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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

Introduction

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

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

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

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

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

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

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

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

Materials and methods

Split-root experiments of *B. napus*

B. napus plants of a commercial cultivar 'ZhongShuang11 (ZS11)' were used in this work. Seeds were firstly surface sterilized in 1.0 % (v/v) NaClO for 20 min, rinsed five times with distilled water and then soaked in a distilled water for 24 h at 4°C. Then, seeds were germinated on a medical gauze attached to a foam board in 0.5 mM $CaCl_2$ at 25°C for 5 days until being transferred to the Hoagland solution. After 5 days, the PR tip was excised to induce the formation of lateral roots. After another 7 days, the seedlings having two first-order lateral roots (1°LRs) about 10 cm long were placed in a split-root experiment device with two separate chambers, which containing 750 mL nutrient solution for a culture period of 15 days. The nutrient solution contained 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 2 mM $MgSO_4$, 0.5 mm K_2SO_4, 46 × 10^{-3}mM H_3BO_3, 9.14 × 10^{-3} mM $MnCl_2$, 0.32 × 10^{-3} mM $CuSO_4$, 0.77 × 10^{-3} mM $ZnSO_4$, 0.37 × 10^{-3} mM Na_2MoO_4 and 50 × 10^{-3} mM Fe-EDTA. Three Pi treatments were applied: nutrient solution with 250 μM KH_2PO_4 in both compartments (homogenous Pi supply; +P/+P), Pi added to only one compartment

(heterogeneous Pi supply; +P/-P) or Pi deprivation treatment (homogenous no Pi supply; -P/-P). To maintain an equimolar K concentration, K_2SO_4 was added to the local and homogenous no Pi treatment. The pH of nutrient solution was adjusted to 5.5, and renewed every 3 d. Plants were grown in a controlled environment with a light/dark regime of 16/8 h at 22~24°C, light intensity of 300-320 μmol $m^{-2}s^{-1}$ and relative humidity of 60-75%. Plants were photographed, and then shoots (S++, S+- and S--) and roots (R++, R+, R- and R--) were separately harvested at 15 days after transplanting (DAT). The JA (1 μM, 10 μM) or DIECA (100 μM; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) were added exogenously as described in our previous study (Li et al. 2022).

Root morphology parameter analysis

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

Determination of Pi concentration

The Pi concentration was measured using the method described by Wang *et al.* (2012), with some modification. Briefly, 50 μg of fresh tissue was homogenized with 50 μL of 5 M H_2SO_4 and 950 μL H_2O. The homogenate was centrifuged at 10000 g for 10 min at 4℃. The supernatant was collected and diluted to an appropriate concentration. The diluted supernatant was mixed with a malachite green reagent in 3:1 ratio and analysed after 30 min. The absorption values for the solution at 650 nm were determined using Multifunctional Enzyme Marker (TECAN infinite M200). Pi concentration was calculated based on a standard curve generated with varying concentrations of KH_2PO_4.

Determination of APase activity in shoot and root-secretory APase activity

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

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

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

Determination of anthocyanins

Extraction of anthocyanins from 0.3 g of fresh leaf samples was carried out with 1 mL of 1% HCl-methanol. The absorption values for the extracting solution at 530 nm and 657 nm were determined according to Ticconi et al. (2001) using Multifunctional Enzyme Marker (TECAN infinite M200). The calculation formula for anthocyanin content was $Q_{anthocyanins}$ = (A530-A657)/fresh weight.

Determination of mineral elements concentration

Five independent replicates were employed in this study, each consisting of four individual plants. These samples were dried in an oven (65 °C) for 72 h for determination of dry weight and then ground to be fine powder using mortar for further analysis. Each sample of about 50 mg (dry weight) was placed in a digestive tube. For determining concentrations of total K, Ca, Mg, Fe, Mn, Zn, Cu, samples were digested with 2 mL of concentrated HNO_3. For determining concentrations of total N and P, samples were digested with 2 mL of concentrated H_2SO_4. In the process of high temperature (100 °C) digestion, H_2O_2 was added until the digestive solution became clear. The digestive solution was then diluted to 50 mL with ultra-pure water and then filtered at 0.45 μm. Concentrations of total K, Na, Ca, Mg and S were determined using

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

Extraction and determination of JA and JA-Ile

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

Root Growth Experiments of *Arabidopsis thaliana*

All *Arabidopsis thaliana* (*A. thaliana*) plants used in this study, including mutants and transgenic plants, were in the Col-0 ecotype. The *coi1-2*, *aos*, *lox2* mutant from the Salk collection and was obtained from the European Arabidopsis Stock Centre (http://arabidopsis.info/). All these seeds were sterilized by 10% sodium hypochlorite for 5 min and immersed in 75 % ethanol for 3 min, then rinsed with sterilized water for 10 min. Sterilized seeds were stratified at 4 °C for 2 d before sown on agar-solidified nutrition medium containing 1/2 MS with 625 μM or 6.25 μM KH_2PO_4 and 1% sucrose at pH 5.8. Subsequently, all seedlings were grown in a growth chamber with a light/dark regime of 16/8 h at 22~24°C. PR length was measured at 10 DAT. Each replication was comprised of at least 10 plants and all experiments were repeated three times.

GUS staining

Ten-day-old *A. thaliana* seedlings with two lateral roots were transferred to a split-root system, which has two compartments, one compartment containing 625 μM KH_2PO_4 (+P), and one compartment containing 0 μM KH_2PO_4 (-P). JA

(10 μM) and DIECA (100 μM) were applied to the -P compartment, respectively. After two days, transgenic seedlings of pBnPht1;4-GUS were submerged in GUS staining solution (0.l mM Na_3PO_4 (pH 7.0), 1 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6$, 10 mM Na_2EDTA (pH 8.0), 0.1% (v/v) Triton X-100 and 2 mM X-Gluc (5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid cyclohexylammonium salt)) at 37 °C for 1 h. The stained seedlings were rinsed with 75% ethanol for 30 min and imaged with the light microscope (Olympus SZX16, Tokyo, Japan).

Detection of expression of PSSI genes

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

Methods for statistical analysis

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

Results

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

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

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

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

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

Difference in ionomic composition of *B. napus* between homogeneous and heterogeneous Pi supplies

The interaction between elements can seriously disrupt the composition of

ionome when one element is deficient (Maillard et al. 2016). In order to investigate the modification of shoot and root ionic composition between homogeneous and heterogeneous Pi supply, the concentration of nine elements (N, P, K, Ca, Mg, Fe, Mn, Zn and Cu) were determined. As shown in Table 1, compared with homogeneous Pi supply (S++ and R++), homogeneous -Pi treatment significantly decreased the concentrations of N, P, K, Ca, Mn in shoot (S--) and N, P, K, Mg, Mn, Zn in roots (R--), but significantly increased the concentrations of Mg, Fe, Zn in S-- and Fe, Cu in R--. No significant effects were reported on Cu content in S-- and Ca content in R--. Heterogeneous Pi supply did not significantly reduce the concentrations of N, P, K, Mg, Fe, Zn and Cu in S+-, but significantly increased and decreased the concentration of Mn and Ca, respectively, when compared to that in S++. Since S+- had similar dry weight to S++ (Figure 1C), we might conclude that heterogeneous Pi supply significantly promoted Pi uptake by R+ when compared with homogeneous Pi supply. Under heterogeneous Pi supply, the two halves of root (R+ and R-) had similar concentration of K, Mg, Fe, Cu, but the concentrations of N, P, Ca, Mn in R+ were significantly higher than that in R-, and only the concentrations of Zn in R+ were lower than that in R- (Table 1). Meanwhile, the concentration of N, P, K, Ca, Mg, Fe, Zn, Cu and Mn in R+ were similar to or higher than that in R++, and R- had higher concentrations of N, P, K, Mg, Mn, Zn and lower concentrations of Ca, Fe, Cu than R-- (Table 1). Since the dry weights of R+ and R- were higher than that of R++ and R--, respectively (Figure 1D), this indicates that heterogeneous Pi supply significantly promote uptake of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu in R+, and N, P, K, Mg, Mn, Zn in R-.

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

JA biosynthesis is systemically regulated by Pi starvation

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

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

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

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

JA systemically regulate Pi uptake and Pi starvation response

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

Conclusion

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

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

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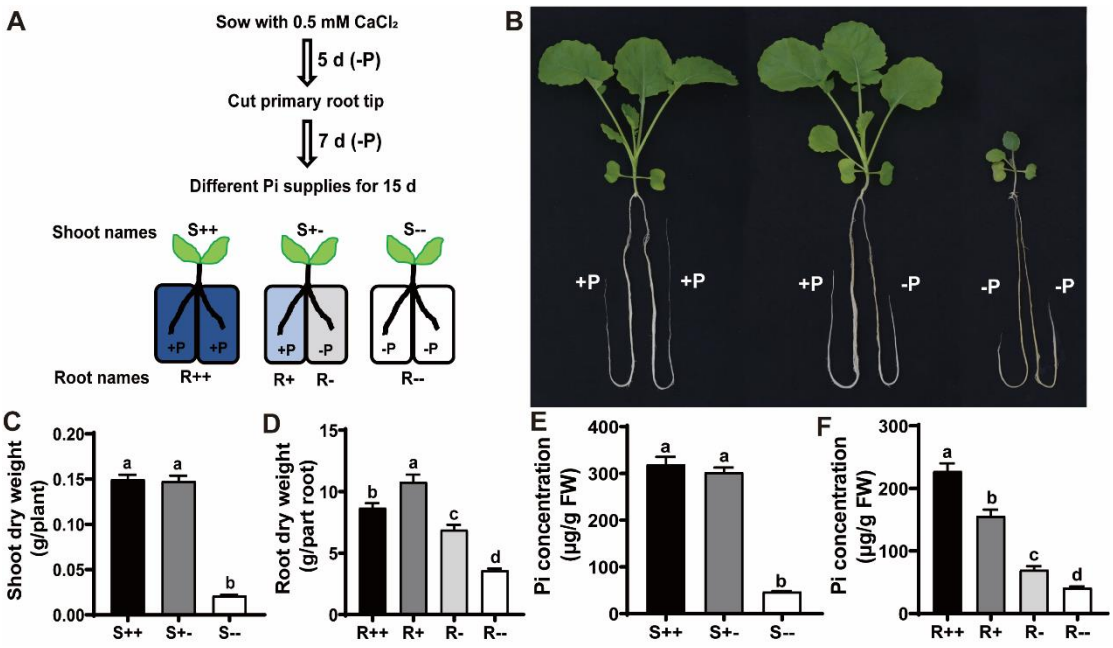


Figure 1. **Growth and Pi concentration of *B. napus* seedlings under homogenous and local Pi supply.** (A) A schematic diagram of the experimental procedure of different Pi supplies. S++ and R++: shoot and root of the plant with homogenous Pi supply (+P/+P); S-- and R--: shoot and root of the plant 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 KH_2PO_4; -P: 0 μM KH_2PO_4. (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.

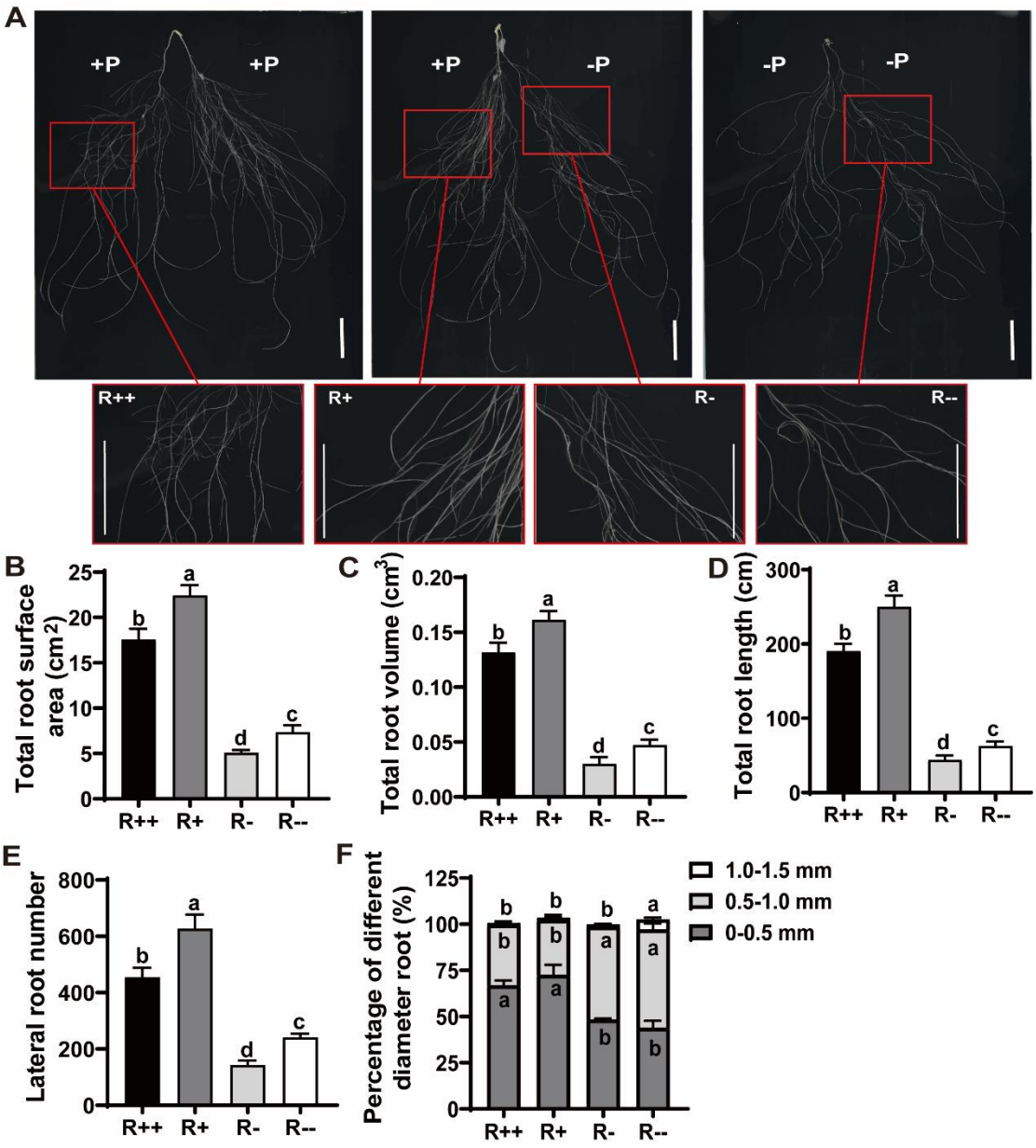


Figure 2. Root system architecture of *B. napus* under homogeneous and local Pi supply. (A) Root phenotype of *B. napus* at 15 DAT. Scale bar = 5 cm. The root in the red boxes were enlarged and shown below, respectively. Effect of homogeneous and local Pi supply on (B) total root surface area, (C) total 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 ± SE (n ≥ 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.

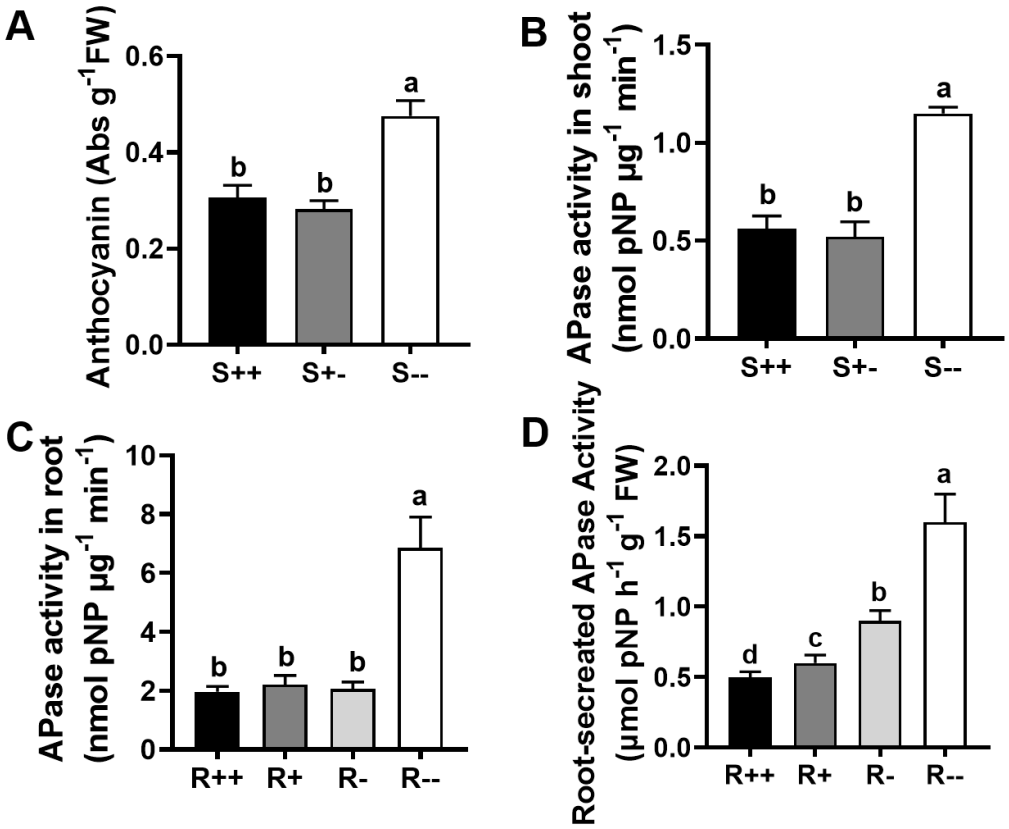
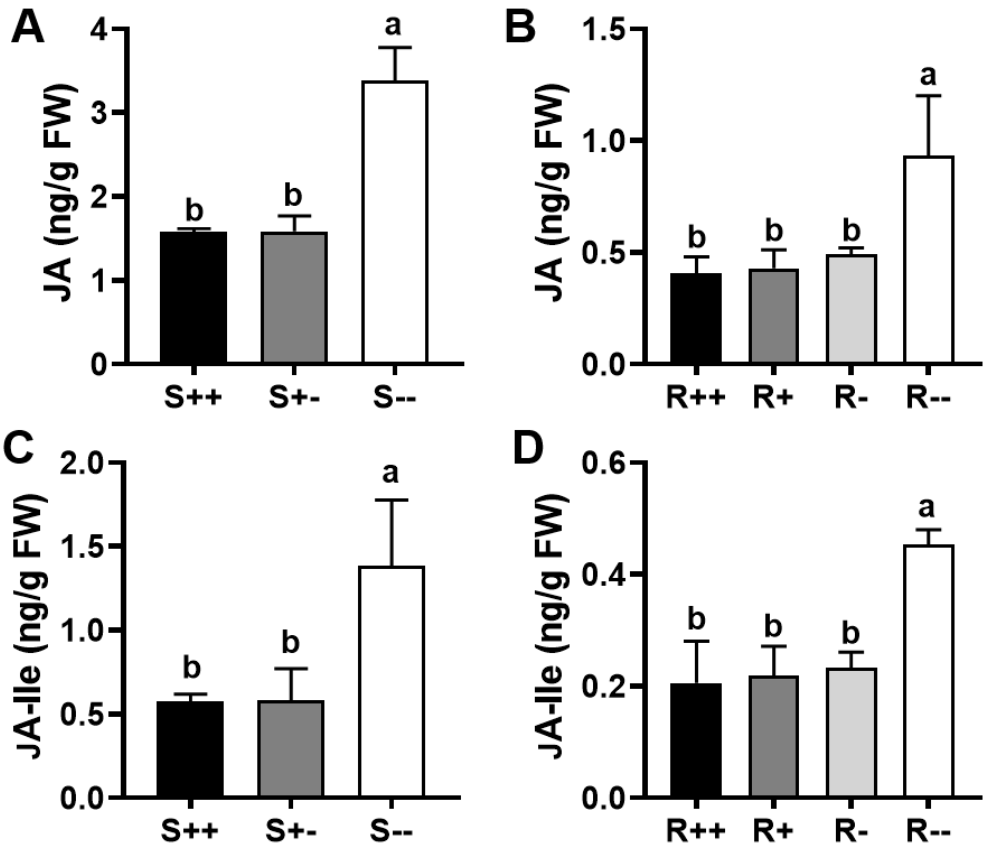


Figure 3. **Effect of homogeneous and local Pi supply on sugars concentration, anthocyanin content and acid phosphate (APase) activity of *B. napus*.** (A) Anthocyanins content in shoot, APase activity in shoot (B) and root (C), root-secreted APase activity (D) after a 15-day treatment. Values are the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

736 **Table 1 The effect of homogeneous and local Pi supply on the ionomic 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.



739

740 **Figure 4**. **Effect of homogeneous and local Pi supply on JA and JA-Ile concentration**
741 **of *B. napus*.** JA (A-B) and JA-Ile (C-D) concentration in shoots and roots at 15 DAT.
742 Values are the means ± SE (n = 5). A one-way ANOVA was carried out for the whole data
743 set, and post hoc comparisons were conducted using the SPSS Tukey HSD test at $P <$
744 0.05 level. Significant differences are indicated by different letters above the bars.

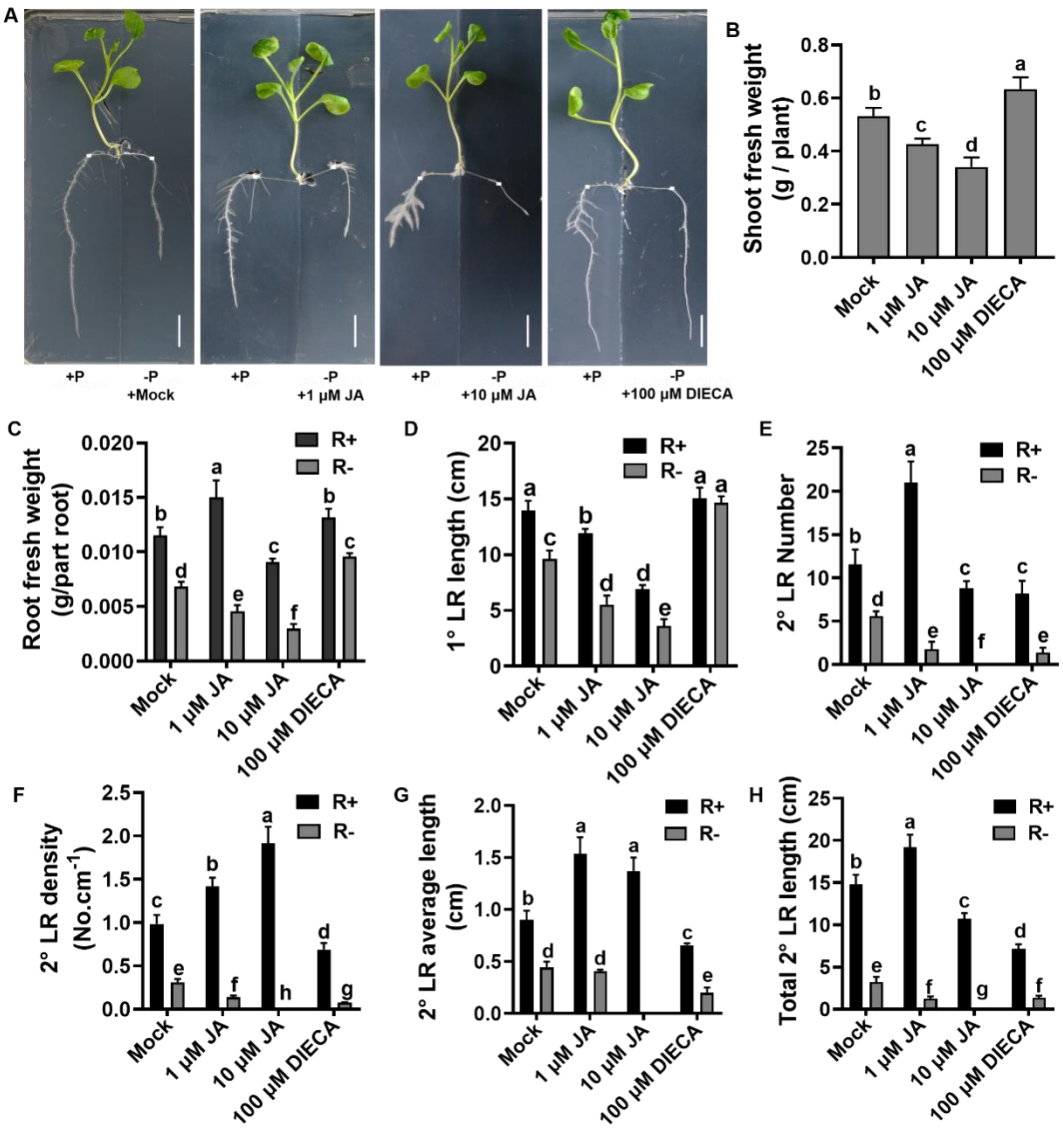


Figure 5. Effects of JA or DIECA on biomass and root morphology of *B. napus* seedlings grown in a split-root system with heterogeneous Pi supply. The split-root system with two compartments, a compartment containing 625 μM KH_2PO_4 (abbreviated as +P), and a compartment containing 0 μM KH_2PO_4 (abbreviated 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.

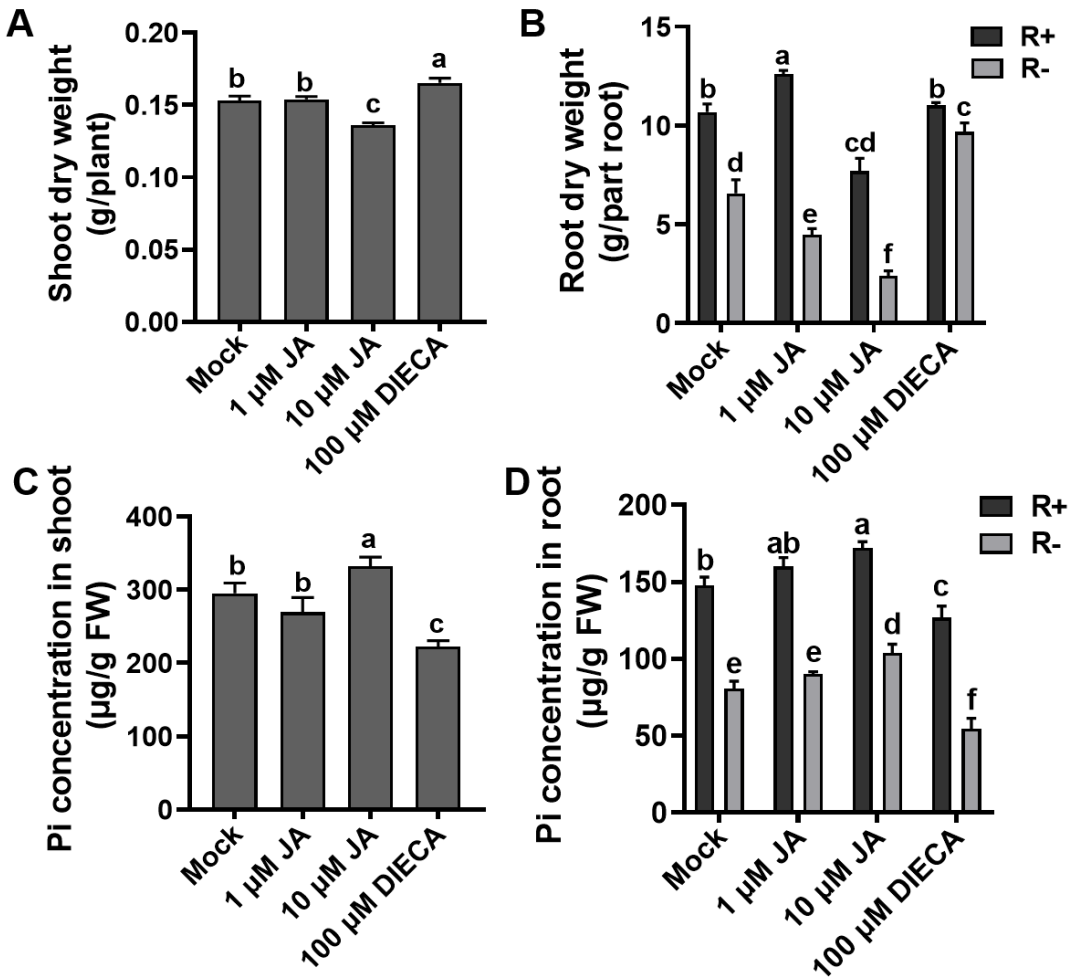


Figure 6. **Shoot and root dry weight and Pi concentration of *B. napus* seedlings under JA or DIECA treatment in hydroponic split-root system.** JA (1 μM, 10 μM) and DIECA (100 μM; diethyldithiocarbamic acid, a JA biosynthesis inhibitor) applied to the -P compartment. (A-B) Dry weight and (C-D) Pi concentration 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.

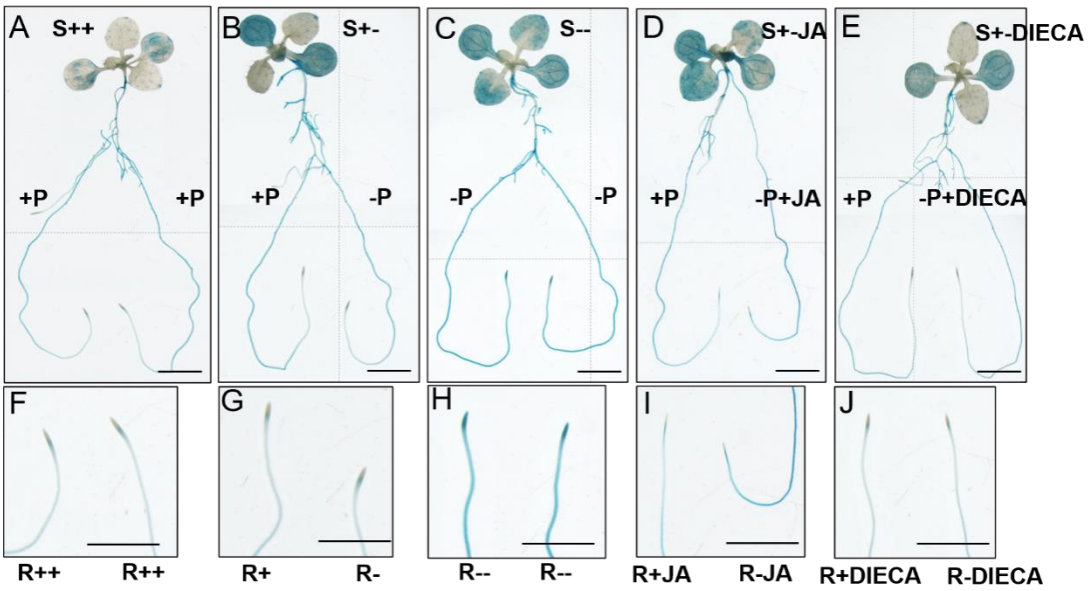


Figure 7. **Effects of JA or DIECA treatment on the activity of *BnPht1;4* prompter in split-root system.** The primary root tip of five-day-old *A. thaliana* seedlings was excised with blade to induce formation of lateral roots. After another 4 d, seedlings with two lateral roots were transferred to split-root system for 2 d. Two compartments in the split-root system, one compartment containing 625 μM KH_2PO_4 (+P), and another one containing 0 μM KH_2PO_4 (-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).

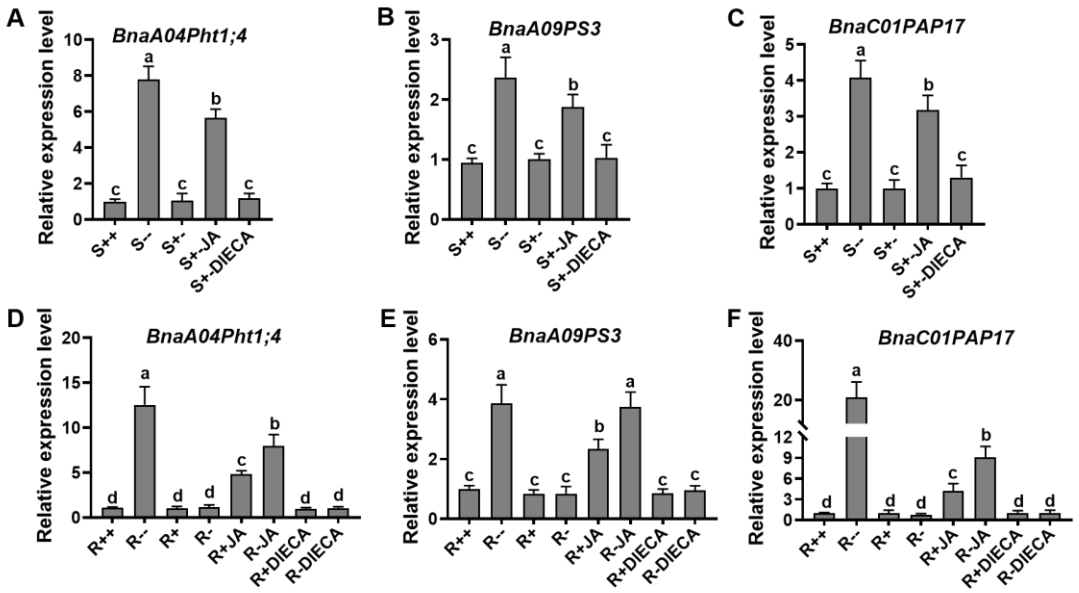


Figure 8. **Effects of JA or DIECA treatment on the expression of Pi-starvation induced genes in split-root system.** Six-day-old *B. napus* seedlings with two lateral roots were transferred to hydroponic split-root system with two compartments, one compartment containing 250 μM KH_2PO_4 (+P), and one 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.