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Jasmonic acid participating in the systemic regulation of phosphate starvation response in *Brassica napus*

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Abstract

Aims The aims of this work were to investigate phosphate starvation responses of *Brassica napus* (*B. napus*) under heterogeneous phosphate (Pi) supply and the regulatory role of jasmonic acid (JA) in the systemic response to Pi starvation.

Methods A split-root system with two separated compartments was employed to mimic heterogeneous Pi distribution in the soil and to examine the effect of heterogeneous Pi supply, and JA or DIECA (JA biosynthesis inhibitor) on growth, root morphology, Pi concentration, Acid phosphatase (APase) activity, nutrition uptake, JA concentration and expression of Pi starvation systemically-induced (PSSI) genes of *B. napus*.

Results Heterogeneous Pi supply systemically modified root morphology that increased the total root surface area (TRSA), total root volume (TRV), total root length (TRL) and total lateral root number (TLRN) of root with local Pi supply (R+) and decreased them of root with local no Pi supply (R-) when compared to root with homogeneous Pi supply (R++) and root devoid of Pi (R--), respectively. Anthocyanin, APase activity and JA concentration in shoot and root of *B. napus* were systemically regulated by heterogeneous Pi supply. In addition, heterogeneous Pi supply significantly promoted nutrient uptake when compared with homogeneous no Pi supply. Root morphology of *B. napus* was significantly changed by exogenous addition of JA or DIECA in a split-root system. JA enhanced Pi starvation response by inducing expression of PSSI genes in shoots and roots.

Conclusions Our results suggest that JA enhances systemic Pi starvation response of *B. napus* by regulating root morphology, Pi homeostasis and inducing expression of PSSI genes under heterogeneous Pi supply.

Keywords Jasmonic acid, *Brassica napus*, systemic Pi starvation response, root morphology, heterogeneous Pi supply

Abbreviations

Pi	Phosphate
JA	Jasmonic acid
DIECA	Diethyldithiocarbamic acid
APase	Acid phosphatase
PSSI	Pi starvation systemically-induced
TRSA	Total root surface area
TRV	Total root volume
TRL	Total root length
TLRN	Total lateral root number
P	Phosphorus
JA-Ile	Jasmonoyl-L-isoleucine
PR	Primary root
LR	Lateral root
1°LR	First-order lateral root
2°LR	Second-order lateral root

1 **Introduction**

2 Phosphorus (P) is an essential macronutrient that is required for plant
3 development and reproduction (Hawkesford et al. 2012). Inorganic phosphate
4 (Pi), the only form of P that can be assimilated by plants, is a highly limited
5 resource, as Pi is immobilized and heterogeneously distributed in the soil
6 (Kirkby and Johnston, 2008; Lynch, 2011; Alewell et al. 2020).

7 Plants have a wide range of adaptive mechanisms under Pi starvation,
8 which can be grouped into two major categories, namely (i) enhance
9 acquisition and (ii) utilization efficiency, via a series of morphological,
10 physiological, metabolic, and molecular alterations (Ham et al. 2018). Plant
11 acquisition of available Pi is greatly influenced by the root exploration capacity.
12 Because of the low mobility and heterogeneous distribution of Pi in the soil,
13 plasticity of root system determines the efficiency of nutrient uptake (Sun et al.
14 2018). Root system architecture (RSA) modified with patchy Pi availability can
15 effectively enhance capacity of foraging and exploitation of available Pi, such
16 as allow root proliferation in Pi-rich zones (Jin et al. 2017; Jia et al. 2018; Wang
17 et al. 2019, Li et al. 2022).

18 To coordinate morphological and molecular responses to Pi starvation,
19 plants require to perceive and integrate information on the external and
20 internal Pi concentrations along with co-ordination between local and systemic
21 signaling pathways. These two signaling pathways co-operate to modulate Pi
22 homeostasis under Pi starvation (Thibaud et al. 2010; Chiou and Lin, 2011).
23 Modification of the RSA in response to Pi starvation were not only regulated by
24 sensing local Pi concentration in the external medium, but also a subject of
25 systemic control (Rüdiger Scheible and Rojas-Triana, 2015; Gutiérrez-Alanís
26 et al. 2018; Oldroyd and Leyser, 2020). Systemic responses for modulating Pi
27 uptake, remobilization and recycling depends on the internal Pi concentration
28 (Thibaud et al. 2010). Many components of the Pi-signaling network have been
29 identified during the past decade (Ham et al. 2018). Systemic signaling
30 between the root and the shoot is complicated (Chien et al. 2018). As an
31 initially acquired molecule, Pi is not only considered to be a nutrient but also a
32 systemic signaling molecule that participate in Pi starvation responses (Jost et
33 al. 2015). Sugars, peptides, microRNAs and hormones, such as strigolactones
34 and cytokinins, also act as systemic Pi signal coordinating shoot growth (Chiou

35 and Lin, 2011; Chien et al. 2018).

36 JA (jasmonic acid) plays a key role in biotic and abiotic stress responses in
37 plants, such as herbivore insect, microbial infection, mechanical damage,
38 drought, salt, low temperature, and nutrient stress (Guo et al. 2018; Koo, 2018;
39 Ali and Baek, 2020; Hu et al. 2023). JA biosynthesis is activated at specific
40 developmental stages and in stress response (Wan and Xin, 2022). The
41 process of JA biosynthesis in plants begins with α -linolenic acid and
42 hexadecatrienoic acid, which are catalysed by a series of enzymes, such as
43 lipoxygenase (LOX), allene oxide synthase (AOS), alleneoxide cyclase (AOC),
44 OPDA reductase 3 (OPR3), and acyl-CoA oxidase1 (ACX1), and converted to
45 JA. Then, JA is conjugated with isoleucine (Ile) by JASMONATE RESISTANT1
46 (JAR1) to form jasmonoyl-L-isoleucine (JA-Ile), the bioactive form of JA
47 (Fonseca et al. 2009; Howe et al. 2018; Wan and Xin, 2022). JA-Ile is primarily
48 perceived by CORONATINE INSENSITIVE1 (COI1) and triggers the complex
49 formation of JASMONATE ZIM (COI1-JAZ), leading to degradation of JAZs
50 and releasing transcription factors to regulate JA-responsive genes (Yan et al.
51 2018; Hu et al. 2023).

52 Hormones are important components that participate in Pi starvation
53 responses (Puga et al. 2017). Compared with other hormones, such as auxin,
54 ethylene, and cytokinins, the role of JA in Pi starvation response was rarely
55 studied. A transcriptomic study between *low phosphorus insensitive 4 (lpi4)*
56 mutant and WT revealed the downregulation of expression levels of several
57 JA-regulated genes in *lpi4* mutant, indicating a potential role of JA in the root
58 tip response to Pi starvation (Chacon-Lopez et al. 2011). JA not only
59 participated in inhibition of primary root (PR) elongation, but also promoted
60 root hair growth (López-Arredondo et al. 2014). OsJAZ11 protein, a
61 transcriptional repressor of JA signaling, regulates Pi homeostasis by
62 interacting with a key Pi sensing protein, OsSPX1 (Pandey et al. 2021). The
63 study by Khan et al. (2016) showed that Pi starvation triggered JA
64 accumulation and enhanced herbivory resistance of Pi-starved plants. A recent
65 studie also found that JA was involved in the root cell wall phosphorus
66 remobilization in response to P deficiency (Tao et al. 2022). The key
67 transcription factor PHR1 (PHOSPHATE STARVATION RESPONSE1)
68 interacts with JAZ and MYC2, a key transcription factor in regulating

69 JA-responsive genes, to regulate Pi starvation-induced JA signaling (He et al.
70 2023). Our previous study also confirmed that genes related to JA metabolism
71 and signalling pathway were systemically induced by Pi starvation (Li et al.
72 2022). However, how is JA involved in the systemic response to Pi starvation
73 remains elusive.

74 *Brassica napus* (*B. napus*) is one of the important oil crops widely planted
75 and its demand of Pi-fertilizer is large and shows very sensitive to Pi deficiency.
76 In this study, heterogeneous Pi supply was used to mimic heterogeneous
77 distribution of Pi in the soil and to investigate the mechanism of JA participating
78 in the systemic response to Pi starvation. Firstly, we investigated the effect of
79 heterogeneous Pi supply on biomass, Pi concentration, root morphology
80 changes, ionic composition, acid phosphatase (APase) activity, JA
81 accumulation in shoots and roots of *B. napus* under heterogeneous Pi supply.
82 Then, systemic regulation of JA on root morphology was studied. Finally, we
83 report that JA also induced expression of Pi starvation-related genes. This
84 work provided new evidence for the involvement of JA in systemic response to
85 Pi starvation.

86 **Materials and methods**

87 **Split-root experiments of *B. napus***

88 *B. napus* plants of a commercial cultivar 'ZhongShuang11 (ZS11)' were used in
89 this work. Seeds were firstly surface sterilized in 1.0 % (v/v) NaClO for 20 min,
90 rinsed five times with distilled water and then soaked in a distilled water for 24
91 h at 4°C. Then, seeds were germinated on a medical gauze attached to a foam
92 board in 0.5 mM CaCl₂ at 25°C for 5 days until being transferred to the
93 Hoagland solution. After 5 days, the PR tip was excised to induce the
94 formation of lateral roots. After another 7 days, the seedlings having two
95 first-order lateral roots (1°LRs) about 10 cm long were placed in a split-root
96 experiment device with two separate chambers, which containing 750 mL
97 nutrient solution for a culture period of 15 days. The nutrient solution contained
98 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, 0.5 mM K₂SO₄, 46 × 10⁻³mM
99 H₃BO₃, 9.14 × 10⁻³ mM MnCl₂, 0.32 × 10⁻³ mM CuSO₄, 0.77 × 10⁻³ mM ZnSO₄,
100 0.37 × 10⁻³ mM Na₂MoO₄ and 50 × 10⁻³ mM Fe-EDTA. Three Pi treatments
101 were applied: nutrient solution with 250 μM KH₂PO₄ in both compartments
102 (homogenous Pi supply; +P/+P), Pi added to only one compartment

103 (heterogeneous Pi supply; +P/-P) or Pi deprivation treatment (homogenous no
104 Pi supply; -P/-P). To maintain an equimolar K concentration, K₂SO₄ was added
105 to the local and homogenous no Pi treatment. The pH of nutrient solution was
106 adjusted to 5.5, and renewed every 3 d. Plants were grown in a controlled
107 environment with a light/dark regime of 16/8 h at 22~24°C, light intensity of
108 300-320 μmol m⁻²s⁻¹ and relative humidity of 60-75%. Plants were
109 photographed, and then shoots (S++, S+- and S--) and roots (R++, R+, R- and
110 R--) were separately harvested at 15 days after transplanting (DAT). The JA (1
111 μM, 10 μM) or DIECA (100 μM; diethyldithiocarbamic acid, a JA biosynthesis
112 inhibitor) were added exogenously as described in our previous study (Li et al.
113 2022).

114 **Root morphology parameter analysis**

115 Roots were photographed with a digital camera (NIKON D750). Root
116 parameter including TRSA, TRV, TRL, TLRN and number of different diameter
117 root were analysed by WinRhizo Pro software (Regent Instruments, Quebec,
118 QC, Canada).

119 **Determination of Pi concentration**

120 The Pi concentration was measured using the method described by Wang *et al.*
121 (2012), with some modification. Briefly, 50 μg of fresh tissue was homogenized
122 with 50 μL of 5 M H₂SO₄ and 950 μL H₂O. The homogenate was centrifuged at
123 10000 g for 10 min at 4°C. The supernatant was collected and diluted to an
124 appropriate concentration. The diluted supernatant was mixed with a malachite
125 green reagent in 3:1 ratio and analysed after 30 min. The absorption values for
126 the solution at 650 nm were determined using Multifunctional Enzyme Marker
127 (TECAN infinite M200). Pi concentration was calculated based on a standard
128 curve generated with varying concentrations of KH₂PO₄.

129 **Determination of APase activity in shoot and root-secretory APase 130 activity**

131 APase activities of shoots were assessed as described previously (Liang et al.
132 2012). Briefly, about 0.1 g of fresh samples were ground and extracted for
133 soluble protein. Reaction mixtures containing 600 μL of 10 mM *p*-nitrophenyl
134 phosphate (pNPP), 50 mM Na-acetate buffer (pH 5.5) and protein extract were
135 incubated at 25°C for 30 min, then halted reactions via the addition of 1.2 mL

136 of 1 M NaOH. Absorbance was measured at 405 nm. The concentration of
137 soluble protein was analysed using Coomassie Brilliant Blue staining, then
138 converted the concentration of soluble protein into fresh weight. Acid
139 phosphatase activity was presented as nanomoles of pNPP hydrolysed per
140 gram of fresh weight.

141 Root-associated APase activity in roots was quantified according to Wang et
142 al. (2011). Roots of three seedlings were washed with distilled water for 2 min
143 to remove Pi on the surface of root system and transferred to 50 mL centrifugal
144 tube with 40 mL incubation solution, which containing 10 mM pNPP substrate
145 and 50 mM Na-acetate buffer (pH 5.5). After an incubation of 30 min at 30°C,
146 0.37 mL of the reaction medium was taken out and mixed with 1.66 mL of 1 M
147 NaOH to halt the reaction in another new 2 mL tube. The absorbance was
148 measured at 405 nm. The fresh weight of the roots was recorded after the
149 determination of APase activity. The APase activity was presented as milligram
150 of pNPP produced per hour per gram of fresh weight.

151 **Determination of anthocyanins**

152 Extraction of anthocyanins from 0.3 g of fresh leaf samples was carried out
153 with 1 mL of 1% HCl-methanol. The absorption values for the extracting
154 solution at 530 nm and 657 nm were determined according to Ticconi et al.
155 (2001) using Multifunctional Enzyme Marker (TECAN infinite M200). The
156 calculation formula for anthocyanin content was $Q_{\text{anthocyanins}} =$
157 $(A_{530} - A_{657}) / \text{fresh weight}$.

158 **Determination of mineral elements concentration**

159 Five independent replicates were employed in this study, each consisting of
160 four individual plants. These samples were dried in an oven (65 °C) for 72 h for
161 determination of dry weight and then ground to be fine powder using mortar for
162 further analysis. Each sample of about 50 mg (dry weight) was placed in a
163 digestive tube. For determining concentrations of total K, Ca, Mg, Fe, Mn, Zn,
164 Cu, samples were digested with 2 mL of concentrated HNO₃. For determining
165 concentrations of total N and P, samples were digested with 2 mL of
166 concentrated H₂SO₄. In the process of high temperature (100 °C) digestion,
167 H₂O₂ was added until the digestive solution became clear. The digestive
168 solution was then diluted to 50 mL with ultra-pure water and then filtered at
169 0.45 µm. Concentrations of total K, Na, Ca, Mg and S were determined using

170 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)
171 (Agilent 5110). Concentrations of total N and P were assayed using the Mobile
172 injection analyser (SEAL AA3).

173 **Extraction and determination of JA and JA-Ile**

174 Extraction and analysis of endogenous plant hormones were conducted
175 according to the method described by Liu et al. (2012). The hormone extract
176 was gathered and injected into UFLC-ESI-MS/MS (ultrafast liquid
177 chromatography-electrospray ionization/tandem-mass spectrometry system)
178 in the National Key Laboratory of Crop Genetic Improvement, Huazhong
179 Agricultural University. Five biological replicates were done for each treatment.
180 The standard of JA and JA-Ile was purchased from Sigma-Aldrich (St. Louis,
181 MO, USA) and OlChemIm (OlChemIm, Olomouc, Czech Republic),
182 respectively. The internal standard was 10-dihydro-JA (DHJA; Olchemin). All
183 these standards and internal standards were kindly provided by Dr. Hongbo
184 Liu from National Key Laboratory of Crop Genetic Improvement, Huazhong
185 Agricultural University.

186 **Root Growth Experiments of *Arabidopsis thaliana***

187 All *Arabidopsis thaliana* (*A. thaliana*) plants used in this study, including
188 mutants and transgenic plants, were in the Col-0 ecotype. The *coi1-2*, *aos*,
189 *lox2* mutant from the Salk collection and was obtained from the European
190 Arabidopsis Stock Centre (<http://arabidopsis.info/>). All these seeds were
191 sterilized by 10% sodium hypochlorite for 5 min and immersed in 75 % ethanol
192 for 3 min, then rinsed with sterilized water for 10 min. Sterilized seeds were
193 stratified at 4 °C for 2 d before sown on agar-solidified nutrition medium
194 containing 1/2 MS with 625 µM or 6.25 µM KH₂PO₄ and 1% sucrose at pH 5.8.
195 Subsequently, all seedlings were grown in a growth chamber with a light/dark
196 regime of 16/8 h at 22~24°C. PR length was measured at 10 DAT. Each
197 replication was comprised of at least 10 plants and all experiments were
198 repeated three times.

199 **GUS staining**

200 Ten-day-old *A. thaliana* seedlings with two lateral roots were transferred to a
201 split-root system, which has two compartments, one compartment containing
202 625 µM KH₂PO₄ (+P), and one compartment containing 0 µM KH₂PO₄ (-P). JA

203 (10 μ M) and DIECA (100 μ M) were applied to the -P compartment, respectively.
204 After two days, transgenic seedlings of pBnPht1;4-GUS were submerged in
205 GUS staining solution (0.1 mM Na₃PO₄ (pH 7.0), 1 mM K₃Fe(CN)₆, 1 mM
206 K₄Fe(CN)₆, 10 mM Na₂EDTA (pH 8.0), 0.1% (v/v) Triton X-100 and 2 mM
207 X-Gluc (5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid
208 cyclohexylammonium salt)) at 37 °C for 1 h. The stained seedlings were rinsed
209 with 75% ethanol for 30 min and imaged with the light microscope (Olympus
210 SZX16, Tokyo, Japan).

211 **Detection of expression of PSSI genes**

212 The hydroponic split-root process was described above, the *B. napus*
213 seedlings with two 1°LRs were treated with heterogeneous Pi supply,
214 meanwhile, 10 μ M JA or 100 μ M DIECA were exogenously added in the -P
215 medium. After 2 days, RNA of shoots and roots was extracted using TRIzol
216 (Takara, Japan). Then, 1 μ g of total RNA was used for the first-strand cDNA
217 synthesis with a HiFiScript cDNA Synthesis Kit (CW BIO, Beijing, China)
218 according to the manufacturer's instructions. For the quantitative RT-PCR
219 analysis, 5 μ L SYBR Green Master Mix (Yeasen, Shanghai, China) was mixed
220 with the primers and a 10-fold dilution of cDNA. Relative expression levels of
221 *BnaA04Pht1;4*, *BnaA09PS3* and *BnaC01PAP17* were measured using the
222 2^{- $\Delta\Delta$ CT} method. Four biological replicates were used for each treatment, and
223 the primers used in this study are listed in Supplementary Table S1.

224 **Methods for statistical analysis**

225 Statistical analysis of the data, shown as means \pm SE, was conducted using
226 one-way analysis of variance (ANOVA), t-test in SPSS (IBM, New York, NY)
227 and Microsoft Office Excel, assuming $p < 0.05$ as a significance threshold.

228 **Results**

229 **Plant growth and P uptake of *B. napus* in a split-root system**

230 Roots of *B. napus* were divided into two halves and placed in separate
231 chambers, such that half root in one compartment was supplied with Pi
232 (abbreviated as R+), and another half of the root in another compartment was
233 deprived Pi (abbreviated as R-), to mimic plants growing in a heterogeneous
234 medium. For control plants, roots in two compartments were both supplied with
235 (R++) or without Pi (R--) to mimic plants growing in a homogeneous medium.

236 Shoots of plant grown under homogeneous, local, and no Pi supply were
237 described as S⁺⁺, S^{+/-} and S⁻⁻, respectively (Figure 1A). Plants grown with
238 heterogeneous Pi supply had similar shoot phenotype as compared to plants
239 receiving a homogeneous Pi supply; however, the shoot growth of plants with
240 two parts of the root being in Pi-deficient solution was inhibited (Figure 1B).
241 Shoot dry weight and Pi concentration of plants grown under heterogeneous Pi
242 supply (S^{+/-}) were similar to that of the plants receiving a homogeneous Pi
243 supply (S⁺⁺) and significantly higher than that of the plants deprived Pi (S⁻⁻)
244 (Figure 1C, E). Under heterogeneous Pi supply, root receiving Pi (R⁺) had
245 higher root dry weight and Pi concentration than the root receiving no Pi (R⁻).
246 R⁺ had greater root dry weight and lower Pi concentration than roots receiving
247 homogeneous Pi supply treatment (R⁺⁺). In addition, root dry weight and Pi
248 concentration of R⁻ both significantly higher than that of R⁻⁻ (Figure 1D, F).

249 **Heterogeneous availability of Pi significantly alters the root morphology** 250 **of *B. napus***

251 Compared to the roots with or without homogenous Pi supply, the morphology
252 of roots with heterogeneous Pi supply was markedly modified. Heterogeneous
253 Pi supply promoted R⁺ proliferation as compared with R⁻ and R⁺⁺. The lateral
254 roots (LRs) of R⁺ were more and longer than that of R⁺⁺. Meanwhile, the LRs
255 of R⁻ were less and shorter than that of R⁻⁻ (Figure 2A, D, E). Under
256 heterogeneous Pi supply, the total root surface area (TRSA), total root volume
257 (TRV), total root length (TRL), and total lateral root number (TLRN) of R⁺ were
258 4.4-, 3.4-, 4.7- and 3.4- fold greater than that of R⁻, respectively. In addition,
259 TRSA, TRV, TRL and TLRN of R⁺ were all significantly increased compared
260 with that of R⁺⁺, but those of R⁻ all significantly decreased compared with R⁻⁻
261 (Figure 2B-E). Furthermore, the percentage of different diameter root of R⁺
262 was similar to that of R⁺⁺. Compared with Pi-sufficient roots (R⁺⁺ and R⁺),
263 Pi-deficient root (R⁻⁻ and R⁻) had higher percentage of the coarse roots
264 (diameter between 0.5-1.0 mm) and a lower percentage of fine roots (diameter
265 between 0-0.5 mm). In addition, the percentage of root diameter between
266 1.0-1.5 mm of R⁻⁻ was the largest (Figure 2F).

267 **Difference in ionic composition of *B. napus* between homogeneous** 268 **and heterogeneous Pi supplies**

269 The interaction between elements can seriously disrupt the composition of

270 ionome when one element is deficient (Maillard et al. 2016). In order to
271 investigate the modification of shoot and root ionic composition between
272 homogeneous and heterogeneous Pi supply, the concentration of nine
273 elements (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) were determined. As shown in
274 Table 1, compared with homogeneous Pi supply (S⁺⁺ and R⁺⁺),
275 homogeneous -Pi treatment significantly decreased the concentrations of N, P,
276 K, Ca, Mn in shoot (S⁻⁻) and N, P, K, Mg, Mn, Zn in roots (R⁻⁻), but significantly
277 increased the concentrations of Mg, Fe, Zn in S⁻⁻ and Fe, Cu in R⁻⁻. No
278 significant effects were reported on Cu content in S⁻⁻ and Ca content in R⁻⁻.
279 Heterogeneous Pi supply did not significantly reduce the concentrations of N, P,
280 K, Mg, Fe, Zn and Cu in S^{+,-}, but significantly increased and decreased the
281 concentration of Mn and Ca, respectively, when compared to that in S⁺⁺.
282 Since S^{+,-} had similar dry weight to S⁺⁺ (Figure 1C), we might conclude that
283 heterogeneous Pi supply significantly promoted Pi uptake by R⁺ when
284 compared with homogeneous Pi supply. Under heterogeneous Pi supply, the
285 two halves of root (R⁺ and R⁻) had similar concentration of K, Mg, Fe, Cu, but
286 the concentrations of N, P, Ca, Mn in R⁺ were significantly higher than that in
287 R⁻, and only the concentrations of Zn in R⁺ were lower than that in R⁻ (Table 1).
288 Meanwhile, the concentration of N, P, K, Ca, Mg, Fe, Zn, Cu and Mn in R⁺
289 were similar to or higher than that in R⁺⁺, and R⁻ had higher concentrations of
290 N, P, K, Mg, Mn, Zn and lower concentrations of Ca, Fe, Cu than R⁻⁻ (Table 1).
291 Since the dry weights of R⁺ and R⁻ were higher than that of R⁺⁺ and R⁻⁻,
292 respectively (Figure 1D), this indicates that heterogeneous Pi supply
293 significantly promote uptake of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu in R⁺, and N, P,
294 K, Mg, Mn, Zn in R⁻.

295 **Heterogeneous availability of Pi significantly alters anthocyanin content** 296 **and APase activity of *B. napus***

297 Anthocyanins accumulation is a typical characteristic of Pi starvation.
298 Compared with S⁺⁺ and S^{+,-}, anthocyanins was more accumulated in S⁻⁻
299 plants, and there was no significant difference between S⁺⁺ and S^{+,-} (Figure
300 3A). APase activity in plants with homogeneous -Pi supply (S⁻⁻ and R⁻⁻) were
301 significantly higher than plants with homogeneous or heterogeneous Pi supply
302 (S^{+,-}, S⁺⁺, and R⁺⁺, R⁺ and R⁻), and APase activity in plants with
303 homogeneous and heterogeneous Pi supply were not significantly different

304 (Figure 3B, C). However, root-secreted APase activity was not consistent with
305 APase activity in the root (Figure 3D). Compared with R⁺⁺, root-secreted
306 APase activity of R⁻⁻ was significantly increased, indicating that Pi starvation
307 activated root-secreted APase activity to increase the availability of Pi in the
308 growth medium. Under heterogeneous Pi supply, root-secreted APase activity
309 in R⁻ was also significantly higher than that in R⁺. In addition, due to one half
310 root receiving no Pi, the root-secreted APase activity in R⁺ was significantly
311 higher than that in R⁺⁺. Similarly, the root-secreted APase activity in R⁺ was
312 significantly lower than that in R⁻⁻ because of the other half root receiving
313 sufficient Pi (Figure 3D).

314 **JA accumulation in shoots and root of *B. napus* under different Pi supply**

315 In order to investigate whether different Pi supplies affect JAs accumulation in
316 plants, concentration of JA and its bioactive metabolite JA-Ile were analysed.
317 Compared with homogeneous Pi supply (S⁺⁺ and R⁺⁺), the concentrations of
318 JA and JA-Ile in plants without homogeneous Pi supply (S⁻⁻ and R⁻⁻) were all
319 significantly increased, indicating that Pi starvation induced JA and JA-Ile
320 accumulation in shoots and roots (Figure 4A-D). However, the concentration of
321 JA and JA-Ile in S⁺⁻ were not significantly different from that in S⁺⁺, but
322 significantly higher than that in S⁻⁻ (Figure 4A, C). Meanwhile, the two halves
323 of the root with heterogeneous Pi supply (R⁺ and R⁻) had similar
324 concentrations of JA and JA-Ile (Figure 4B, D). There were no significant
325 differences in the concentration of JA and JA-Ile of R⁺ and R⁻ when compared
326 with R⁺⁺ (Figure 4B, D), indicating that JA may act as a systemic signaling in
327 response to Pi starvation.

328 **JA involved in regulation of root morphology in response to Pi starvation**

329 We further explored the role of JA in a systemic response of *B. napus* to Pi
330 starvation by exogenous addition of JA or DIECA (diethyldithiocarbamic acid,
331 a JA biosynthesis inhibitor) in an agar split-root system. Shoot growth of plants
332 with heterogeneous Pi supply was inhibited by exogenous JA (1 μ M and 10
333 μ M) but promoted by DIECA (100 μ M) (Figure 5A, B). Compared with control
334 (mock-treated plant), root fresh weight of R⁺ and R⁻ was increased and
335 decreased by 1 μ M JA, respectively. However, root growth of both R⁺ and R⁻
336 were significantly inhibited by 10 μ M JA, and promoted by DIECA, especially
337 for R⁻ (Figure 5C). In addition, the root morphology noticeably changed when

338 R- was treated with exogenous JA or DIECA. First-order lateral root (1°LR)
339 elongation of both R+ and R- were significantly inhibited by JA (1 μM and 10
340 μM), but 1°LR of R- restored to the same length as R+ when R- was treated
341 with DIECA (Figure 5D), indicating that JA involved in the regulation of 1°LR
342 elongation in response to heterogeneous Pi supply. Second-order lateral root
343 (2°LR) number, 2°LR density and total 2°LR length of R- were significantly
344 decreased by JA and DIECA when compared to the control (-P + mock)
345 (Figure 5E, F, H). However, 2°LR density and 2°LR average length of R+ under
346 JA and DIECA treatment showed a completely opposite difference when
347 compared with the control (Figure 5F, G). This suggested that JA also
348 participated in 2°LR growth in response to heterogeneous Pi supply.

349 **JA involved in regulation of Pi homeostasis and systemic response to** 350 **heterogeneous Pi supply**

351 The alteration of exogenous of JA or DIECA (a JA biosynthesis inhibitor) on Pi
352 homeostasis was further investigated. Shoot biomass of *B. napus* was
353 significantly decreased upon 10 μM JA treatment and increased by DIECA,
354 when compared to control (mock-treated plant) (Figure 6A). Growth of R+ was
355 promoted by 1 μM JA, but inhibited by 10 μM JA. Growth of R- was
356 significantly inhibited by JA, but promoted by DIECA (Figure 6B). Pi
357 concentration in shoot and root was both significantly increased by 10 μM JA
358 and decreased by DIECA (Figure 6C, D) when compared to mock-treated plant.
359 This indicates that JA promoted Pi uptake and systemically regulated Pi
360 homeostasis.

361 It has been reported that *BnPht1;4*, encoding a high affinity Pi transporter,
362 was strongly induced by Pi starvation (Ren et al. 2014). Subsequently,
363 histochemical assay of GUS activity in transgenic *A. thaliana pBnPht1;4-GUS*
364 was further analysed. As shown in Figure 7, GUS signals were detected in
365 shoot (S--) and root (R--) of Pi-starved plants, but weak in shoot (S++) and root
366 tip (R++) of plants with homogeneous Pi supply, indicating that the activity of
367 *BnPht1;4* promoter was induced by Pi starvation (Figure 7A, C, F, H).
368 Compared with homogeneous Pi supply, GUS signal in the cotyledon (S+-)
369 was significantly enhanced by heterogeneous Pi supply. The signal was rather
370 weak in leaves and root tips of both R+ and R-, suggesting that the expression
371 of *BnPht1;4* was systemically regulated by Pi starvation (Figure 7B, G). GUS

372 signal in roots and shoots was significantly enhanced by exogenous JA and
373 weakened by DIECA (Figure 7D-E, I-J), suggesting that the activity of
374 *BnPht1;4* promoter was regulated by JA.

375 According to our previous transcriptome data of *B. napus* under
376 homogeneous and heterogenous Pi supply, *BnaA04Pht1;4* was systemically
377 induced by Pi starvation that the transcription level of *BnaA04Pht1;4* among
378 R++, R+ and R- were not different and significantly lower than that in R-- (Li et
379 al. 2022). The expression level of *BnaA04Pht1;4* in shoot and root of *B. napus*
380 was further measured under exogenous JA or DIECA treatments. We found
381 that the expression of *BnaA04Pht1;4* in both shoots (S+-JA) and roots (R+JA,
382 R-JA) was significantly induced by JA, but not affected by DIECA (Figure 8 A,
383 D). *BnaA09PS3* (*phosphate starvation-induced gene 3*) and *BnaC01PAP17*
384 (*purple acid phosphatase 17*) are involved in regulation of Pi homeostasis and
385 belong to Pi starvation systemically-induced genes (Li et al. 2022). They were
386 also measured and the expression level of them were also significantly
387 induced by JA but not affected by DIECA (Figure 8B-C, E-F). This indicates
388 that JA is involved in the systemic regulation of Pi starvation.

389 Discussion

390 Changes in the root system architecture contributes to Pi acquisition 391 and shoot growth of *B. napus* under heterogeneous Pi supply

392 P is often heterogeneously distributed in the soil because of its immobility, and
393 root proliferation into Pi-enrich zones is an important strategy for efficient
394 absorption of Pi (White et al. 2013; Lynch and Wojciechowski, 2015;
395 Gutiérrez-Alanís et al. 2018). In our study, heterogeneous Pi supply (split-root
396 experiment) was used to mimic heterogeneous Pi distribution in the soil. The
397 root dry weight of R+ was higher than that of R- and R++, indicating that local
398 Pi deprivation supply stimulated root growth in Pi-enriched zone when
399 compared with homogeneous Pi supply (Figure 1D). These findings agree with
400 the earlier studies in maize that reported that plants give a preferential
401 partitioning of the biomass to the place with greater Pi availability, under
402 heterogenous Pi distribution (Li et al. 2014; Wang et al. 2019). Meanwhile, R+
403 displayed a lower Pi concentration than R++, and R- displayed a higher Pi
404 concentration than R-- (Figure 1F), illustrating there might exist Pi
405 translocation from R+ to R- via the shoot. The lower Pi concentration and dry

406 weight in R- than in R+ (Figure 1D, F) indicated the Pi translocation from R+ to
407 R- through Pi cycling in phloem sap is limited, because the Pi take-up by R+ is
408 mainly supplied to shoot for maintaining shoot growth. Additionally, another
409 possibility is that more Pi was translocated from R+ to shoot than R-. Thus,
410 plants grown with heterogeneous Pi supply achieved a similar shoot dry weight
411 and Pi concentration to the plants receiving homogeneous Pi supply (Figure
412 1C, E). These findings suggest an elaborate distribution of Pi happened in
413 plant when they confronted with uniform Pi distribution. In addition, the TRSA,
414 TRV, TRL and TLRN were greater in R+ than in R++ (Figure 2A-E), thus
415 enhancing Pi acquisition efficiency and contributing to Pi uptake and biomass
416 production. Previous studies have also shown that greater root proliferation in
417 Pi-rich zones enhanced root uptake capacity to maintain Pi uptake and
418 biomass production (Shen et al. 2005; Liu et al. 2013). Meanwhile, R-
419 processed smaller TRSA, TRV, TRL and TLRN than R+ (Figure 2B-E), which
420 allowed plants to allocate more carbon to the root enriched with Pi (R+) and
421 proliferate for enhanced Pi uptake. The TLRN in R+ was more than that in R++,
422 and that in R- was less than that in R-- (Figure 1D). This suggests that some
423 signals were transduced between R+ and R- and systemically regulated lateral
424 root formation. In addition, the percentage of root with small diameter (0-0.5
425 mm) was greater in roots exposed to sufficient Pi (R++ and R+) than roots
426 deprived of Pi (R-- and R-) (Figure 1F), which is beneficial for Pi uptake.

427 **Impact of the heterogeneous Pi supply on nutrient uptake**

428 P is a critical macronutrient and required for many biochemical processes.
429 Compared with homogeneous Pi supply, Pi starvation reduced the uptake of N,
430 P, K, Mg, Mn, Zn in roots of R--, and N, P, K, Ca, Mn in shoots of S-- (Table 1).
431 This agreed with an earlier study that N, K, Ca, Mg, Mn, Zn uptake were
432 decreased under Pi-starvation in *B. napus* (Maillard et al. 2016). However,
433 when one half of the root was supplied with Pi (R+), the uptake of N, P, K, Mg,
434 Mn, Zn were increased in the other half of the root (R-); but the uptake of Ca,
435 Fe, Cu in R- were decreased when compare to Pi-starved root (R--). This
436 indicates that the increase of systemic-regulated Pi uptake also promoted
437 uptake of N, K, Mg, Mn, Zn and decreased uptake of Ca, Fe and Cu in R-
438 (Table 1). Meanwhile, one half root without Pi supply (R-) stimulated root
439 growth of other half root, which promoted the uptake of Pi in R+ compared to

440 R⁺⁺ (Figure 2A-E, Table 1). The uptake of N, P, K, Mg, Fe, Zn and Cu were
441 similar between homogeneous Pi supply (S⁺⁺) and heterogeneous Pi supply
442 (S^{+/-}) (Table 1), resulting in a similar shoot biomass between these two
443 treatments (Figure 1C). These results indicate that heterogeneous Pi supply
444 promoted the uptake of nutrients by systemic regulation of root morphology, so
445 even although plants received only half of total Pi of that for homogeneous Pi
446 supply, the biomass and nutrient accumulation of shoot was not reduced
447 (Figure 1C and Table 1).

448 **Physiological adaptation of *B. napus* in response to heterogeneous Pi** 449 **supply**

450 Plants undergo a series of changes in physiological adaptation when exposed
451 to Pi starvation, including accumulation of anthocyanin and secretion of
452 phosphatase (Lopez-Arredondo et al. 2014; Leong et al. 2018). In our study,
453 anthocyanin accumulation in S⁻⁻ was induced by Pi starvation, and S^{+/-} and
454 S⁺⁺ had lower anthocyanin than S⁻⁻ (Figure 3A), which were consistent with
455 the difference of Pi and total P concentration among S⁺⁺, S^{+/-} and S⁻⁻ (Figure
456 1E and Table 1). JA also induces the biosynthesis of anthocyanin (An et al.
457 2021). Interestingly, anthocyanin accumulation and Pi concentration were
458 consistent with JA and JA-Ile concentration in shoots (Figure 1C, Figure 3A,
459 and Figure 4A, C). These indicated that anthocyanin accumulation was
460 systemically regulated by Pi starvation and JA might be function as a systemic
461 signal involved in this process.

462 In addition, increasing activity and secretion of APase is a universal
463 response of plants to Pi starvation, and which promote remobilization and
464 reutilization of P (Baker et al. 2015). The APase activity in shoots depended on
465 Pi or total P concentration in shoot, and APase activity was in S^{+/-} was similar
466 to S⁺⁺, but both were significantly higher than that in S⁻⁻ (Figure 3B, Figure 1E
467 and Table 1). Similarly, roots with homogeneous (R⁺⁺) or heterogeneous Pi
468 supply (R⁺ and R⁻) had similar APase activity, but they all higher than that in
469 R⁻⁻ (Figure 3C). However, root-secreted APase activity had a negative
470 correlation with the trend of Pi concentration in root (Figure 3D), these
471 indicated that root-secreted APase activity was dependent on Pi concentration
472 in the root but not Pi concentration in the medium (Figure 1E and Figure 3H).
473 Thus, the root -secreted APase activity was also systemically regulated by Pi

474 starvation. In addition, when the root sensed the decrease of intracellular Pi
475 level, the activity of secretory APase preferentially increased, instead of the
476 APase in roots.

477 **JA biosynthesis is systemically regulated by Pi starvation**

478 Pi starvation-induced genes expression display a reduction in JA synthesis and
479 signaling mutants under Pi starvation, suggesting that JA plays an important
480 role in response to Pi starvation (Khan et al. 2016; Paz-Ares et al. 2022). In our
481 study, S⁻ and R⁻ had higher JA and JA-Ile concentration compared with S⁺
482 and R⁺ (Figure 4A-D); this was consistent with Pi concentration in shoots and
483 roots (Figure 1E-F), and also agrees with the earlier study that Pi starvation
484 raised the concentration of JA (Khan et al. 2016; Tao et al. 2022). Meanwhile,
485 the concentration of JA and JA-Ile in shoots and roots was not different
486 between homogeneous and heterogeneous Pi supply, respectively (Figure
487 4A-D). This indicated that JA biosynthesis was not regulated by local Pi level in
488 medium but systemically regulated by Pi starvation.

489 **JA is involved in systemic regulation of root morphology and Pi 490 homeostasis**

491 Plant exposed to Pi deficiency produce local signals that lead to inhibition of
492 primary root (PR) elongation (Péret et al. 2011; He et al. 2023). JA has also
493 been reported to negatively regulate PR growth (Huang et al. 2017). Our
494 results found that Pi starvation induces JA accumulation in the root (Figure 4B,
495 D). JA is involved in systemic signaling associated with the response of plant to
496 wounding responses and light stress (Takahashi and Shinozaki, 2019). In
497 order to understand whether JA act as systemic signal in response to Pi
498 starvation, we analysed the modification of root morphology in the split-root
499 system by either adding JA exogenously, or inhibiting its biosynthesis in -P
500 medium. Interestingly, JA enhanced Pi starvation response by inhibiting 1°LR
501 elongation of R⁺ and R⁻ and promoting 2°LR growth of R⁺, especially under 10
502 μM JA treatment (Figure 5A, D-H). If JA biosynthesis was blocked by DIECA,
503 Pi starvation status of R⁻ is significantly weakened, because 1°LR elongation
504 of R⁻ was restored to same length as R⁺ and 2°LR growth of R⁺ was
505 dramatically inhibited (Figure 5A, D-H). This also indicated that 1°LR might
506 function as the primary root after splitting the root system. These significant
507 root morphological changes confirmed the involvement of JA in a systemic Pi

508 starvation response. It has been reported that Pi concentration in *A. thaliana*
509 *coi1* and *aos* mutants was significantly lower than WT under Pi deficiency
510 (Khan et al. 2016). Similarly, exogenous JA promoted Pi uptake by inducing
511 expression of *OsPT2* in rice under Pi-deficient condition (Tao et al. 2022). Our
512 results also demonstrated that Pi uptake was promoted by JA and decreased
513 by blocking JA biosynthesis (Figure 6C-D). The Pi content in R+ (100 μ M
514 DIECA) is lower than that of R+ (Mock) probably because that the inhibition of
515 the synthesis of JA in R- weakened the systemic-Pi starvation signaling to R+
516 (100 μ M DIECA) which led to the decrease in Pi uptake capacity of R+ (100
517 μ M DIECA) (Figure 6C-D), suggesting that JA involved in a systemic regulation
518 of Pi homeostasis.

519 **JA systemically regulate Pi uptake and Pi starvation response**

520 PHOSPHATE TRANSPORTER 1 (PHT1) proteins are high affinity Pi
521 transporters, responsible for Pi homeostasis under Pi starvation (Chen et al.
522 2015; Ham et al. 2018). Earlier studies have shown that expression of
523 *BnPht1;4*, encoding a phosphate transporter of PHT1 family, was remarkably
524 induced by Pi starvation (Ren et al. 2014). In order to verify whether JA
525 regulates Pi homeostasis by controlling expression of Pi transporter, the
526 activity of *BnPht1;4* promoter was analyzed under JA or DIECA treatment.
527 GUS staining results showed that GUS signals from R+ and R- were
528 significantly enhanced by JA and attenuated by DIECA (Figure 7A-I). This
529 further indicating that JA activated Pi starvation response. Our previous
530 transcriptome data showed that *BnaA04Pht1;4*, *BnaA09PS3* and
531 *BnaC01PAP17* belong to Pi starvation systemically-induced genes (Li et al.
532 2022). Their expression levels in R+ and R- were both strongly induced when
533 JA was added to the -P medium (Figure 8 A-F), indicating JA enhanced the Pi
534 starvation response of both R+ and R-. These further illustrated the
535 involvement of JA in a systemic regulation of Pi starvation response.

536 **Conclusion**

537 The present study describes the morphological, and physiological response of
538 *B. napus* to heterogeneous Pi supply. Root morphology, anthocyanin content,
539 APase activity, and JA and JA-Ile concentration, were all systemically
540 regulated by Pi starvation. Heterogeneous Pi supply promote the uptake of

541 nutrients by systemic regulation of root morphology. JA systemically regulated
542 root morphology under conditions of heterogeneous Pi supply.

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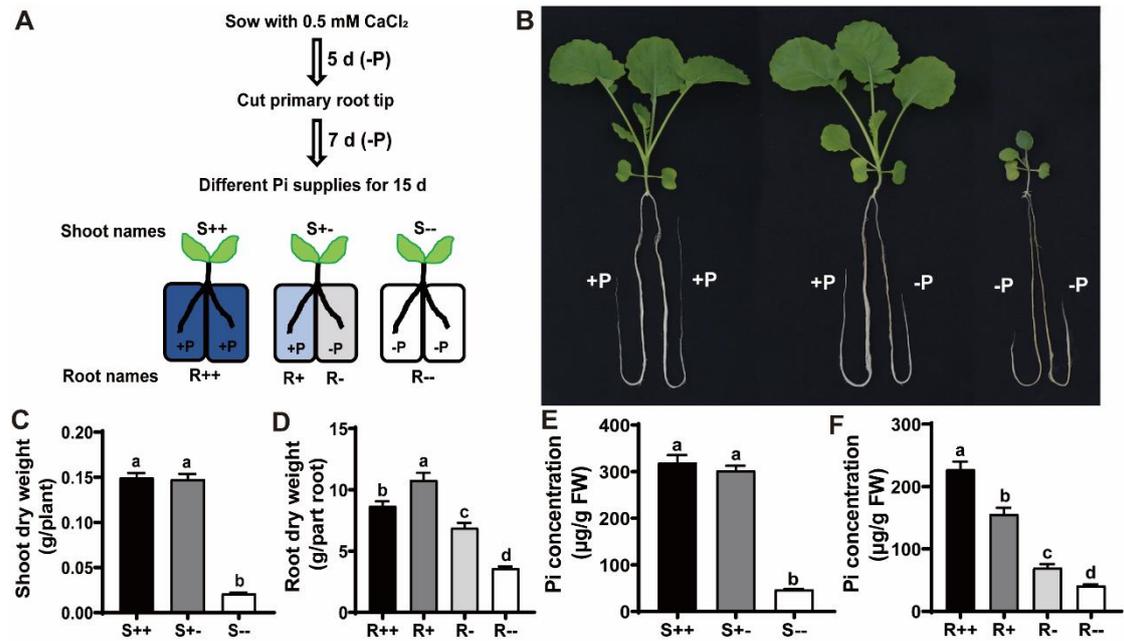
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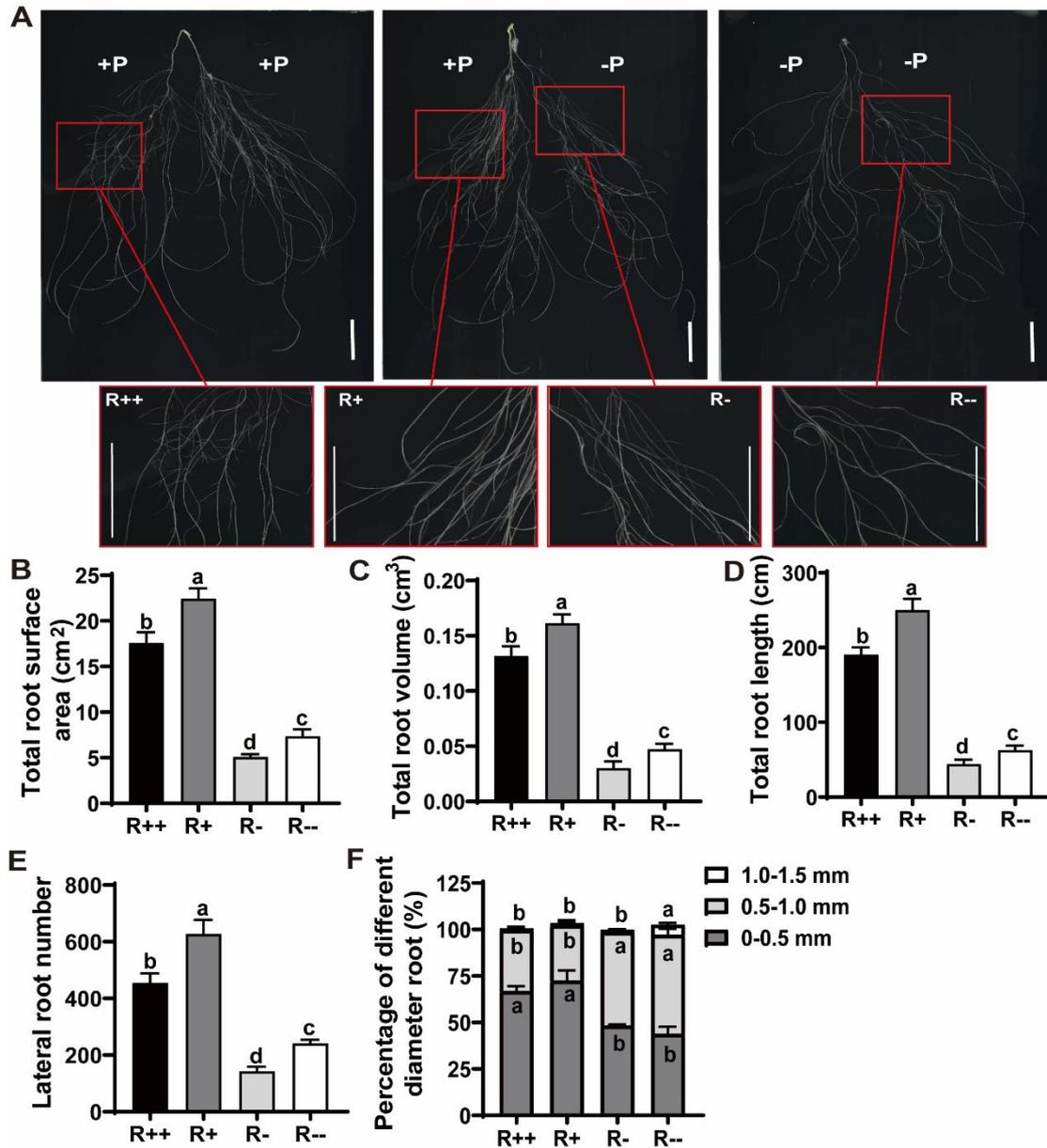
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Figure 1. Growth and Pi concentration of *B. napus* seedlings under homogenous and local Pi supply. (A) A schematic diagram of the experimental procedure of different Pi supplies. S++ and R++: shoot and root of the plant with homogenous Pi supply (+P/+P); S-- and R--: shoot and root of the plant fully deprived of Pi supply (-P/-P); S+-: shoot of the plant with local Pi supply (+P/-P); R+ and R-: root receiving local Pi and no Pi supply, respectively; +P: 250 µM KH₂PO₄; -P: 0 µM KH₂PO₄. (B) Growth phenotype of the seedlings at 15 DAT (day after transplantation). Scale bar = 5 cm. (C-D) Dry weight of shoots and roots. (E-F) Pi concentration of shoots and roots. Root dry weight from each compartment were analysed separately. Values are the means ± SE (for dry weight, n ≥ 20; for Pi concentration, n=5). A one-way ANOVA was carried out for the 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.



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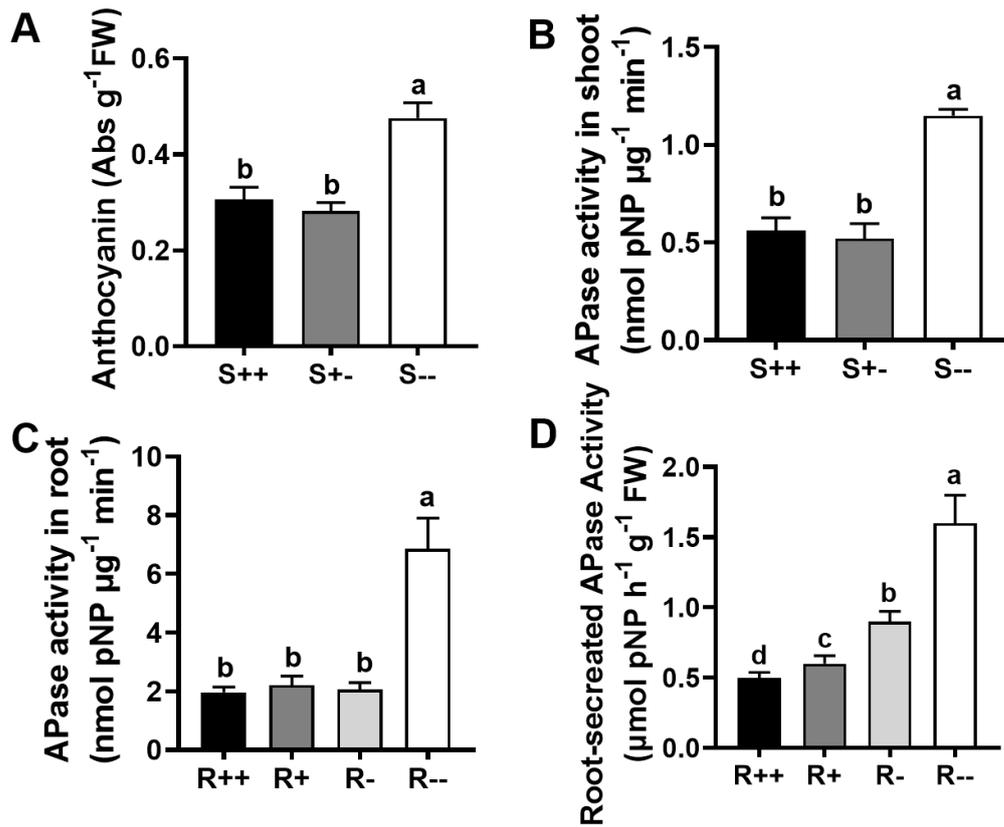
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Figure 2. Root system architecture of *B. napus* under homogeneous and local Pi supply. (A) Root phenotype of *B. napus* at 15 DAT. Scale bar = 5 cm. The root in the red boxes were enlarged and shown below, respectively. Effect of homogeneous and local Pi supply on (B) total root surface area, (C) total root volume, (D) total root length, (E) lateral root number and (F) percentage of different diameter root of *B. napus* at 15 DAT. Roots from each compartment were analysed separately. Values are the means \pm SE ($n \geq 7$). A one-way ANOVA was carried out for the 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.



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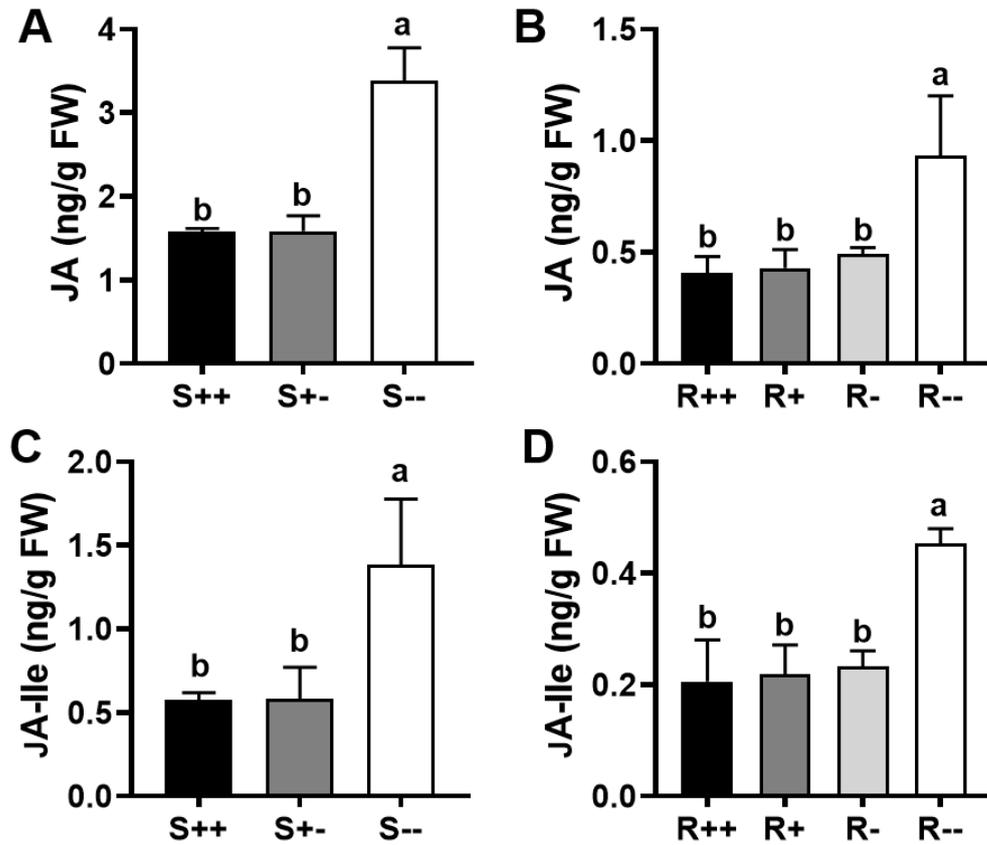
Figure 3. Effect of homogeneous and local Pi supply on sugars concentration, anthocyanin content and acid phosphatase (APase) activity of *B. napus*. (A) Anthocyanins content in shoot, APase activity in shoot (B) and root (C), root-secreted APase activity (D) after a 15-day treatment. Values are the means \pm SE (n = 5). 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.

Table 1 The effect of homogeneous and local Pi supply on the ionic composition in the shoot and root of *B. napus*

Elemental content (mg g ⁻¹ DW)	Shoot			Root			
	S++	S+-	S--	R++	R+	R-	R--
N	56.137±0.67 a	58.733±2.70 a	49.212±0.34 b	43.262±1.19 a	43.914±0.86 a	40.461±1.11 b	33.321±1.31 c
P	6.840±0.17 a	6.525±0.17 a	0.563±0.01 b	12.198±0.17 a	12.027±0.17 a	5.543±0.08 b	1.759±0.02 c
K	43.690±0.92 a	44.613±0.92 a	14.135±0.46 b	28.431±4.53 a	30.787±1.47 a	31.621±1.83 a	11.973±0.51 b
Ca	37.884±0.77 a	35.111±0.71 b	33.377±0.45 b	9.915±0.39 a	9.285±0.19 a	7.409±0.21 b	9.140±0.17 a
Mg	4.627±0.03 b	4.661±0.08 b	5.579±0.06 a	2.786±0.04 a	2.889±0.03 a	2.969±0.06 a	2.041±0.10 b
Fe	0.098±0.004 b	0.112±0.010 ab	0.128±0.008 a	10.071±0.773 b	10.042±0.148 b	11.542±0.326 b	31.959±0.822 a
Mn	0.145±0.004 b	0.172±0.005 a	0.058±0.001 c	0.627±0.027 b	1.194±0.027 a	0.614±0.020 b	0.068±0.002 c
Zn	0.051±0.003 b	0.048±0.002 b	0.062±0.003 a	0.142±0.008 b	0.127±0.006 b	0.173±0.009 a	0.089±0.003 c
Cu	0.005±0.000 a	0.005±0.000 a	0.005±0.000 a	0.031±0.002 b	0.023±0.001 b	0.022±0.001 b	0.094±0.005 a

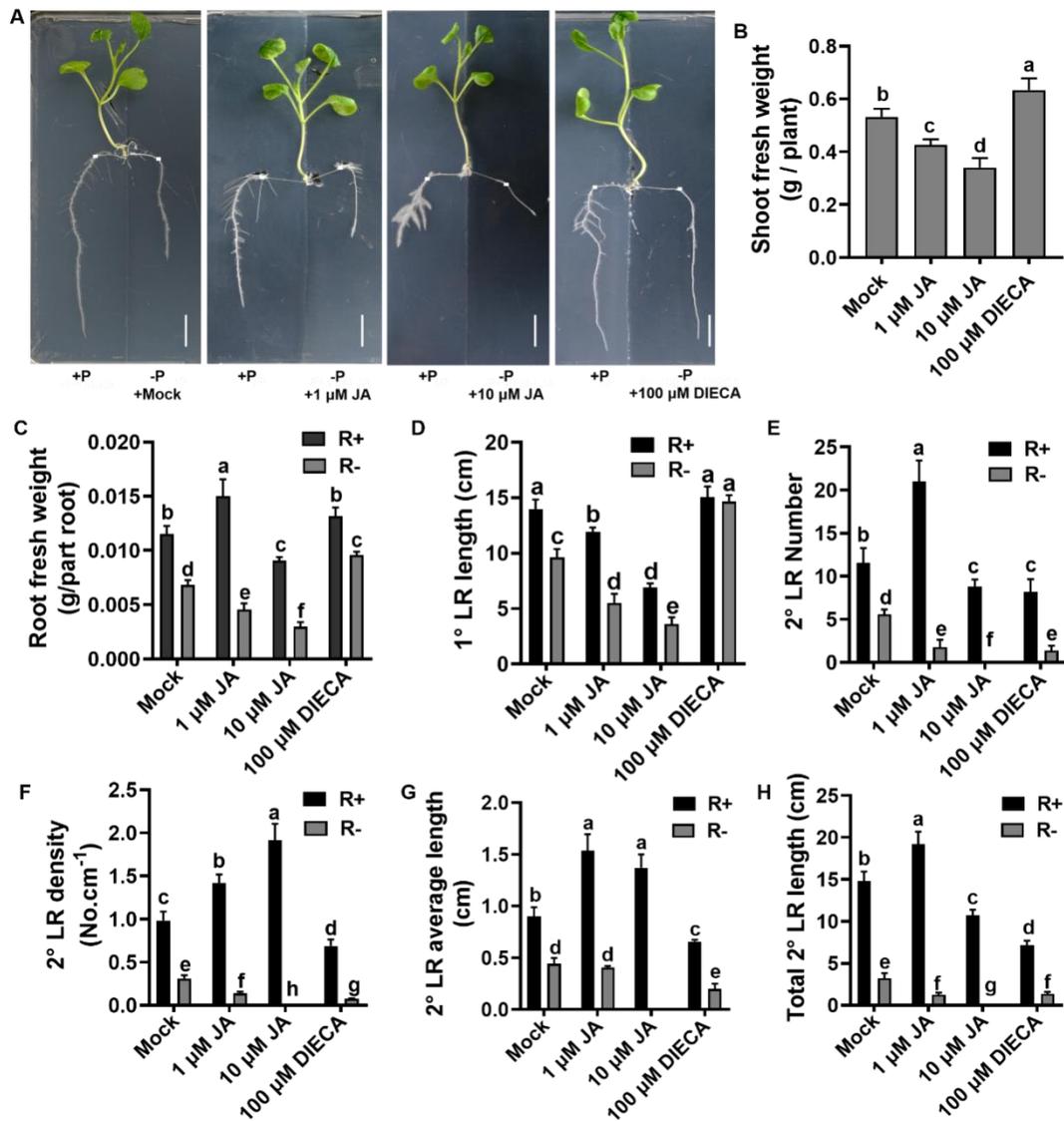
737 Content (mg g⁻¹ DW) of total N, P, K, Ca, Mg, Fe, Mn, Zn and Cu in shoots and roots of *B. napus* under homogeneous and local Pi supply for 15 days.

738 Values are the means ± SE (n = 5). The means with different letters are significantly different among Pi treatments at *P* < 0.05 level.



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Figure 4. Effect of homogeneous and local Pi supply on JA and JA-Ile concentration of *B. napus*. JA (A-B) and JA-Ile (C-D) concentration in shoots and roots at 15 DAT. Values are the means \pm SE ($n = 5$). 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.



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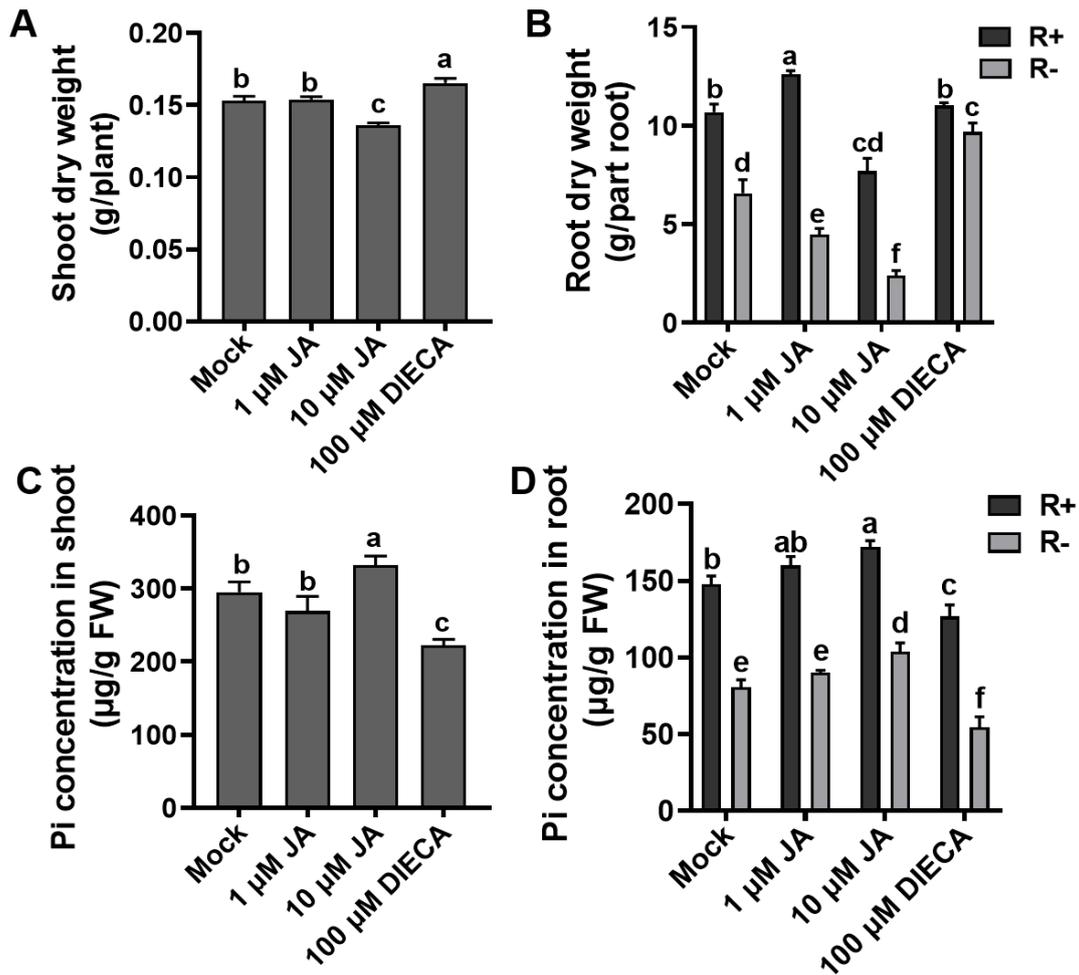
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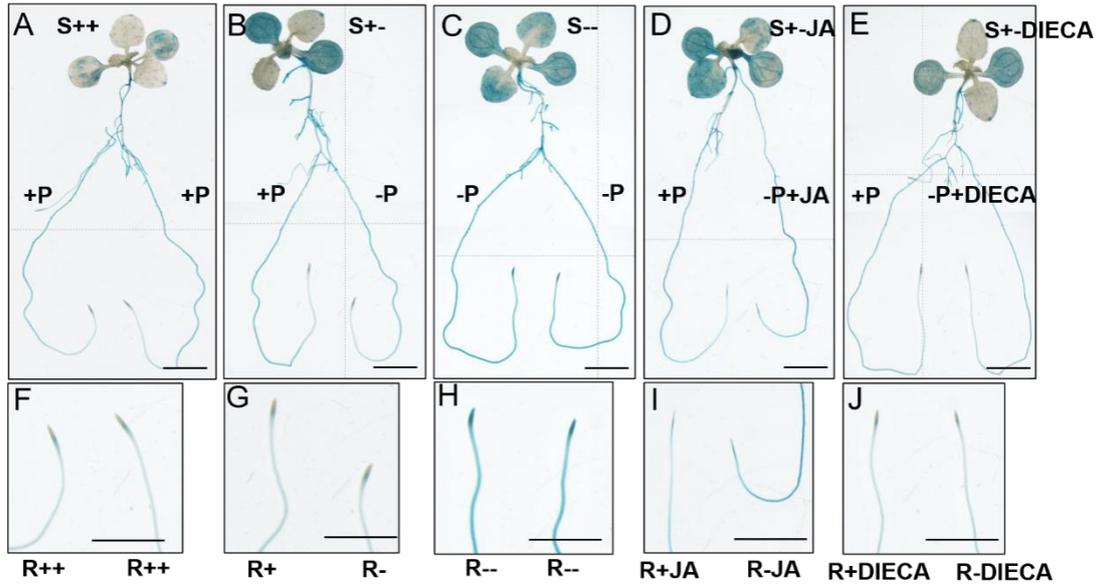
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Figure 5. Effects of JA or DIECA on biomass and root morphology of *B. napus* seedlings grown in a split-root system with heterogeneous Pi supply. The split-root system with two compartments, a compartment containing 625 μM KH₂PO₄ (abbreviated as +P), and a compartment containing 0 μM KH₂PO₄ (abbreviated as -P). JA (1 μM, 10 μM) and DIECA (100 μM; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) applied to the -P compartment. The first-order lateral root and second-order lateral root were abbreviated as 1°LR and 2°LR, respectively. (A) Phenotype of the seedlings after treatment for 9 days. The white horizontal lines show the root tip 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 days after transfer to the treatment. Values are means ±SE (n=20). A one-way ANOVA was carried out for the whole dataset, and post-hoc comparisons were conducted using the SPSS Tukey HSD test at the P<0.05 level. Significant differences are indicated by different letters above the bars.



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Figure 6. Shoot and root dry weight and Pi concentration of *B. napus* seedlings under JA or DIECA treatment in hydroponic split-root system. JA (1 µM, 10 µM) and DIECA (100 µM; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) applied to the -P compartment. (A-B) Dry weight and (C-D) Pi concentration of shoots and roots were determined after a 15-day treatment. Root dry weight from each compartment were analysed separately. Values are the means ± SE (for dry weight, n ≥ 10; for Pi concentration, n=5). A one-way ANOVA was carried out for the 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.



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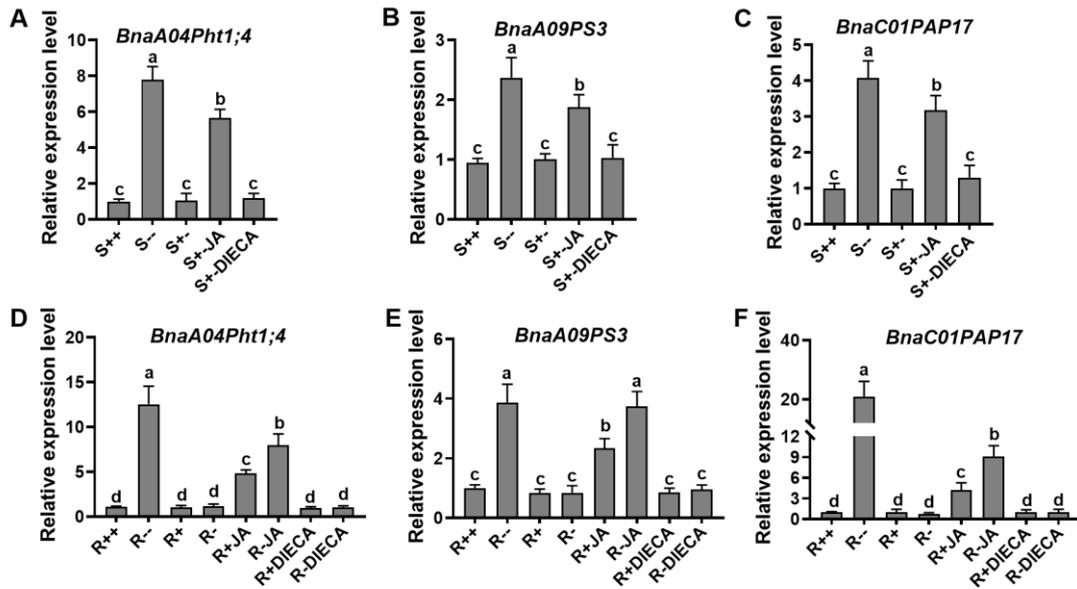
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Figure 7. Effects of JA or DIECA treatment on the activity of *BnPht1;4* promoter in split-root system. The primary root tip of five-day-old *A. thaliana* seedlings was excised with blade to induce formation of lateral roots. After another 4 d, seedlings with two lateral roots were transferred to split-root system for 2 d. Two compartments in the split-root system, one compartment containing 625 μM KH₂PO₄ (+P), and another one containing 0 μM KH₂PO₄ (-P). JA (10 μM) and DIECA (100 μM; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) applied to the -P compartment. Two days after transfer, transgenic *pBnPht1;4-GUS* seedlings (>10) were stained by GUS solution and their whole seedling (A-E) were imaged by light microscope. (F-J) The local enlarged images of root tips in (A-E).



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Figure 8. Effects of JA or DIECA treatment on the expression of Pi-starvation induced genes in split-root system. Six-day-old *B. napus* seedlings with two lateral roots were transferred to hydroponic split-root system with two compartments, one compartment containing 250 μM KH_2PO_4 (+P), and one compartment containing 0 μM KH_2PO_4 (-P). JA (10 μM) or DIECA (100 μM) applied to the -P compartment. The relative expression of *BnaA04Pht1;4* (A, D), *BnaA09PS3* (PHOSPHATE STARVATION-INDUCED GENES 3) (B, E) and *BnaC01PAP17* (PURPLE ACID PHOSPHATASE 17) (C, F) was detected in shoots and roots after 2 days. A one-way ANOVA was carried out for the 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.