* **177**, 90–104.
- Sun L, Sun Y, Zhang M, et al.** 2012. Suppression of 9-*cis*-epoxycarotenoid dioxygenase, which encodes a key enzyme in abscisic acid biosynthesis, alters fruit texture in transgenic tomato. *Plant Physiology* **158**, 283–298.
- Svistoonoff S, Creff A, Reymond M, Sigoillot-Claude C, Ricaud L, Blanchet A, Nussaume L, Desnos T.** 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genetics* **39**,

792–796.

Takahashi F, Suzuki T, Osakabe Y, Betsuyaku S, Kondo Y, Dohmae N, Fukuda H, Yamaguchi-Shinozaki K, Shinozaki K. 2018. A small peptide modulates stomatal control via abscisic acid in long-distance signaling. *Nature* **556**, 235–238.

Thibaud MC, Arrighi JF, Bayle V, Chiarenza S, Creff A, Bustos R, Paz-Ares J, Poirier Y, Nussaume L. 2010. Dissection of local and systemic transcriptional responses to phosphate starvation in *Arabidopsis*. *Plant Journal* **64**, 775–789.

Ticconi CA, Lucero RD, Sakhonwasee S, Adamson AW, Creff A, Nussaume L, Desnos T, Abel S. 2009. ER-resident proteins PDR2 and LPR1 mediate the developmental response of root meristems to phosphate availability. *Proceedings of the National Academy of Sciences* **106**, 14174–14179.

Trachsel S, Kaeppler SM, Brown KM, Lynch JP. 2011. Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil* **341**, 75–87.

Tyburski J, Dunajska K, Tretyn A. 2009. Reactive oxygen species localization in roots of *Arabidopsis thaliana* seedlings grown under phosphate deficiency. *Plant Growth Regulation* **59**, 27–36.

Vanacker H, Carver TLW, Foyer CH. 2000. Early H₂O₂ accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. *Plant Physiology* **123**, 1289–1300.

van der Bom FJT, Williams A, Bell MJ. 2020. Root architecture for improved resource capture: trade-offs in complex environments. *Journal of Experimental Botany* **71**, 5752–5763

Wang X, Feng J, White PJ, Shen J, Cheng L. 2019. Heterogeneous phosphate supply influences maize lateral root proliferation by regulating auxin redistribution. *Annals of Botany* **125**, 119–130.

Wang C, Huang W, Ying Y, Li S, Secco D, Tyerman S, Whelan J, Shou H. 2012. Functional characterization of the rice *SPX-MFS* family reveals a key role of *OsSPX-MFS1* in controlling phosphate homeostasis in leaves. *New Phytologist* **196**, 139–148.

- Wang W Bin, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS.** 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiology and Biochemistry* **47**, 570–577.
- Wang J, Qin Q, Pan J, Sun L, Sun Y, Xue Y, Song K.** 2019. Transcriptome analysis in roots and leaves of wheat seedlings in response to low-phosphorus stress. *Scientific Reports* **9**, 19802.
- Wang Q, Wang J, Yang Y, Du W, Zhang D, Yu D, Cheng H.** 2016. A genome-wide expression profile analysis reveals active genes and pathways coping with phosphate starvation in soybean. *BMC Genomics* **17**, 1–11.
- Williamson LC, Ribrioux SPCP, Fitter AH, Ottoline Leyser HM.** 2001. Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiology* **126**, 875–882.
- Woo J, MacPherson CR, Liu J, Wang H, Kiba T, Hannah MA, Wang XJ, Bajic VB, Chua NH.** 2012. The response and recovery of the *Arabidopsis thaliana* transcriptome to phosphate starvation. *BMC Plant Biology* **12**, 1–22.
- Xue Y, Zhuang Q, Zhu S, Xiao B, Liang C, Liao H, Tian J.** 2018. Genome wide transcriptome analysis reveals complex regulatory mechanisms underlying phosphate homeostasis in soybean nodules. *International Journal of Molecular Sciences* **19**, 2924.
- Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H.** 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. *Plant Physiology* **128**, 63–72.
- Yang DL, Yao J, Mei CS, et al.** 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America* **109**, E1192–E1200.
- Zhang T, Hong Y, Wen XP.** 2020. Over-expression of masson pine *PmPT1* gene in transgenic tobacco confers tolerance enhancement to Pi deficiency by ameliorating P level and the antioxidants. *Plant Molecular Biology Reporter* **38**, 238–249.
- Zhang M, Leng P, Zhang G, Li X.** 2009. Cloning and functional analysis of 9-*cis*-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. *Journal of Plant*

Physiology **166**, 1241–1252.

Zhang Y, Zhou Z, Yang Q. 2013. Genetic variations in root morphology and phosphorus efficiency of *Pinus massoniana* under heterogeneous and homogeneous low phosphorus conditions. Plant and Soil **364**, 93–104.

Zhao L, Zhou T, Li X, Fan S, You L. 2013. Expression and characterization of GH3 β -Glucosidase from *Aspergillus niger* NL-1 with high specific activity, glucose inhibition and solvent tolerance. Microbiology **82**, 356–363.

Zhou L, Hou H, Yang T, Lian Y, Sun Y, Bian Z, Wang C. 2018. Exogenous hydrogen peroxide inhibits primary root gravitropism by regulating auxin distribution during *Arabidopsis* seed germination. Plant Physiology and Biochemistry **128**, 126–133.

Zhu JK. 2016. Abiotic stress signaling and responses in plants. Cell **167**, 313–324.

844 **Figure 1.** Growth, biomass, Pi concentration in shoot and root, and lateral root
 845 morphology of *B. napus* seedlings in the split-root experiments. (a) A
 846 schematic diagram of the experimental procedure of the split-root experiment.
 847 R++: roots exposed to homogenous P treatment (P+/P+); R--: homogenous
 848 treatment without P given to roots (P-/P-); R+: heterogenous P treatment, with
 849 a part of the roots system receiving adequate P supply (on the P+ side); R-:
 850 heterogenous P treatment, with a part of the roots system receiving no P. S++:
 851 Shoots grown on P+/P+ dishes; S+-: Shoots grown on P+/P- dishes; S--:
 852 Shoots grown on P-/P- dishes. (b) Shoot and root growth of seedlings 9 DAT
 853 (days after transplantation) to the split-root system. The white horizontal lines
 854 show the root tips when the seedlings were transplanted to the split-root
 855 system. The scale bar = 2 cm. (c) Fresh weights of shoots and roots. (d) Pi
 856 concentrations of shoots and roots. (e) 1°LR (first-order lateral root) elongation
 857 rates, (f) 1°LR lengths, (g) 2°LR (second-order lateral root) numbers, (h) 2°LR
 858 density (number of 2°LR per 1°LR cm), (i) 2°LR average lengths, and (j) total
 859 2°LR lengths 9 DAT. Values are the means \pm SE ($n = 20$ biological replicates,
 860 except for Pi concentration where $n = 5$ biological replicates, each replicate
 861 being a composite sample of 10 plants). In (e) asterisks indicate a significant
 862 difference in 1°LR elongation rate between R++ and R--, and between R-- and
 863 R- ($*P < 0.05$, $**P < 0.01$; Student's *t*-test). A one-way ANOVA was carried out
 864 for the other data, and post hoc comparisons were conducted using the SPSS
 865 Tukey HSD test at $P < 0.05$ level. Significant differences are indicated by
 866 different letters above the bars.

867 **Figure 2.** Transcriptional analysis of roots of *B. napus* grown in the split-root
868 system shown in Figure 1. (a) Number of up-regulated and down-regulated
869 differentially expressed genes (DEGs) in the pairwise comparisons of R++ vs.
870 R--, R+ vs. R++, R- vs. R-- and R+ vs. R-. (b-g) Venn diagrams showing the
871 number of genes that were locally-induced (b) locally-repressed (c)
872 systemically-induced (d, e) and systemically-repressed (f, g) by Pi starvation.
873 The number of locally or systemically regulated genes is highlighted in red.
874 The height of columns on the schematic histograms in b-g show relative
875 expression levels of genes involved in the local or systemic regulation in roots.
876 (h) The proportion of the genes whose promoter contains PHR1 binding site
877 (P1BS, GNATATNC) in different groups of genes.

878 **Figure 3.** Distribution and function of the genes regulated locally (a) or
879 systemically (b) by Pi starvation in roots of *B. napus* detailed in Tables 1 and 2.
880 The number of genes in the corresponding function is shown. Grey and black
881 arrows outside the circle and semicircle at the center of the circle indicate
882 locally- or systemically- induced and repressed genes, respectively.

883 **Figure 4.** Differentially expressed genes (DEGs) induced systemically by Pi
884 starvation in roots of *B. napus*. (a-b) Venn diagrams and heat map of
885 systemically induced DEGs. (c) The top 20 GO terms in the category of
886 biological process enriched in systemically-induced DEGs. (d) The top 10
887 KEGG pathways enriched in systemically induced DEGs. The X-axis indicates
888 the enrichment factor. The dot color and size indicate the *q*-value and gene
889 number as shown on the right, respectively.

890 **Figure 5.** ABA and JA-mediated signalling pathway components in roots of *B.*
891 *napus* implicated in the response to Pi starvation. (a) A heatmap showing
892 expression levels, based on relative FPKM values, of 18 genes in the GO
893 terms of both the ABA-activated and JA-mediated signalling pathways (the top
894 frame), 23 genes in the GO terms of the ABA-activated signalling pathway (the
895 middle frame) and 25 genes in the GO terms of the JA-mediated signalling
896 pathway (the bottom frame). The ID of *B. napus* genes are shown on the right.
897 The color gradient scale on the right represents the normalized FPKM values.
898 (b, c) ABA, (d, e) JA and (f, g) JA-Ile concentrations in shoots and roots 9 DAT
899 to the split-root system. Values are the means \pm SE (n = 6 biological replicates,
900 each replicate being a composite sample of 10 plants). A one-way ANOVA was
901 carried out for the whole data set, and post hoc comparisons were conducted
902 using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences are
903 indicated by different letters above the bars.

904 **Figure 6.** Effects of JA (1 μ M) and DIECA (10 μ M; diethyldithiocarbamic acid, a
905 JA biosynthesis inhibitor) applied to the -P compartment on the biomass and
906 lateral root morphology of *B. napus* seedlings grown in a split-root system with
907 heterogeneous Pi availability. (a) Shoot and root growth of the seedlings 9 DAT
908 to the treatments. The white horizontal lines show the root tips position when
909 the seedlings were transplanted to the split-root system. Scale bar = 2 cm. (b-c)
910 Fresh weights of shoots and roots. (d) 1°LR lengths, (e) 2°LR numbers, (f)
911 2°LR density, (g) 2°LR average lengths, and (h) total 2°LR lengths 9 DAT to the
912 treatments. Values are the means \pm SE (n = 20 biological replicates). A
913 one-way ANOVA was carried out for the whole data set, and post hoc
914 comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$
915 level. Significant differences are indicated by different letters above the bars.

916 **Figure 7.** Effects of ABA (5 μ M) and FLD (3 μ M; fluridone, an ABA biosynthesis
917 inhibitor) applied to the -P compartment on the biomass and lateral root
918 morphology of *B. napus* seedlings grown in a split-root system with
919 heterogeneous Pi availability. (a) Shoot and root growth of the seedlings 9 DAT
920 to the treatments. The white horizontal lines show the root tips position when
921 the seedlings were transplanted to the split-root system. Scale bar = 2 cm. (b-c)
922 Fresh weights of shoots and roots. (d) 1°LR lengths, (e) 2°LR numbers, (f)
923 2°LR density, (g) 2°LR average lengths, and (h) total 2°LR lengths 9 DAT to the
924 treatments. Values are the means \pm SE (n = 20 biological replicates).
925 A one-way ANOVA was carried out for the whole data set, and post hoc
926 comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level.
927 Significant differences are indicated by different letters above the bars.

928 **Figure 8.** The response of sugar metabolism in roots of *B. napus* to Pi
 929 starvation. (a) A heatmap showing the expression of differentially expressed
 930 genes (DEGs) induced systemically by Pi starvation in the KEGG pathway of
 931 starch and sucrose metabolism. The gene ID and gene function in *B. napus*
 932 are shown on the left and right, respectively. The gradient color barcode in the
 933 top right corner represents the normalized FPKM values. Concentrations of
 934 total soluble sugars (e.g., glucose, fructose, sucrose; b-c) and, specifically,
 935 sucrose (as an important systemic signal of plant P status; d-e) in shoots and
 936 roots 9 DAT to the split-root system. Values are the means \pm SE ($n = 5$
 937 biological replicates, each replicate being a composite sample of 10 plants).
 938 A one-way ANOVA was carried out for the whole data set, and post hoc
 939 comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level.
 940 Significant differences are indicated by different letters above the bars.

941 **Figure 9.** Effects of sucrose (1%) applied to the -P compartment on the
942 biomass and lateral root morphology of *B. napus* seedlings grown in a
943 split-root system with heterogeneous P availability. (a) Shoot and root growth
944 of the seedlings 9 DAT to the split-root system. The white horizontal lines show
945 the root tips position when the seedlings were transplanted to the split-root
946 system. Scale bar = 2 cm. (b-c) Fresh weights of shoots and roots. (d) 1°LR
947 lengths, (e) 2°LR numbers, (f) 2°LR density, (g) 2°LR average lengths, and (h)
948 total 2°LR lengths 9 DAT to the split-root system. Values are the means \pm SE (n
949 = 20 biological replicates). A one-way ANOVA was carried out for the whole
950 data set, and post hoc comparisons were conducted using the SPSS Tukey
951 HSD test at $P < 0.05$ level. Significant differences are indicated by different
952 letters above the bars.

953 **Figure 10.** Modulation of the antioxidant system in roots of *B. napus* in
 954 response to Pi starvation. (a) A heatmap showing the expression of 28
 955 differentially expressed genes (DEG), based on relative FPKM values, in the
 956 GO term of response to oxidative stress. The gene ID and gene function in *B.*
 957 *napus* are shown on the left and right, respectively. The gradient colour
 958 barcode in the top right corner represents normalized FPKM values. (b) In situ
 959 accumulation of H₂O₂ (the upper row), O₂⁻ (the middle row) and callose (the
 960 bottom row) in the root tips as revealed by histochemical staining with DAB,
 961 DHE and aniline blue, respectively. Scale bar = 200 µm. (c) Quantification of
 962 DAB reactive staining intensity, relative fluorescent intensity of DHE and
 963 aniline blue in the root, respectively (n ≥ 20). (d, e) Activities of POD (d) and
 964 SOD (e) enzymes and (f) H₂O₂, (g) O₂⁻, (h) callose and (i) MDA content in the
 965 root measured 9 DAT to the split-root system. Values are the means ± SE (n =
 966 5 biological replicates, each replicate being a composite sample of 10 plants).
 967 A one-way ANOVA was carried out for the whole data set, and post hoc
 968 comparisons were conducted using the SPSS Tukey HSD test at *P* < 0.05
 969 level. Significant differences are indicated by different letters above each
 970 column.

971 **Figure 11.** Schematic model for local and systemic signalling involved in the
972 response of RSA of *B. napus* to homogeneous and heterogeneous Pi
973 availability in a split-root system. We speculate that systemic signals for P
974 supply and P demand regulate RSA when plants are exposed to
975 heterogeneous Pi availability: systemic P-demand (–P) signalling promotes
976 2°LR growth of R+ (in blue), systemic P-supply (+P) signalling inhibits 2°LR
977 growth of R- (in orange). Hormones (ABA and JA) and sugars are involved in
978 the systemic response of RSA to Pi starvation. These systemic signals likely
979 act in combination with local signals to regulate root development. “=” and “≠”
980 indicate that the expression level of genes are either similar or significant
981 different in roots of the two groups of plants.

982 **Table 1.** Function and differential expression of genes regulated locally by Pi-starvation in *Brassica napus* roots

Classification	FC		
	FC > 8	4 < FC < 8	2 < FC < 4
Locally-induced gene			
Hormone-related			
Gibberellin-regulated family protein			<i>BnA09g0343580.1</i>
Ethylene-responsive element binding factor 15 (<i>ERF15</i>)			<i>BnC03g0556860.1</i>
Ethylene response factor 1 (<i>ERF1</i>)			<i>BnA02g0074220.1</i> <i>BnC03g0556860.1</i>
Ethylene response DNA binding factor 3 (<i>EDF3</i>)			<i>BnA02g0077520.1</i>
Jasmonate-zim-domain protein 10 (<i>JAZ10</i>)		<i>BnA10g0414670.1</i>	
Ethylene-forming enzyme (<i>EFE</i>)			<i>BnC05g0688270.1</i>
Zinc-finger protein 2 (<i>ZF2</i>)			<i>BnA03g0132100.1</i>
Indole-3-acetic acid 7 (<i>IAA7</i>)			<i>BnC02g0502030.1</i>
Indole-3-acetic acid inducible 29 (<i>IAA29</i>)			<i>BnA03g0154430.1</i>
Auxin efflux carrier family protein (<i>PIN</i>)	<i>BnA07g0287850.1</i>		
Metal-related			
Matrixin family protein		<i>BnUnng1003600.1</i>	<i>BnA06g0236950.1</i>
Heavy metal transport/detoxification superfamily protein		<i>BnC07g0792430.1</i>	<i>BnC01g0425370.1</i>
Ferritin 3 (<i>FER3</i>)			<i>BnUnng0950990.1</i> <i>BnA09g0373290.1</i>
			<i>BnC08g0865140.1</i>
YELLOW STRIPE like 2 (<i>YSL2</i>)			<i>BnC07g0813840.1</i>
Zinc transporter precursor (<i>ZIP</i>)		<i>BnUnng0946810.1</i>	<i>BnA01g0028880.1</i>
Copper chaperone (<i>CCH</i>)			<i>BnA09g0373430.1</i>
Farnesylated protein 3 (<i>FP3</i>)			<i>BnC09g0929170.1</i>

Classification	FC			
	FC > 8	4 < FC < 8	2< FC < 4	
Stress-related				
Cytochrome P450	BnC03g0530870.1	BnA08g0313800.1	BnC08g0863810.1	BnA08g0317030.1
	BnC04g0672330.1	BnA09g0386110.1	BnC03g0609930.1	BnC01g0450440.1
Disease resistance protein family		BnC03g0560400.1	BnC01g0452050.1	BnC02g0477310.1
Response to stress protein			BnC04g0626250.1	BnA03g0140120.1
			BnA08g0314910.1	
Transcription factors				
WRKY family transcription factor	BnC05g0711350.1	BnC02g0475750.1	BnC01g0441220.1	BnA08g0317280.1
	BnC03g0540540.1	BnA10g0414810.1	BnA04g0181090.1	BnA03g0147830.1
			BnC03g0561760.1	BnA06g0250710.1
			BnC07g0789510.1	BnC03g0542280.1
			BnUnng0945570.1	BnA02g0052450.1
			BnA03g0095590.1	BnC09g0928650.1
NAC domain containing protein	BnA05g0214880.1	BnC09g0889430.1	BnUnng1000260.1	BnC04g0658660.1
	BnC03g0581630.1	BnC05g0723420.1	BnA01g0035980.1	BnA02g0045140.1
	BnA02g0056610.1		BnA02g0088360.1	BnA10g0414720.1
			BnC03g0538780.1	
NAC-like		BnC06g0763770.1	BnA07g0294910.1	
Myb domain protein	BnC01g0427060.1	BnA05g0186360.1	BnC03g0569850.1	BnC08g0880160.1
		BnA02g0083870.1	BnA09g0382840.1	BnC04g0634540.1
		BnC09g0889670.1	BnC08g0854560.1	BnA08g0322200.1
			BnA02g0058610.1	BnC07g0814160.1
		BnA07g0272050.1	BnC07g0786310.1	

Classification	FC			
	FC > 8	4 < FC < 8	2 < FC < 4	
Myb-like transcription factor family protein			<i>BnA03g0143670.1</i>	<i>BnC02g0491140.1</i>
Homeodomain-like superfamily protein	<i>BnC08g0880540.1</i>	<i>BnA07g0284190.1</i>	<i>BnC03g0534210.1</i>	<i>BnA02g0046790.1</i>
			<i>BnC03g0536670.1</i>	
Homeobox-leucine zipper protein 3 (<i>HAT3</i>)			<i>BnC08g0868870.1</i>	
C2H2-type zinc finger family protein	<i>BnA02g0058540.1</i>	<i>BnA02g0058550.1</i>	<i>BnC03g0547480.1</i>	<i>BnC04g0676810.1</i>
Basic helix-loop-helix (bHLH) DNA-binding superfamily protein			<i>BnA09g0337080.1</i>	<i>BnA04g0162960.1</i>
			<i>BnC01g0464080.1</i>	<i>BnA05g0188540.1</i>
Integrase-type DNA-binding superfamily protein	<i>BnC07g0815890.1</i>	<i>BnC06g0757280.1</i>	<i>BnUnng0963640.1</i>	<i>BnC05g0714210.1</i>
PLATZ transcription factor family protein			<i>BnA07g0300540.1</i>	<i>BnC09g0892170.1</i>
Dof-type zinc finger DNA-binding family protein	<i>BnA02g0089590.1</i>		<i>BnA02g0067780.1</i>	
WUSCHEL related homeobox		<i>BnA02g0087930.1</i>		
		<i>BnUnng0960070.1</i>		
Transporter or traffic facilitator				
Nodulin MtN21/EamA-like transporter family protein		<i>BnC04g0652060.1</i>	<i>BnC08g0865700.1</i>	<i>BnC06g0747440.1</i>
			<i>BnC04g0678440.1</i>	
Major facilitator superfamily protein (<i>MFS</i>)	<i>BnA03g0112530.1</i>		<i>BnUnng0946560.1</i>	<i>BnA02g0050060.1</i>
			<i>BnA06g0228180.1</i>	<i>BnC02g0472890.1</i>
			<i>BnA08g0333700.1</i>	<i>BnA06g0250430.1</i>
			<i>BnA05g0211860.1</i>	<i>BnC02g0475290.1</i>
			<i>BnUnng0977560.1</i>	
Phosphate transporter 3;1 (<i>PHT3;1</i>)			<i>BnC09g0927650.1</i>	
Phosphate transporter traffic facilitator1 (<i>PHF1</i>)			<i>BnC08g0861530.1</i>	<i>BnC04g0654240.1</i>
Amino acid permease 4 (<i>AAP4</i>)		<i>BnA06g0249500.1</i>	<i>BnC09g0929350.1</i>	<i>BnA08g0308700.1</i>
MATE efflux family protein			<i>BnA03g0094220.1</i>	<i>BnA09g0384810.1</i>

Classification	FC		
	FC > 8	4 < FC < 8	2 < FC < 4
Sugar transporter (<i>STP</i>)		<i>BnC08g0883620.1</i> <i>BnA09g0358900.1</i>	<i>BnA09g0387420.1</i>
Cation/H ⁺ exchanger (<i>CHX</i>)		<i>BnC08g0862990.1</i>	<i>BnA08g0321080.1</i>
ARM repeat superfamily protein	<i>BnC07g0837090.1</i>		
Detoxifying efflux carrier 35 (<i>DTX35</i>)			<i>BnUnng0944580.1</i>
Dicarboxylate carrier 3 (<i>DIC3</i>)	<i>BnC03g0538850.1</i>		
Nucleotide-sugar transporter family protein	<i>BnC06g0755290.1</i>		
Non-intrinsic ABC protein 12 (<i>NAP12</i>)			<i>BnC03g0546880.1</i>
NOD26-like intrinsic protein 1;2 (<i>NIP1;2</i>)			<i>BnA01g0011110.1</i>
Oligopeptide transporter 9 (<i>OPT9</i>)	<i>BnC09g0913620.1</i>		
SNARE-like superfamily protein	<i>BnA07g0303360.1</i>		
Locally-repressed gene			
Growth, development			
Expansin	<i>BnA05g0190670.1</i>		<i>BnC06g0766230.1</i> <i>BnA02g0044620.1</i> <i>BnA07g0294990.1</i> <i>BnA02g0060570.1</i> <i>BnA04g0175660.1</i> <i>BnC02g0517740.1</i> <i>BnC04g0669040.1</i> <i>BnC07g0827020.1</i> <i>BnA03g0152010.1</i>
Cellulase 3 (<i>CEL3</i>)			<i>BnA07g0295860.1</i>
Cellulose synthase like (<i>CSLA7</i>)			<i>BnC04g0643300.1</i>
Leucine-rich receptor-like protein kinase family protein			<i>BnA09g0379090.1</i> <i>BnC08g0872370.1</i>
Cysteine-rich RLK (RECEPTOR-like protein kinase) (<i>CRK</i>)			<i>BnC03g0572510.1</i> <i>BnC04g0656580.1</i>
Leucine-rich repeat (LRR) family protein			<i>BnA05g0209450.1</i> <i>BnC09g0928830.1</i>

Classification	FC		
	FC > 8	4 < FC < 8	2 < FC < 4
Leucine-rich repeat protein kinase family protein	<i>BnA05g0204990.1</i>		<i>BnC07g0783470.1</i>
Lateral root primordium (LRP) protein-related			<i>BnC01g0452890.1</i> <i>BnA01g0027570.1</i>
			<i>BnA07g0281960.1</i>
Root hair defective 6-like 4 (<i>RSL4</i>)			<i>BnC05g0711090.1</i>
Plant regulator RWP-RK family protein			<i>BnA03g0151900.1</i>
Hormone-related			
Gibberellin-regulated family protein		<i>BnA02g0058510.1</i>	
Gibberellin-oxidase	<i>BnA02g0063550.1</i>		<i>BnC04g0621660.1</i>
BURP domain-containing protein			<i>BnC09g0891360.1</i>
SAUR-like auxin-responsive protein family			<i>BnC01g0428740.1</i>
Phosphatase 2C5 (<i>PP2C5</i>)		<i>BnC08g0856910.1</i>	
Indoleacetic acid-induced protein 16 (<i>IAA16</i>)			<i>BnUnng1013730.1</i>
Metal-related			
2-oxoglutarate (2OG) and Fe (II)-dependent Oxygenase superfamily protein	<i>BnC03g0580110.1</i>		<i>BnA02g0072340.1</i> <i>BnA06g0252990.1</i>
Root FNR 1 (<i>RFNR1</i>)			<i>BnUnng0944290.1</i>
Heavy metal transport/detoxification superfamily protein		<i>BnA03g0136570.1</i>	
Vacuolar iron transporter (<i>VIT</i>) family protein			<i>BnA02g0072100.1</i>
FER-like regulator of iron uptake			<i>BnC04g0640590.1</i> <i>BnA07g0282910.1</i>
Copper transporter 2 (<i>COPT2</i>)			<i>BnC01g0456180.1</i>
Potassium channel 3 (<i>AKT3</i>)			<i>BnA03g0154620.1</i>
Metal tolerance protein A2 (<i>MTPA2</i>)			<i>BnC02g0476650.1</i>
Sodium/calcium exchanger family protein/calcium-binding EF hand family protein			<i>BnA06g0224840.1</i>

Classification	FC		
	FC > 8	4 < FC < 8	2 < FC < 4
Pi recycle			
HAD superfamily, subfamily IIIB acid phosphatase			<i>BnA03g0111260.1</i> <i>BnC03g0561880.1</i>
Purple acid phosphatase 15 (<i>PAP15</i>)			<i>BnA01g0012910.1</i>
Transcription factors			
Myb domain protein			<i>BnC03g0580070.1</i> <i>BnA09g0340680.1</i> <i>BnC09g0928930.1</i> <i>BnC09g0893670.1</i>
Myb-like HTH transcriptional regulator family protein			<i>BnA10g0419160.1</i>
Basic helix-loop-helix (bHLH) DNA-binding family protein	<i>BnC04g0621110.1</i>	<i>BnA02g0079260.1</i> <i>BnA07g0290920.1</i> <i>BnA09g0374140.1</i>	<i>BnC03g0568170.1</i>
Homeobox protein			<i>BnA10g0413110.1</i>
Homeodomain-like superfamily protein	<i>BnC09g0913780.1</i>		
Duplicated homeodomain-like superfamily protein			<i>BnA03g0110860.1</i>
Integrase-type DNA-binding superfamily protein			<i>BnA06g0255510.1</i> <i>BnC03g0544530.1</i> <i>BnC09g0891290.1</i>
NAC domain containing protein	<i>BnC04g0619940.1</i>	<i>BnA02g0049060.1</i>	<i>BnA03g0139350.1</i>
K-box region and MADS-box transcription factor family protein	<i>BnA03g0106510.1</i>		<i>BnC09g0925990.1</i>
P-loop containing nucleoside triphosphate hydrolases superfamily protein			<i>BnC04g0657250.1</i> <i>BnA04g0160160.1</i>
AGAMOUS-like			<i>BnA02g0069020.1</i> <i>BnA03g0096130.1</i> <i>BnA01g0032040.1</i>
WRKY family transcription factor			<i>BnUnng0955050.1</i>
GATA type zinc finger transcription factor family protein			<i>BnA03g0112190.1</i>

Classification	FC			
	FC > 8	4 < FC < 8	2 < FC < 4	
TBP-associated factor 5 (<i>TAF5</i>)			<i>BnA02g0085290.1</i>	
HY5-homolog (<i>HYH</i>)			<i>BnUnng0959450.1</i>	
C2H2-type zinc finger family protein			<i>BnC08g0866970.1</i>	
Basic leucine-zipper 7 (<i>bzip7</i>)			<i>BnC01g0426160.1</i>	
RAD-like 6 (<i>RL6</i>)	<i>BnA07g0299340.1</i>			
Transporter				
Major facilitator superfamily protein	<i>BnA07g0289640.1</i>	<i>BnC03g0561070.1</i>	<i>BnA07g0282080.1</i>	<i>BnA07g0289620.1</i>
			<i>BnUnng0966600.1</i>	<i>BnC04g0620600.1</i>
			<i>BnA10g0404070.1</i>	<i>BnC09g0893830.1</i>
H ⁺ -ATPase 1 (<i>HA1</i>)		<i>BnA03g0153330.1</i>	<i>BnC07g0829770.1</i>	
Cation/H ⁺ exchanger 20 (<i>CHX20</i>)			<i>BnA09g0341200.1</i>	
Na ⁺ /H ⁺ exchanger 1 (<i>NHX1</i>)			<i>BnC01g0453250.1</i>	
ABC transporter family protein	<i>BnC09g0904070.1</i>		<i>BnA06g0260600.1</i>	
ABC2 homolog 13 (<i>ATH13</i>)			<i>BnA06g0250820.1</i>	
ABC-2 type transporter family protein			<i>BnA04g0165490.1</i>	
Non-intrinsic ABC protein 14 (<i>NAP14</i>)			<i>BnC09g0927590.1</i>	
MATE efflux family protein		<i>BnA09g0383220.1</i>	<i>BnUnng1012830.1</i>	<i>BnC07g0805390.1</i>
Plasma membrane intrinsic protein (<i>PIP</i>)	<i>BnC09g0920100.1</i>		<i>BnA01g0036970.1</i>	
Transmembrane amino acid transporter family protein			<i>BnC02g0475860.1</i>	<i>BnC08g0838640.1</i>
Amino acid permease (<i>AAP</i>)			<i>BnC04g0645270.1</i>	<i>BnUnng1012390.1</i>
Inositol transporter (<i>INT</i>)			<i>BnC04g0622990.1</i>	<i>BnA01g0022340.1</i>
Atpase E1-E2 type family protein/haloacid dehalogenase-like hydrolase family protein		<i>BnC06g0744860.1</i>		
Tonoplast intrinsic protein 2;2 (<i>TIP2;2</i>)			<i>BnA01g0021060.1</i>	

Classification	FC		
	FC > 8	4 < FC < 8	2 < FC < 4
Proton gradient regulation 5 (<i>PGR5</i>)			<i>BnA03g0137840.1</i>
Ammonium transporter 1;3 (<i>AMT1;3</i>)			<i>BnA07g0272070.1</i>
Nitrate transporter 1.1 (<i>NRT1.1</i>)		<i>BnA06g0232010.1</i>	
CBL-interacting protein kinase 23 (<i>CIPK23</i>)			<i>BnC05g0711790.1</i>
Sulfate transporter 1;2 (<i>SMLTR1;2</i>)			<i>BnC02g0499990.1</i>
Photosynthetic electron transfer C (<i>PETC</i>)			<i>BnC03g0572990.1</i>
CBS domain-containing protein			<i>BnA03g0099600.1</i>
Nodulin MtN21 /EamA-like transporter family protein		<i>BnA09g0350870.1</i>	
NOD26-like intrinsic protein 3;1 (<i>NIP3;1</i>)			<i>BnA08g0312780.1</i>
Dicarboxylate transporter 1 (<i>DiT1</i>)			<i>BnA10g0414990.1</i>

983 **Note:** Genes are classified according to their level of induction or repression (FC > 8, 4 < FC < 8 or 2 < FC < 4; FC, fold change). For induced genes, FC = R--/R++; for

984 repressed genes, FC = R++/R--

Figure 1

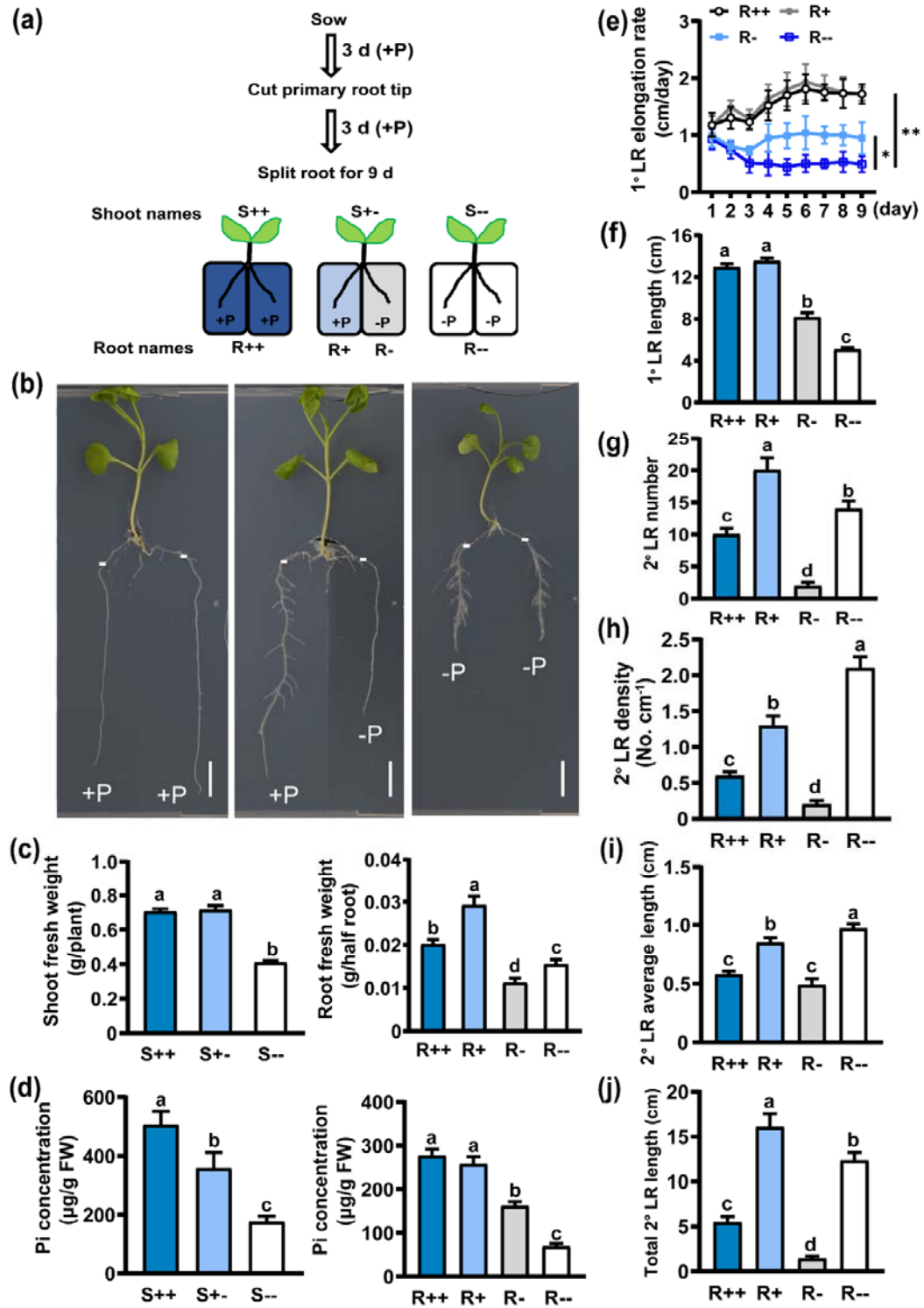


Figure 1. Growth, biomass, Pi concentration in shoot and root, and lateral root morphology of *B. napus* seedlings in the split-root experiments. (a) A

schematic diagram of the experimental procedure of the split-root experiment. R++: roots exposed to homogenous P treatment (P+/P+); R--: homogenous treatment without P given to roots (P-/P-); R+: heterogenous P treatment, with a part of the roots system receiving adequate P supply (on the P+ side); R-: heterogenous P treatment, with a part of the roots system receiving no P. S++: Shoots grown on P+/P+ dishes; S+-: Shoots grown on P+/P- dishes; S--: Shoots grown on P-/P- dishes. (b) Shoot and root growth of seedlings 9 DAT (days after transplantation) to the split-root system. The white horizontal lines show the root tips when the seedlings were transplanted to the split-root system. The scale bar = 2 cm. (c) Fresh weights of shoots and roots. (d) Pi concentrations of shoots and roots. (e) 1°LR (first-order lateral root) elongation rates, (f) 1°LR lengths, (g) 2°LR (second-order lateral root) numbers, (h) 2°LR density (number of 2°LR per 1°LR cm), (i) 2°LR average lengths, and (j) total 2°LR lengths 9 DAT. Values are the means \pm SE (n = 20 biological replicates, except for Pi concentration where n = 5 biological replicates, each replicate being a composite sample of 10 plants). In (e) asterisks indicate a significant difference in 1°LR elongation rate between R++ and R--, and between R-- and R- (* P < 0.05, ** P < 0.01; Student's t -test). A one-way ANOVA was carried out for the other data, and post hoc comparisons were conducted using the SPSS Tukey HSD test at P < 0.05 level. Significant differences are indicated by different letters above the bars.

Figure 2

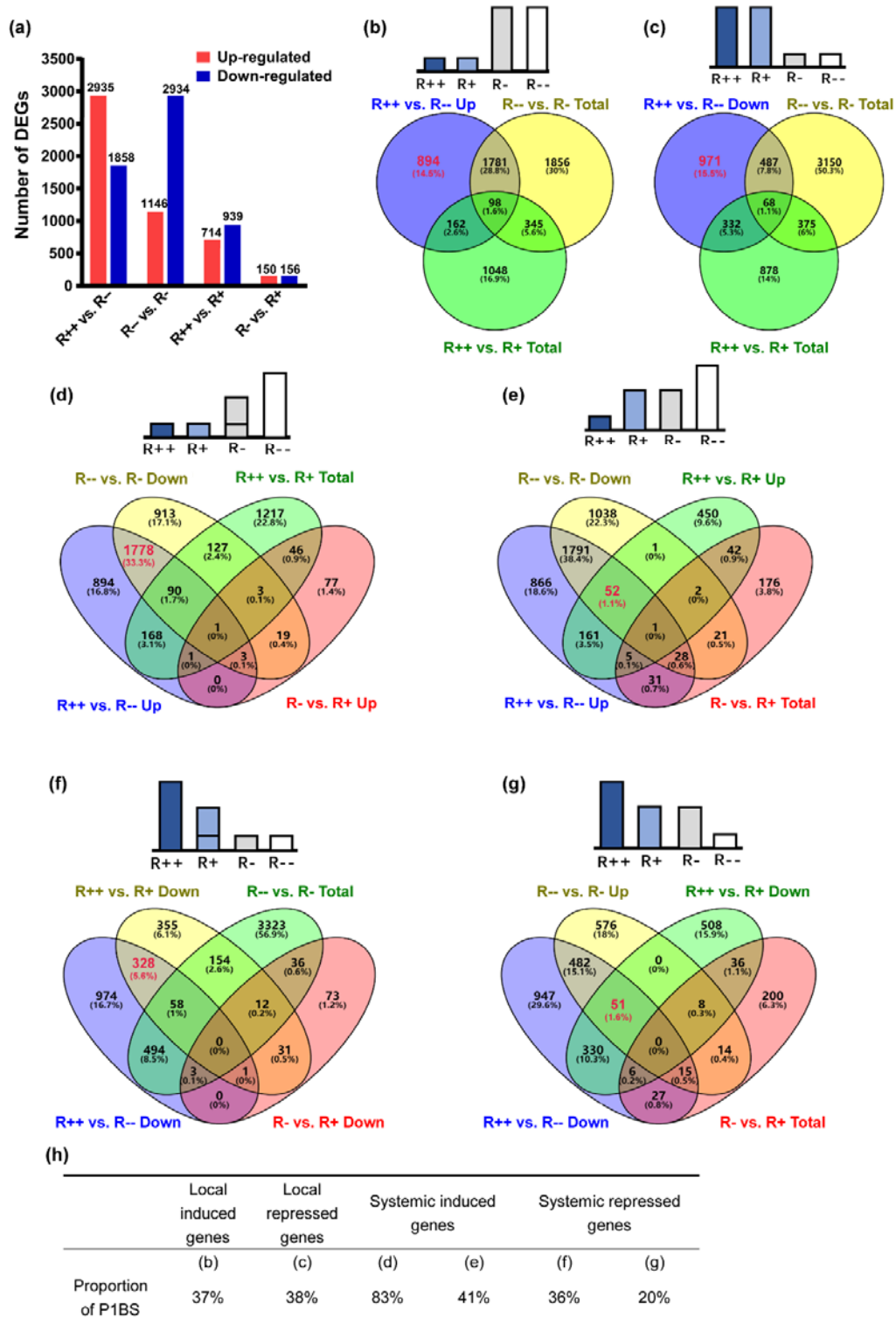


Figure 2. Transcriptional analysis of roots of *B. napus* grown in the split-root system shown in Figure 1. (a) Number of up-regulated and down-regulated differentially expressed genes (DEGs) in the pairwise comparisons of R++ vs.

R--, R+ vs. R++, R- vs. R-- and R+ vs. R-. (b-g) Venn diagrams showing the number of genes that were locally-induced (b) locally-repressed (c) systemically-induced (d, e) and systemically-repressed (f, g) by Pi starvation. The number of locally or systemically regulated genes is highlighted in red. The height of columns on the schematic histograms in b-g show relative expression levels of genes involved in the local or systemic regulation in roots. (h) The proportion of the genes whose promoter contains PHR1 binding site (P1BS, GNATATNC) in different groups of genes.

Figure 3

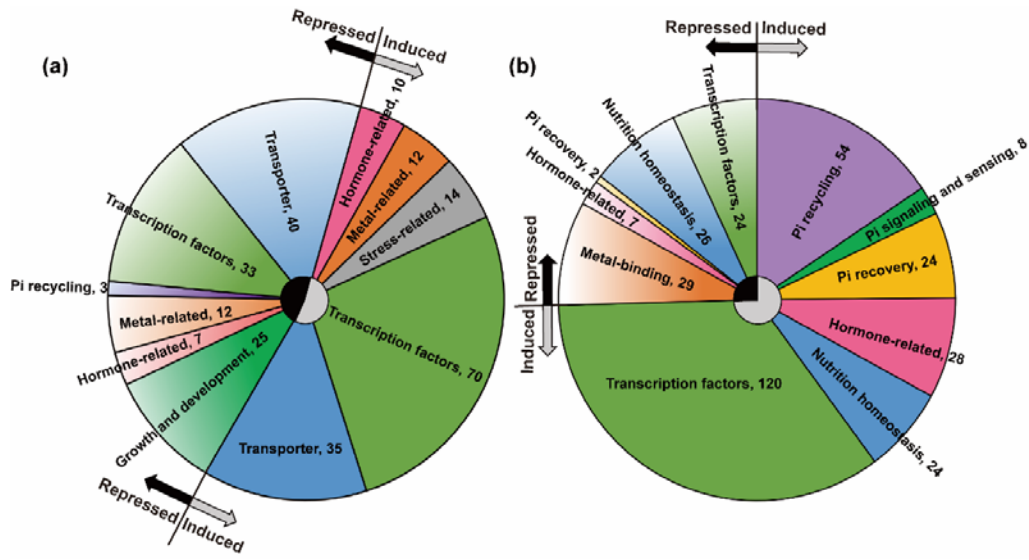


Figure 3. Distribution and function of the genes regulated locally (a) or systemically (b) by Pi starvation in roots of *B. napus* detailed in Tables 1 and 2. The number of genes in the corresponding function is shown. Grey and black arrows outside the circle and semicircle at the center of the circle indicate locally- or systemically- induced and repressed genes, respectively.

Figure 4

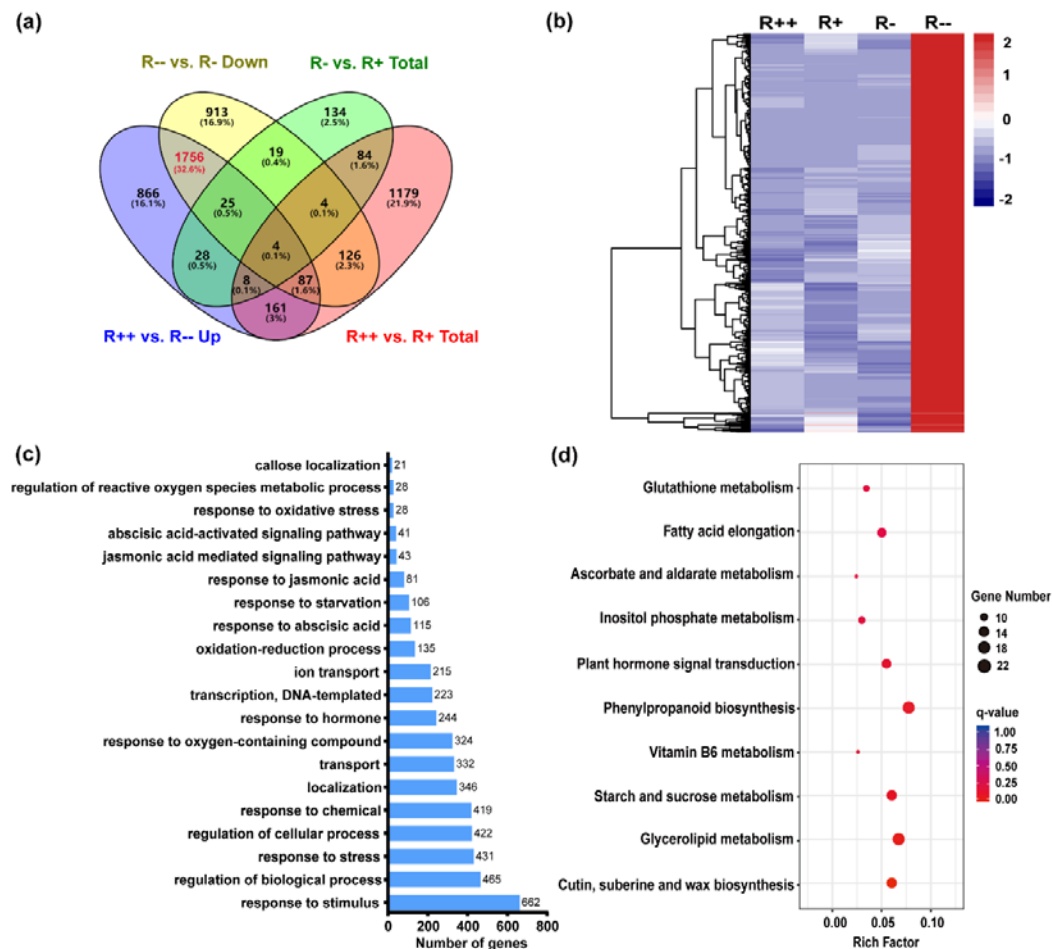


Figure 4. Differentially expressed genes (DEGs) induced systemically by Pi starvation in roots of *B. napus*. (a-b) Venn diagrams and heat map of systemically induced DEGs. (c) The top 20 GO terms in the category of biological process enriched in systemically-induced DEGs. (d) The top 10 KEGG pathways enriched in systemically induced DEGs. The X-axis indicates the enrichment factor. The dot color and size indicate the q -value and gene number as shown on the right, respectively.

Figure 5

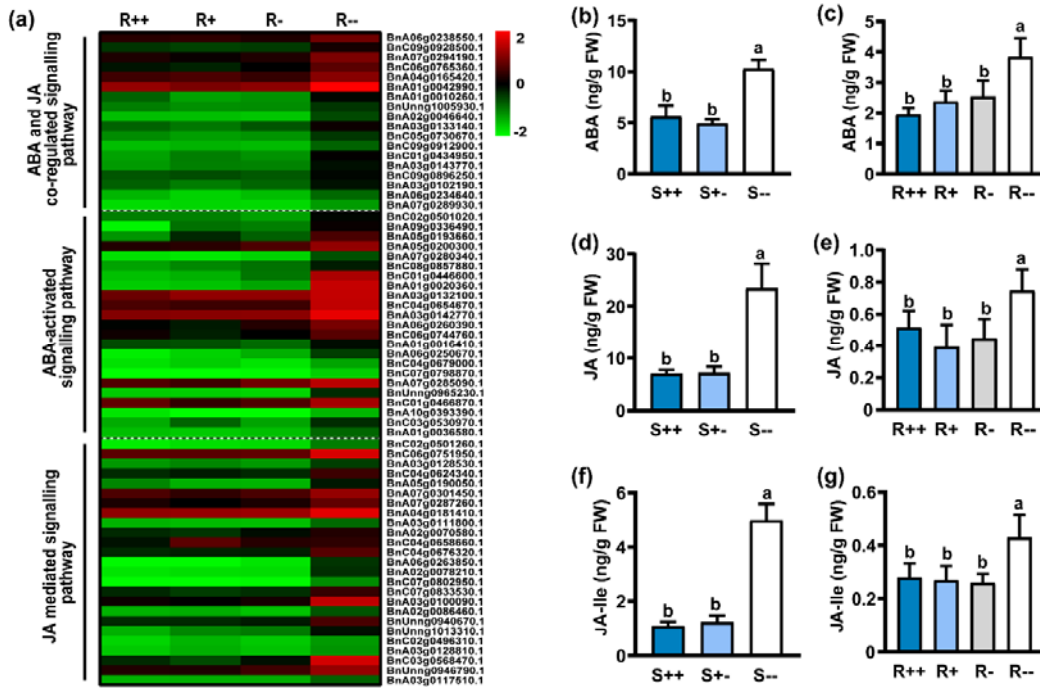


Figure 5. ABA and JA-mediated signalling pathway components in roots of *B. napus* implicated in the response to Pi starvation. (a) A heatmap showing expression levels, based on relative FPKM values, of 18 genes in the GO terms of both the ABA-activated and JA-mediated signalling pathways (the top frame), 23 genes in the GO terms of the ABA-activated signalling pathway (the middle frame) and 25 genes in the GO terms of the JA-mediated signalling pathway (the bottom frame). The ID of *B. napus* genes are shown on the right. The color gradient scale on the right represents the normalized FPKM values. (b, c) ABA, (d, e) JA and (f, g) JA-Ile concentrations in shoots and roots 9 DAT to the split-root system. Values are the means \pm SE ($n = 6$ biological replicates, each replicate being a composite sample of 10 plants). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences were indicated by different letters above the bars.

Figure 6

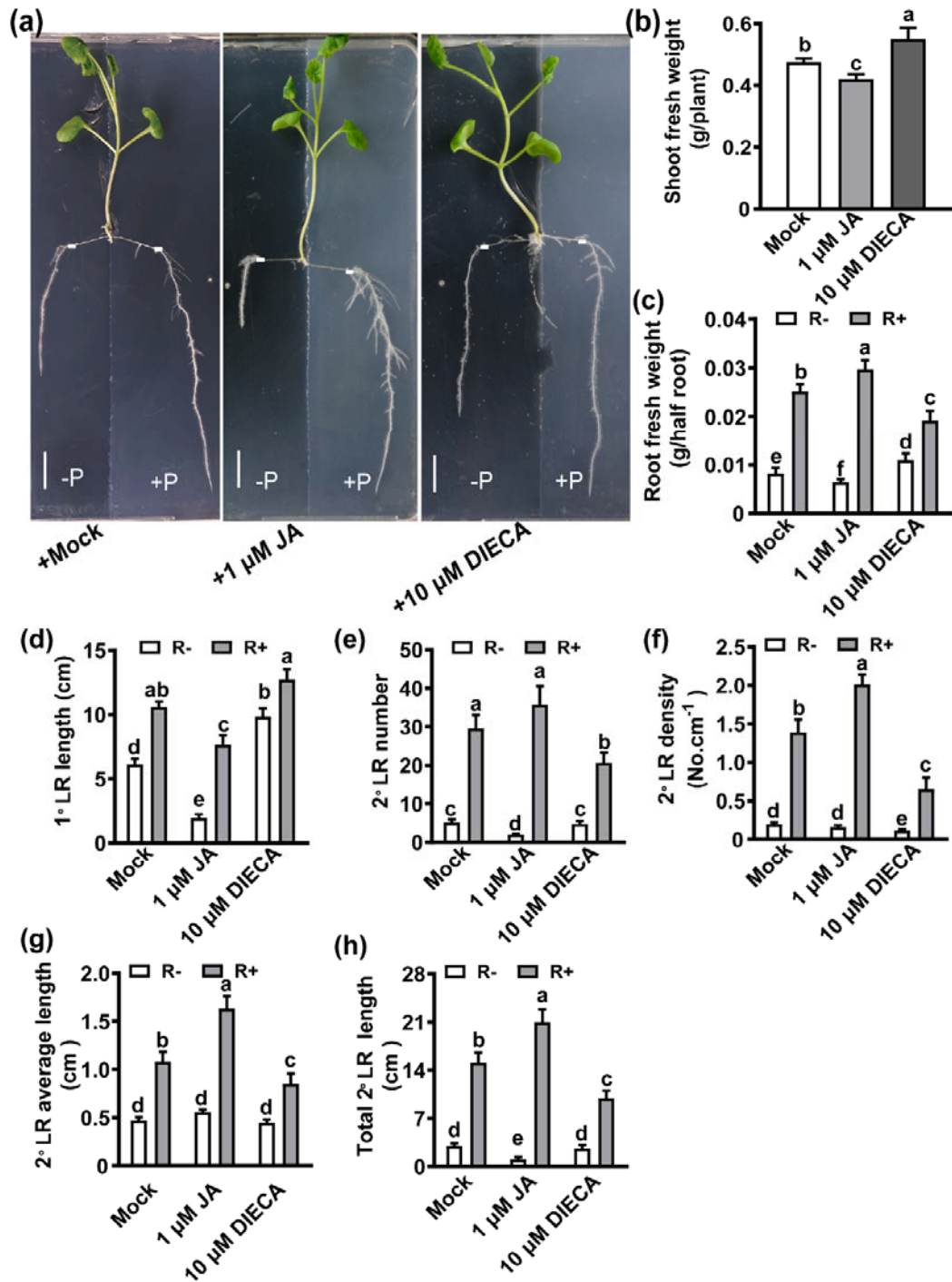


Figure 6. Effects of JA (1 μ M) and DIECA (10 μ M; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) applied to the -P compartment on the biomass and lateral root morphology of *B. napus* seedlings grown in a split-root system with heterogeneous Pi availability. (a) Shoot and root growth of the seedlings 9 DAT to the treatments. The white horizontal lines show the root tips position when the seedlings were transplanted to the split-root system. Scale bar = 2 cm. (b-c)

Fresh weights of shoots and roots. (d) 1°LR lengths, (e) 2°LR numbers, (f) 2°LR density, (g) 2°LR average lengths, and (h) total 2°LR lengths 9 DAT to the treatments. Values are the means \pm SE (n = 20 biological replicates). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences are indicated by different letters above the bars.

Figure 7

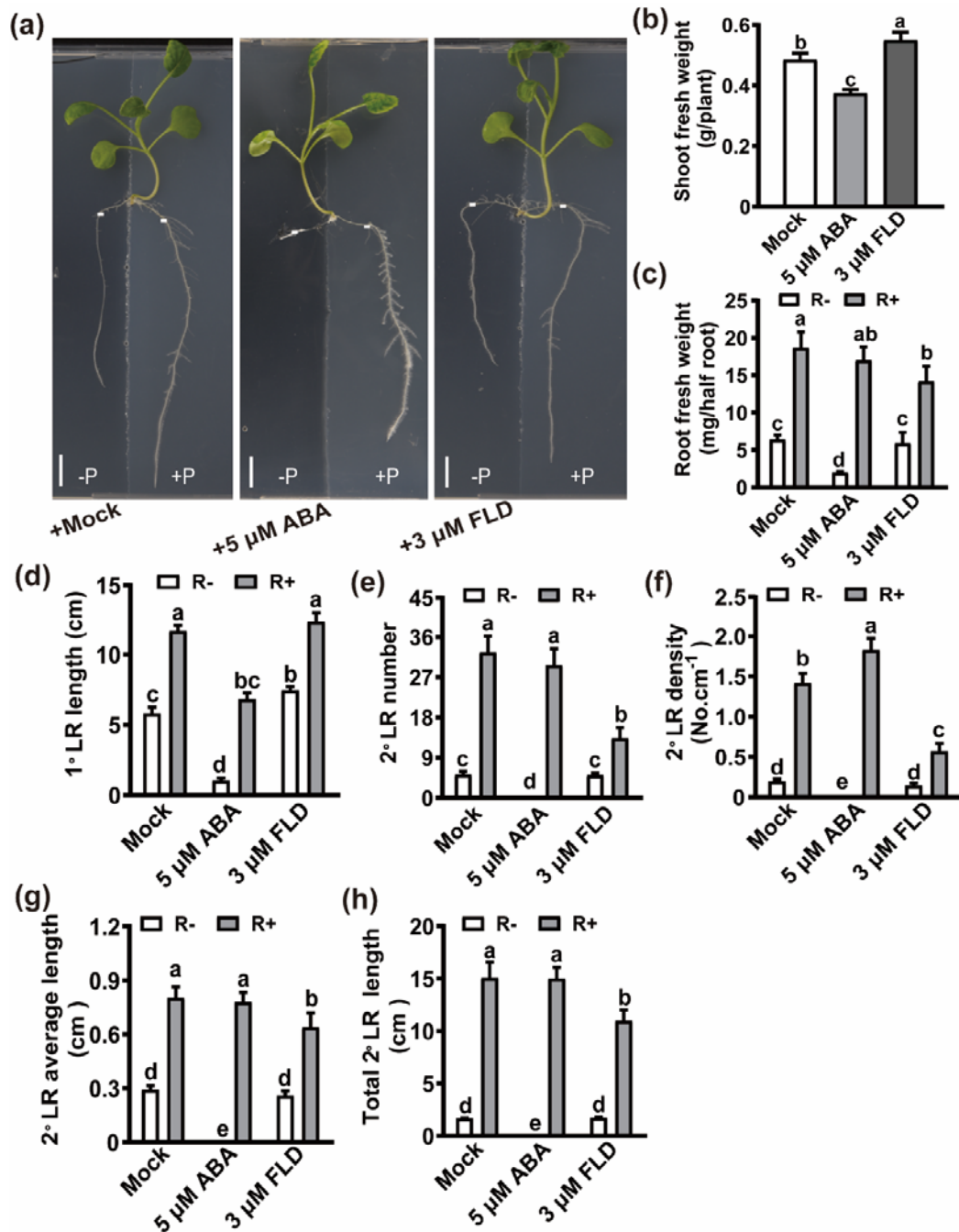


Figure 7. Effects of ABA (5 μM) and FLD (3 μM; fluridone, an ABA biosynthesis inhibitor) applied to the -P compartment on the biomass and lateral root morphology of *B. napus* seedlings grown in a split-root system with heterogeneous Pi availability. (a) Shoot and root growth of the seedlings 9 DAT to the treatments. The white horizontal lines show the root tips position when the seedlings were transplanted to the split-root system. Scale bar = 2 cm. (b-c)

Fresh weights of shoots and roots. (d) 1°LR lengths, (e) 2°LR numbers, (f) 2°LR density, (g) 2°LR average lengths, and (h) total 2°LR lengths 9 DAT to the treatments. Values are the means \pm SE (n = 20 biological replicates). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences are indicated by different letters above the bars.

Figure 8

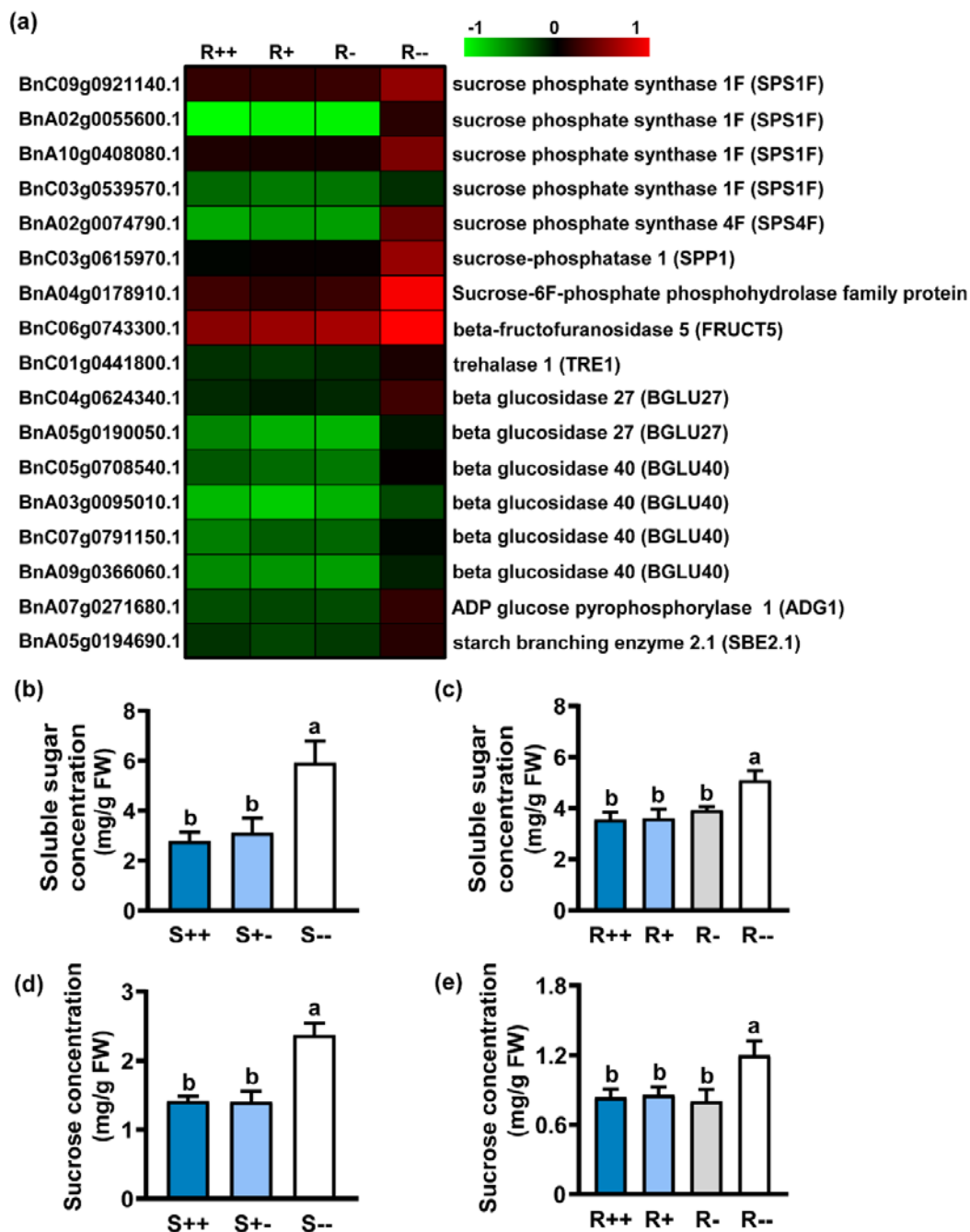


Figure 8. The response of sugar metabolism in roots of *B. napus* to Pi starvation. (a) A heatmap showing the expression of differentially expressed genes (DEGs) induced systemically by Pi starvation in the KEGG pathway of starch and sucrose metabolism. The gene ID and gene function in *B. napus* are shown on the left and right, respectively. The gradient color barcode in the top right corner represents the normalized FPKM values. Concentrations of total soluble sugars (e.g, glucose, fructose, sucrose; b-c) and, specifically,

sucrose (as an important Pi signalling; d-e) in shoots and roots 9 DAT to the split-root system. Values are the means \pm SE ($n = 5$ biological replicates, each replicate being a composite sample of 10 plants). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences were indicated by different letters above the bars.

Figure 9

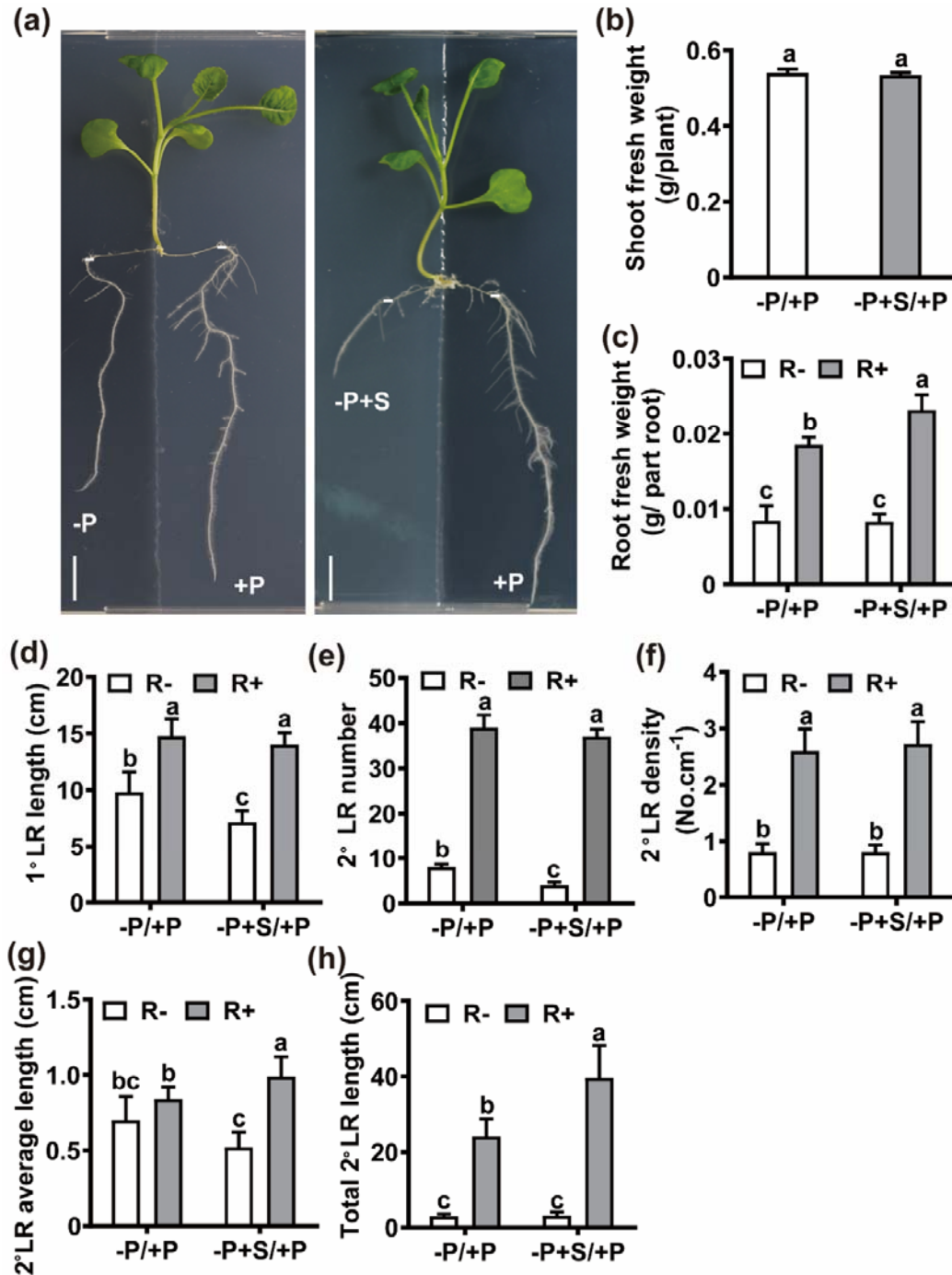


Figure 9. Effects of sucrose (1%) applied to the -P compartment on the biomass and lateral root morphology of *B. napus* seedlings grown in a split-root system with heterogeneous P availability. (a) Shoot and root growth of the seedlings 9 DAT to the split-root system. The white horizontal lines show the root tips position when the seedlings were transplanted to the split-root system. Scale bar = 2 cm. (b-c) Fresh weights of shoots and roots. (d) 1°LR lengths, (e) 2°LR numbers, (f) 2°LR density, (g) 2°LR average lengths, and (h)

total 2°LR lengths 9 DAT to the split-root system. Values are the means \pm SE (n = 20 biological replicates). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences are indicated by different letters above the bars.

Figure 10

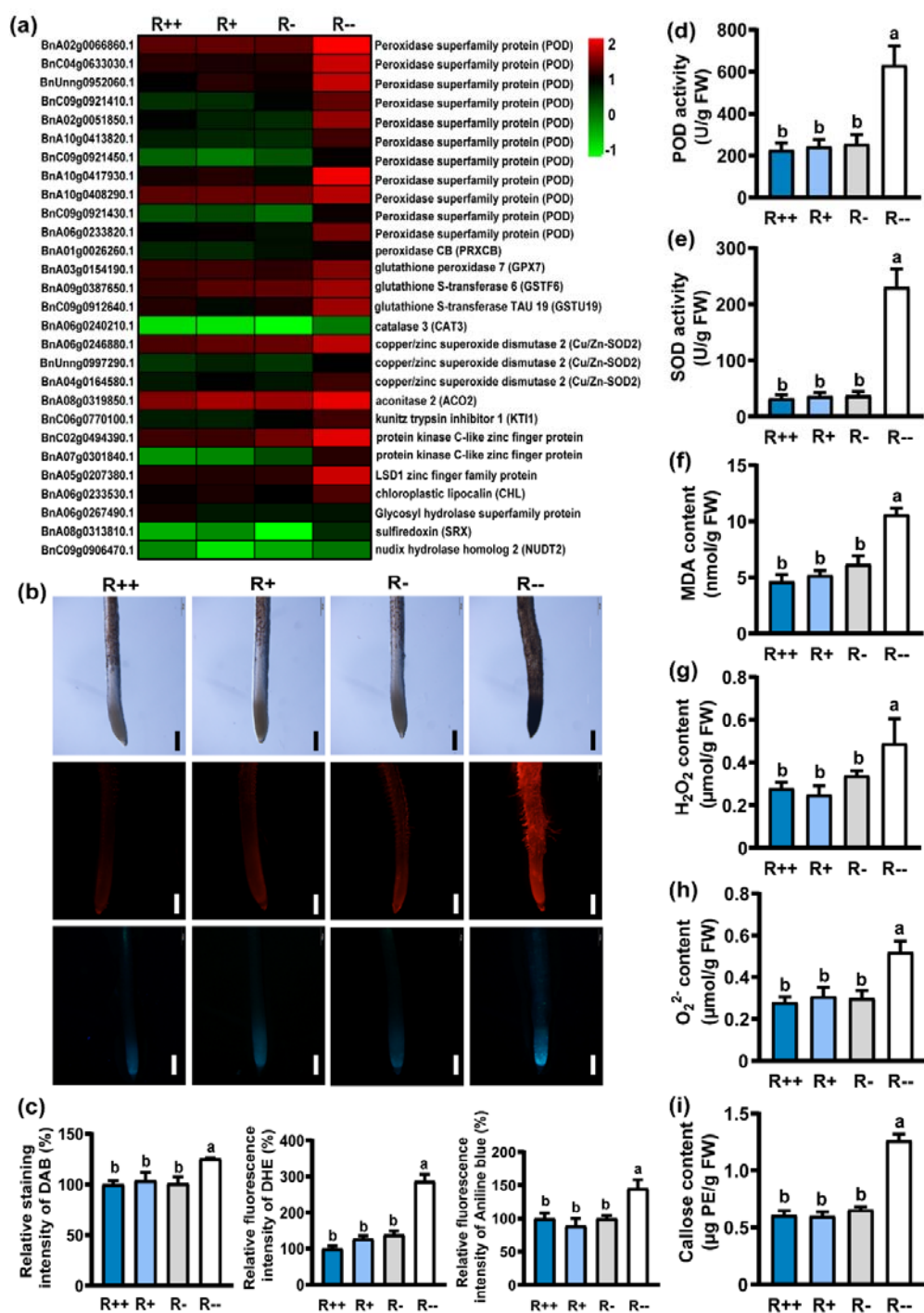


Figure 10. Modulation of the antioxidant system in roots of *B. napus* in response to Pi starvation. (a) A heatmap showing the expression of 28 differentially expressed genes (DEG), based on relative FPKM values, in the GO term of response to oxidative stress. The gene ID and gene function in *B.*

napus are shown on the left and right, respectively. The gradient colour barcode in the top right corner represents normalized FPKM values. (b) In situ accumulation of H_2O_2 (the upper row), O_2^- (the middle row) and callose (the bottom row) in the root tips as revealed by histochemical staining with DAB, DHE and aniline blue, respectively. Scale bar = 200 μm . (c) Quantification of DAB reactive staining intensity, relative fluorescent intensity of DHE and aniline blue in the root, respectively ($n \geq 20$). (d, e) Activities of POD (d) and SOD (e) enzymes and (f) H_2O_2 , (g) O_2^- , (h) callose and (i) MDA content in the root measured 9 DAT to the split-root system. Values are the means \pm SE ($n = 5$ biological replicates, each replicate being a composite sample of 10 plants). A one-way ANOVA was carried out for the whole data set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P < 0.05$ level. Significant differences were indicated by different letters above each column.

Figure 11

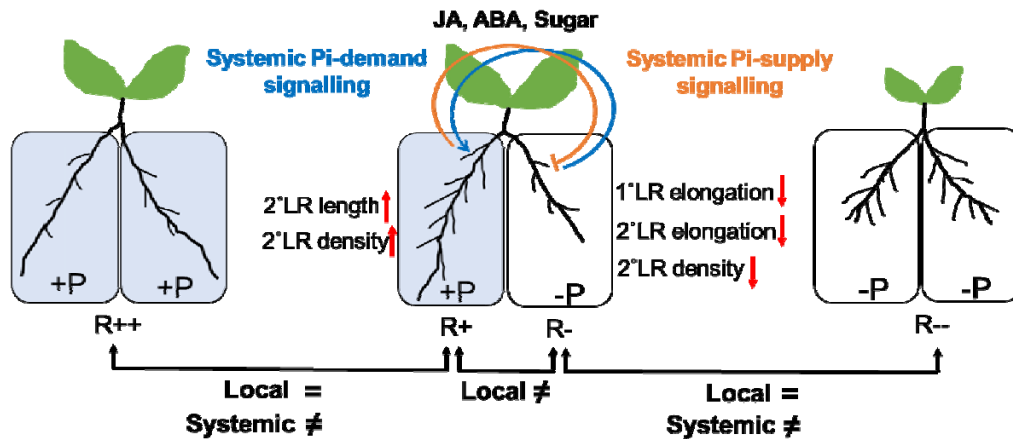


Figure 11. Schematic model for local and systemic signalling involved in RSA of *B. napus* response to homogeneous and heterogeneous Pi availability in split-root system. We speculate the existence of systemic signalling for P supply and P demand that regulate RSA exposed to heterogeneous Pi availability: systemic P-demand (-P) signalling promote 2°LR growth of R+ (in blue), systemic P-supply (+P) signalling inhibit 2°LR growth of R- (in orange) in split-root plants. Hormones (ABA and JA) and sugars are involved in the systemic response of RSA to Pi starvation. These systemic signalings act likely in combination with local signalings to regulate root development. “=” and “≠” indicate the expression level of genes between two groups roots are similar and significant different, respectively.