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Root morphological adaptation and leaf lipid remobilization drive differences in phosphorus use efficiency in rapeseed seedlings

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ABSTRACT

With the objective of investigating the basis of phosphorus (P) utilization efficiency (PUE), physiological and morphological traits, two P-efficient and two P-inefficient rapeseed (*Brassica napus* L.) cultivars were compared at the seedling stage. P-efficient cultivars showed root morphological adaptation, high P uptake activity, and greater phospholipid degradation under low P stress. Improving root morphological adaptation and reducing lipid-P allocation could allow increasing PUE in rapeseed seedlings.

1. Introduction

Phosphorus (P) is a macronutrient that frequently limits plant productivity in agricultural and natural ecosystems. P functions as an essential component of macromolecular structures including DNA, RNA, ATP, and phospholipids in membranes [1]. Over 70% of the world's agricultural land suffers from P deficiency and phosphate (Pi) fertilizer is widely used to address these deficiencies [2]. Inorganic Pi fertilizers are mostly derived from rock phosphate, which is a non-renewable resource, with global reserves of Pi rock predicted to run out in as little as 40 years, but potentially last for 300–400 years [3,4].

Rapeseed (*Brasica napus* L.) is very sensitive to Pi deficiency [5,6]. Application of Pi fertilizer is therefore used to maintain the seed yield of rapeseed [7,8]. However, over-application of Pi fertilizers leads to serious P loss via run-off from farmland, resulting in off-site environment problems such as the eutrophication of waterways [9,10]. The development of P-efficient cultivars is therefore an effective way to reduce P fertilizer inputs and improve seed yield in rapeseed [11].

P acquisition efficiency is mainly dependent on plant root system architecture (RSA), root exudates and Pi transporters [12]. Plants are able to respond to P starvation by changing their RSA, including root morphology, topology, and distribution patterns. Increases in root/shoot ratio, root branching, root elongation, root topsoil foraging, and root hairs are commonly observed in P-deficient plants, while the formation of specialized roots such as cluster roots occurs in a limited number of species [15]. For example, seed yield, shoot dry weight and total P content were strongly correlated with root morphological traits at the leaf development and flowering stages, especially with the coarse root length and root surface area in the surface soil [6]. Root length of rapeseed at the seedling stage in glasshouse studies also shows a positive correlation with the seed yield in field trials [13,14]. The rhizosphere environment is heavily influenced by root exudates, which are associated with P acquisition, especially under Pi deficiency [16,17]. Under Pi deficiency, the roots of rapeseed release large amounts of organic acids, such as malic and citric acid, and phosphatases to mineralize the insoluble inorganic and organic P, and thus increase the plant-available Pi in the soil [18,19]. Pi acquisition by higher plants from the soil solution is mainly mediated by Pi/H^+ co-transporters that belong to the Pi transporter 1 (PHT1) family [20]. In *Arabidopsis*, the PHT1 family consists of nine

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members expressed predominantly in roots and in shoot organs (e.g., leaves and flowers), regulating Pi uptake and remobilization [21–23]. In rapeseed, 49 PHT1 family genes have been identified, and some of them have been functionally characterized [24]. BnPT10, BnPT11 and BnPT35 positively regulate Pi uptake [25,26]. These strategies are required to facilitate Pi acquisition of plants from the environment under low Pi availability.

While most crops exhibit effective Pi uptake capacity, they exhibit limited capacity for P translocation and redistribution [27,28]. Increasing P use efficiency (PUE) has emerged as a strategy for increasing P efficiency in crops cultivated in intensive cropping systems. A critical aspect of PUE in plants, such as maintaining rapid photosynthetic rates and high photosynthetic P utilization efficiency, requires a delicate balance of the five Pi fractions in leaves, including inorganic P (Pi), phospholipids (P_L), metabolite P (P_M), nucleic acid P (P_N), and residual P (P_R) [29–32]. In response to low Pi availability, plants can adapt to low P stress by lipid remobilization with replacing phospholipids on the plasma membrane with P-free glycolipids to reduce allocation to the P_L fraction and increase the allocation to the P_N and Pi fractions [33,34]. Phospholipids are replaced with P-free glycolipids in P-efficient rice varieties compared to P-inefficient rice varieties, suggesting there is a positive correlation between P efficiency and lipid remobilization [35]. BnNPC4 has been reported to promote the degradation of phospholipids and increase Pi fraction in plants [36]. Thus, altering P fractions in agricultural species such as the reduction in P_L fraction through lipid remobilization and the increase in P_N and Pi investments can also be a desirable strategy to improve the P efficiency in crops [34,37,38].

In this study, solution culture experiment were used to assess the contribution of root morphology, root exudates, and leaf P fractions to P acquisition and use efficiency in rapeseed by comparing the difference in root morphological and physiological traits between P-efficient and P-inefficient rapeseed cultivars.

2. Materials and methods

2.1. Plant materials and growth conditions

Zhongshuang 11 (ZS11), Zhongyouza 19 (ZYZ19), Huayouza 9 (HYZ9) and Shengguang 168 (SG168) are semi-winter rapeseed cultivars. HYZ9 and SG168 are P-efficient and ZS11 and ZYZ19 are P-inefficient cultivars [11].

Rapeseed seedlings germinated in sterile conditions on absorbent medium transplanted into black plastic containers holding 10 L Hoagland's solution [39] with 250 μmol L^{-1} Pi (NP, normal P treatment) and 5 μmol L^{-1} Pi (LP, low P treatment) for 25 days unless otherwise stated. The nutrient solutions were refreshed every three days. The plants were cultured in a growth chamber with temperature 20–22 °C, photoperiod 16 h light/ 8 h dark, light density 300–320 mol m^{-2} s^{-1}, and relative humidity 50%–70%.

2.2. Pi depletion experiments

We collected 23-day-old seedlings from the LP and NP treatments. The roots were cleaned in a 0.1 mol L^{-1} $CaSO_4$ solution and the seedlings were cultured in nutrition solution without P for 2 d and then moved to pots with 40 mL of nutrient solution with 60 μmol L^{-1} Pi. At 0, 0.5, 3, 6, 10, 20, 26, and 34 h, a 0.5 mL aliquot of solution from each pot was removed, and replaced with 0.5 mL distilled H_2O [40]. Samples were kept at 4 °C for further determination of Pi concentration as described [41].

The kinetic parameters C_{min}, V_{max} and K_m were estimated as described [40] by regression of solution Pi concentration on depletion time.

2.3. Collection and identification of organic acids in root exudates

Organic acids in root exudates were collected and measured by HPLC as described [42]. Firstly, we collected 23-day-old seedlings and transferred them to a zero P solution for 2 d. Then, the roots were washed four times with distilled H_2O and the seedlings were transferred to 40 mL flasks containing the normal nutrient solution (20 μmol L^{-1} Pi). Each flask contained three seedlings. The seedlings were grown in the plant growth chamber for 6 h. Each sample had five replications. The collected solutions were filtered through a 0.45 nm membrane, and aliquots of the exudates were plated on LB plates and incubated at 30 °C for 24 h to determine if they were infected with bacteria. The filtered root exudates were lyophilized to a volume of 2 mL (concentrated by 20 times) and stored at −80 °C for further study. A 0.5 mL filtered supernatant of each sample was injected to HPLC. Organic acids were separated on an ion exclusion column with 25 mmol L^{-1} KH_2PO_4 and methanol (v/v, 98:2) as mobile phases at a constant flow rate of 1 mL min^{-1} and at operating temperature of 25 °C. The retention time of each test signal was recorded at a wavelength of 210 nm.

2.4. Characterizing root morphology

Seedlings were cultivated in nutrient solution under contrasting P supplies for 25 days. The roots were imaged and their morphological parameters extracted with WinRHIZO software (Regent Instruments Inc., Quebec, Canada) [43].

2.5. Measuring photosynthetic parameters

Gas exchange of leaves grown in LP and NP nutrient solution for 25 d under a light-saturating photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} (90% red light, 10% blue light) was measured with a portable open infrared gas analysis system from 10:00 AM to 3:00 PM. The CO_2 concentration in the leaf chamber was set to 400 μmol mol^{-1}, and the leaf temperature was maintained at 20 °C. The relative humidity was maintained at 50%–60%, and the flow rate was set at 300 μmol s^{-1}. Gas-exchange measurements were recorded after acclimation to steady-state photosynthesis for 10 min.

2.6. Measurement of nutrient concentrations

Some seedlings were grown in LP and NP nutrient solution for 25 d. The sampled plants were divided into root, hypocotyl, cotyledon, old leaves (OL, the first leaf from below at LP or the first two leaves from below at NP), mature leaves (ML, the second leaf from below to top at LP or the second and third leaves from below to top at NP), new leaves (NL, the first leaf from top at LP or the first two leaves from top at NP). Among them, the roots and mature leaves of subsamples were used to determine P fractions concentration at 4 °C and RNA extraction at −80 °C, and the remaining samples were dried at 80 °C.

The total P of the mature leaves was divided into five P fractions following a modified P fractionation assay [44]. The five P fractions were P_L (phospholipids), P_M (soluble P-containing small metabolites such as sugar phosphates and ATP), Pi (soluble inorganic phosphate), P_N (RNA and DNA) and P_R (phosphoproteins, and other recalcitrant complexes). First, the P_L, P_M–Pi (P_M and Pi combined), P_N and P_R fractions were extracted and quantified as described [30,45]. Then, the Pi fraction was extracted and quantified separately [46].

For measurement of total P and total N concentrations, the samples were dried at 80 °C and ground to a homogeneous powder. Around 0.05 g powder were predigested in glass tubes with concentrated sulfuric acid overnight. The tubes were heated to 120 °C for 1 h

with 5–6 drops of 30% H_2O_2 every 30 min until the solution turned colorless. The colorless solution was boiled for an additional 1 h and allowed to cool. The digested solution was diluted to the appropriate concentration to measure total P concentration by molybdenum blue colorimetry method [47]. The H_2SO_4-H_2O_2 digestion solution was also used to measure the total N concentration with a continuous flow analyzer (AA3, SEAL Analytical GmbH) [48].

2.7. RNA extraction, reverse transcription, and qRT-PCR

The total RNA of the roots and mature leaves were extracted and then, cDNAs were synthesized and amplified. The *BnACTIN-2* was used as an internal control, and the fold change was expressed using the $2^{-\Delta\Delta CT}$ method [49–51]. The gene-specific primers used in the quantitative real-time PCR (qRT-PCR) analysis were listed in Table S1.

2.8. Statistical analysis

A principal component analysis was carried out using the 'factoMineR' and 'factoextra' packages in R4.2.2 to assess the relationship between genotypes and investigated traits. Structural equation modeling (SEM) was conducted by using the "lavaan" package in R. The model fit was examined with a maximum likelihood (χ^2) goodness-of-fit test, *P* values and the root mean square error of approximation (RMSEA). The "glmulti" package in R was used to investigate all possible combinations of predictor variables in the mixed-effects *meta*-regression model [52]. The relative importance value of each predictor variable was presented by the total of the Akaike weights. A threshold value of 0.5 was adopted to distinguish between major and nonessential predictors [53].

3. Results

3.1. Difference in plant growth and P uptake of contrasting P-efficient rapeseed cultivars under LP and NP treatments

Compared with normal P treatment (NP, 250 μmol L^{-1} KH_2PO_4), the low P treatment (LP, 5 μmol L^{-1} KH_2PO_4) inhibited the root and shoot growth of all rapeseed cultivars (Fig. 1A). The cotyledons became yellow and the lower true leaves became purple at LP, consistent with plant P deficiency symptoms (Fig. 1A). Under LP, the shoot and root dry weight of all cultivars were significantly reduced by 55.8%–66.9% and by 21.8%–33.6%, respectively (Fig. 1B, C). The shoot and root dry weight of P-efficient cultivars were significantly greater than those of P-inefficient cultivars under both P treatments, with the exception of the shoot dry weights (SDW) of HYZ9 and ZYZ19 under NP, which were not significantly different (Fig. 1B, C). The dry weights of hypocotyl (r^2 = 0.962, P (P treatment): $P < 0.001$, C (cultivar): $P < 0.001$), old leaves (r^2 = 0.913, P: $P < 0.001$, C: $P < 0.05$), mature leaves (r^2 = 0.942, P: $P < 0.001$, C: $P < 0.01$) and new leaves (r^2 = 0.856, P: $P < 0.001$, C: $P < 0.001$) in seedlings were significantly reduced in LP vs. NP across cultivars, with lower weights in P-inefficient cultivars. Conversely, the dry weight of cotyledon (r^2 = 0.937, P: $P < 0.001$, C: $P < 0.001$) was significantly increased in LP vs. NP, higher in P-efficient cultivars (Fig. 1D; Table S2). The allocation of dry weights in root (r^2 = 0.964, $P < 0.001$), cotyledon (r^2 = 0.965, $P < 0.001$) and new leaves (r^2 = 0.932, P: $P < 0.001$) of all cultivars increased in LP vs. NP, while that in hypocotyl (r^2 = 0.955, P: $P < 0.001$) and mature leaves (r^2 = 0.924, P: $P < 0.001$) decreased significantly (Fig. 1E; Table S2). Among them, the reduction of biomass allocation of mature (r^2 = 0.924, C: $P < 0.01$) leaves towards new leaves (r^2 = 0.924, C: $P < 0.001$) is more pronounced in P-efficient cultivars than P-inefficient cultivars (Fig. 1E; Table S2).

Leaf P and N concentrations showed an upward trend from cotyledons to new leaves, and their concentrations were lower in P-efficient cultivars compared to P-inefficient cultivars, particularly under LP conditions (Table S3). P was mainly distributed to mature leaves and hypocotyls under NP (Fig. 1F). However, Pi uptake was severely inhibited under P deficiency, which resulted in distributing more P to the root and new leaves (Fig. 1F). The P distribution ratio (r^2 = 0.483, C: $P < 0.001$) of new leaves of P-efficient cultivars were significantly larger than that of P-inefficient cultivars at LP (Fig. 1F; Table S2). All rapeseed cultivars showed reduced N and P acquisition efficiency and increased N:P ratios in LP vs. NP. P-efficient cultivars exhibited 10.8% and 12.6% higher values of N and P acquisition efficiency than P-inefficient cultivars only under low P conditions, respectively (Fig. 1G, H, I).

3.2. Leaf P fraction allocations correlate with transcript abundance of genes involved in plant Pi status

Compared with P-inefficient cultivars, P-efficient cultivars had higher Pi concentration, but lower total P concentration in mature leaves at both LP and NP (Table S4). Consequently, the P fractions in mature leaves were further investigated (Fig. 2B, C). The average concentrations of Pi, P_L, P_M, P_R and P_N were 76.7%, 64.6%, 36.1%, 35.9% and 34.5% lower in LP vs. NP across all cultivars, respectively. The Pi, P_N and P_M concentrations of the P-efficient cultivar SG168 were significantly higher than that of the two P-inefficient cultivars at both LP and NP, whilst those of the P-efficient cultivar HYZ9 were slightly, but not significantly higher than those of the inefficient cultivar ZYZ19 (Fig. 2B, C). In LP, P-efficient cultivars allocated more total P to P_M, P_N, and Pi, but less to P_L and P_R compared to P-inefficient cultivars (Fig. 2D).

The genes *BnCnPHT5;1b*, *BnPT10*, *BnC2.CRF8*, *BnNPC4* and *BnC3.SPX3* have been reported previously in relation to rapeseed responses to low Pi availability [36,54,55]. In this study, the relative abundance of *BnPT10*, *BnC2.CRF8*, *BnNPC4* and *BnC3.SPX3* in mature leaves of all cultivars were significantly induced at LP, while that of *BnCnPHT5;1b* was significantly reduced at LP (Fig. 2E–I). At LP, the expression of *BnPT10*, *BnNPC4*, and *BnC3.SPX3* in the mature leaves was significantly higher in the P-efficient cultivar SG168 than in the two P-inefficient cultivars (Fig. 2F, H, I). In contrast, the expression of BnC2.CRF8 was significantly lower in SG168 compared to the two P-inefficient cultivars (Fig. 2G). The relative abundance of these genes except for *BnC2.CRF8* was significantly positively correlated (*, $P < 0.05$) with the allocations of all P_N, Pi and P_M in mature leaves, especially at LP (Fig. S1A, B).

The N:P ratios of all cultivars were increased at LP (Table S4). Among them, the P-efficient cultivars maintained a higher N concentrations and lower total P concentrations in the mature leaves, and had significantly higher N:P ratios compared to P-inefficient cultivars (Table S4). To further clarify the effect of increased N storage per unit of P on carbon assimilation during photosynthesis, we measured the photosynthetic parameters of these rapeseed cultivars under LP and NP conditions. Compared with NP, the physiological traits of mature leaves, such as the net CO_2 assimilation rate (A_{area}), inorganic phosphorus (Pi), P and N concentrations, as well as leaf dry weight (LDW), were significantly lower under LP conditions. These traits were significantly higher in the P-efficient cultivar SG168 than in two P-inefficient cultivars, with the exception of P concentration, which was significantly lower in the former than in the latter (Table S4). The reduction of stomatal conductance (g_s) and the increases of the stomatal limiting factor (Ls) and non-stomatal limiting factor (nLs) of the mature leaves resulted in a notable decrease in A_{area} at LP compared to NP. However, under LP conditions, P-efficient cultivars exhibited 66% higher g_s, as well as 40% and 30% lower photosynthetic limiting factors (Ls and nLs), respectively, compared to P-inefficient cultivars

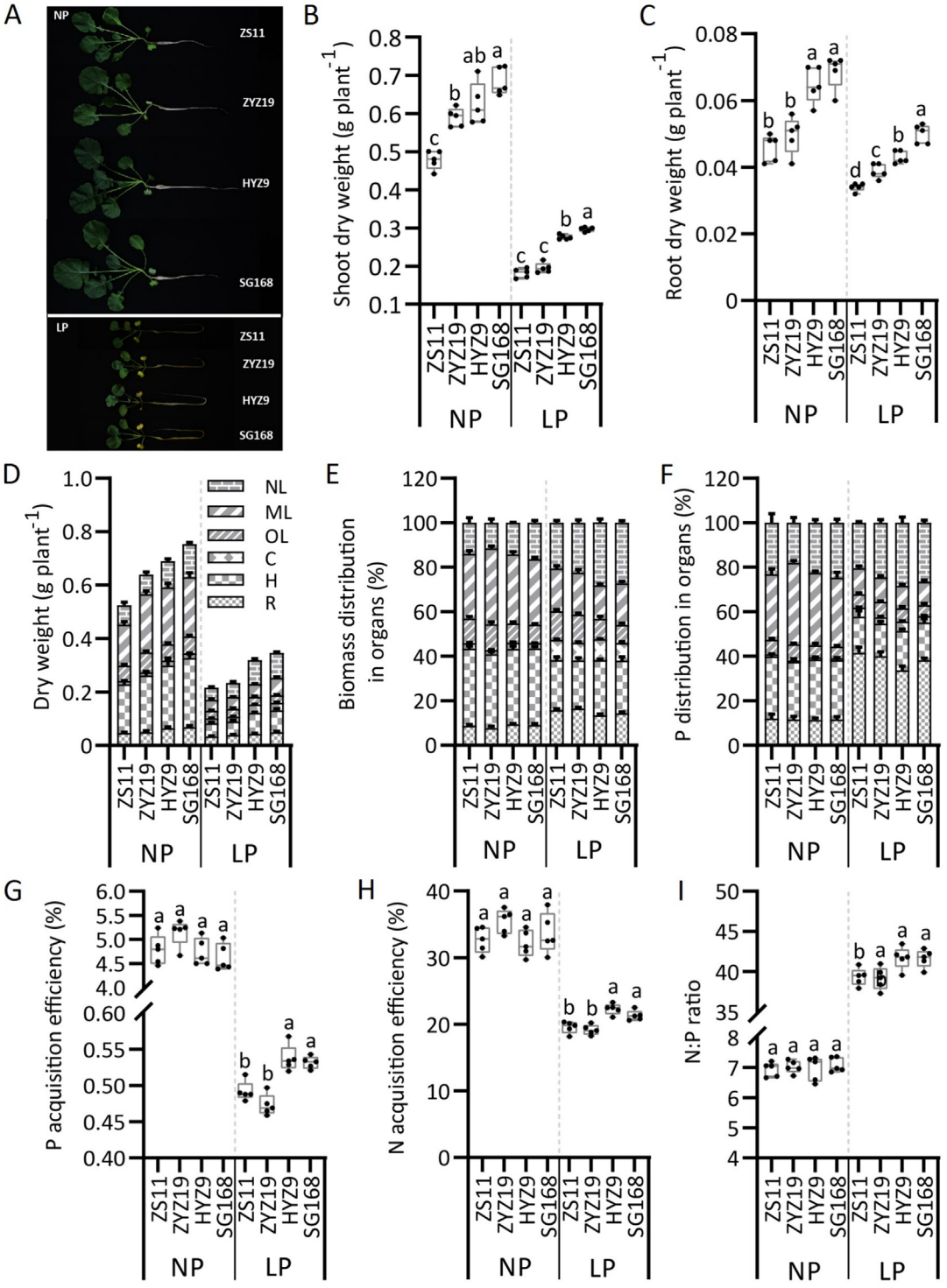

Fig. 1. The plant growth and nutrient uptake related traits of rapeseed cultivars with contrasting P use efficiencies grown under normal-P (NP) and low-P (LP) treatments. (A) Plant growth. (B) Shoot dry weight. (C) Root dry weight. (D) Dry weight in organs. (E) Biomass distribution in organs. (F) P distribution in organs. (G) P acquisition efficiency. (H) N acquisition efficiency. (I) N:P ratio. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168. R, root; H, hypocotyl; C, cotyledon; OL, old leaves; ML, mature leaves; NL, new leaves; Low-P (LP), 5 μmol L^{-1} Pi; Normal-P (NP), 250 μmol L^{-1} Pi. Values are means ± SD of five replicates. Different lowercase letters indicate significant differences between cultivars at the same Pi treatment by the Tukey's HSD test ($P < 0.05$).

(Table S4). Therefore, A_{area}, PPUE and PPUEp of P-efficient cultivars were increased by 15%, 18% and 78%, respectively, compared to P-inefficient cultivars at LP (Table S4).

There were significantly positive correlations (*, $P < 0.05$) between P fractions of Pi, P_M and P_N in mature leaves, and SDW, leaf dry weight, the N and P contents, and photosynthetic parameters (A_{area}, PPUE and PPUEp) at both P levels, however, negative correlations (*, $P < 0.05$) were found between P fractions of P_L and P_R in mature leaves and the above traits (Fig. S2A, C). At LP, P-efficient cultivars maintained higher concentrations of P in P_N, Pi, and P_M fractions compared to P-inefficient cultivars. This resulted in increased N assimilation per unit of P, leading to an improved N:P ratio in P-efficient cultivars at the seedling stage (Fig. 2B, C; Table S4).

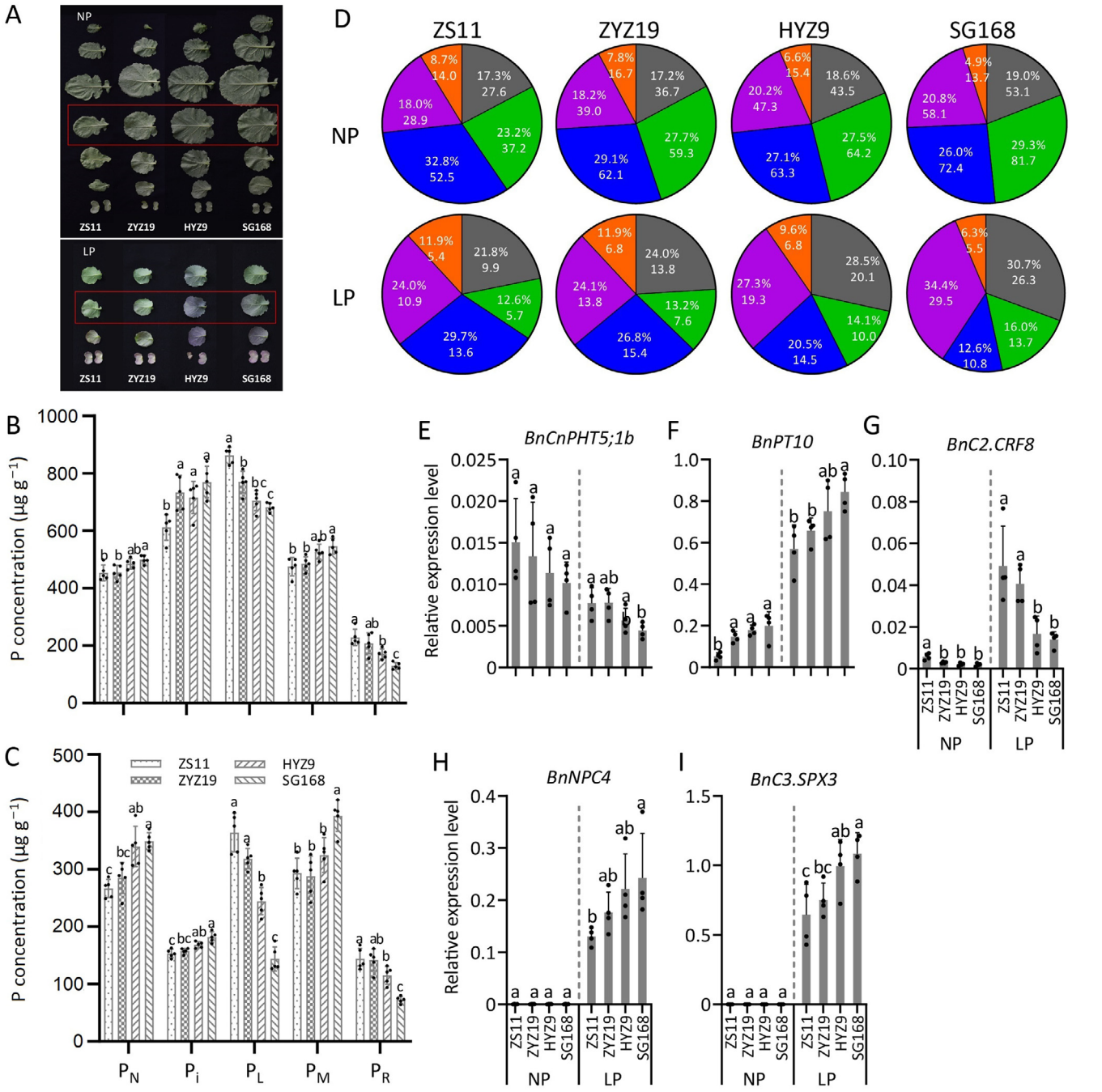

Fig. 2. P fractions and the expression of genes involved in P acquisition and transport in mature leaves (red box) of rapeseed cultivars with contrasting P use efficiencies grown under normal-P (NP) and low-P (LP) treatments. (A) henotype of leaves of rapeseed at LP and NP; (B, C) the concentration of five P fractions in the selected leaves of rapeseed at NP and LP, respectively; (D) P fraction allocation of nucleic acid-P (P_N), inorganic-P (Pi), lipid-P (P_L), metabolite-P (P_M), residual-P (P_R) (percentage of total P allocated to each fraction) in the leaves of rapeseed; the expression of genes of *BnCnPHT5;1b* (E), *BnPT10* (F), *BnC2.CRF8* (G), *BnNPC4* (H) and *BnC3.SPX3* (I) in the leaves of rapeseed at LP and NP. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168. Low-P, 5 μmol L^{-1} Pi; Normal-P, 250 μmol L^{-1} Pi. Values are means ± SD of five replicates. Different lowercase letters indicate significant differences between cultivars at the same Pi treatment and the same P fraction by the Tukey's HSD test ($P < 0.05$).

3.3. Higher allocation of P to P_N, Pi, and P_M and lower allocations to P_L, P_R are associated with P-efficient cultivars, supporting PPUE and plant growth

Principal component analysis (PCA) of the investigated traits revealed a clear clustering of genotypes into two groups (Fig. 3A, D). P-inefficient cultivars were separated from P-efficient cultivars along the first principal component (PCA1) that explained 76.8% and 59.5% genotypic variations at LP and NP, respectively (Fig. 3A, D). This was primarily due to the differences in the allocation between P_L and P_N, PPUEp, PPUEb and PPUE at LP (Fig. 3E). P-inefficient cultivars had lower P_N allocation, PPUEp, PPUEb and PPUE, and higher P_L allocation, while P-efficient cultivars were the opposite (Figs. 2D, 3B; Table S4). The allocation of P_L and P_R,

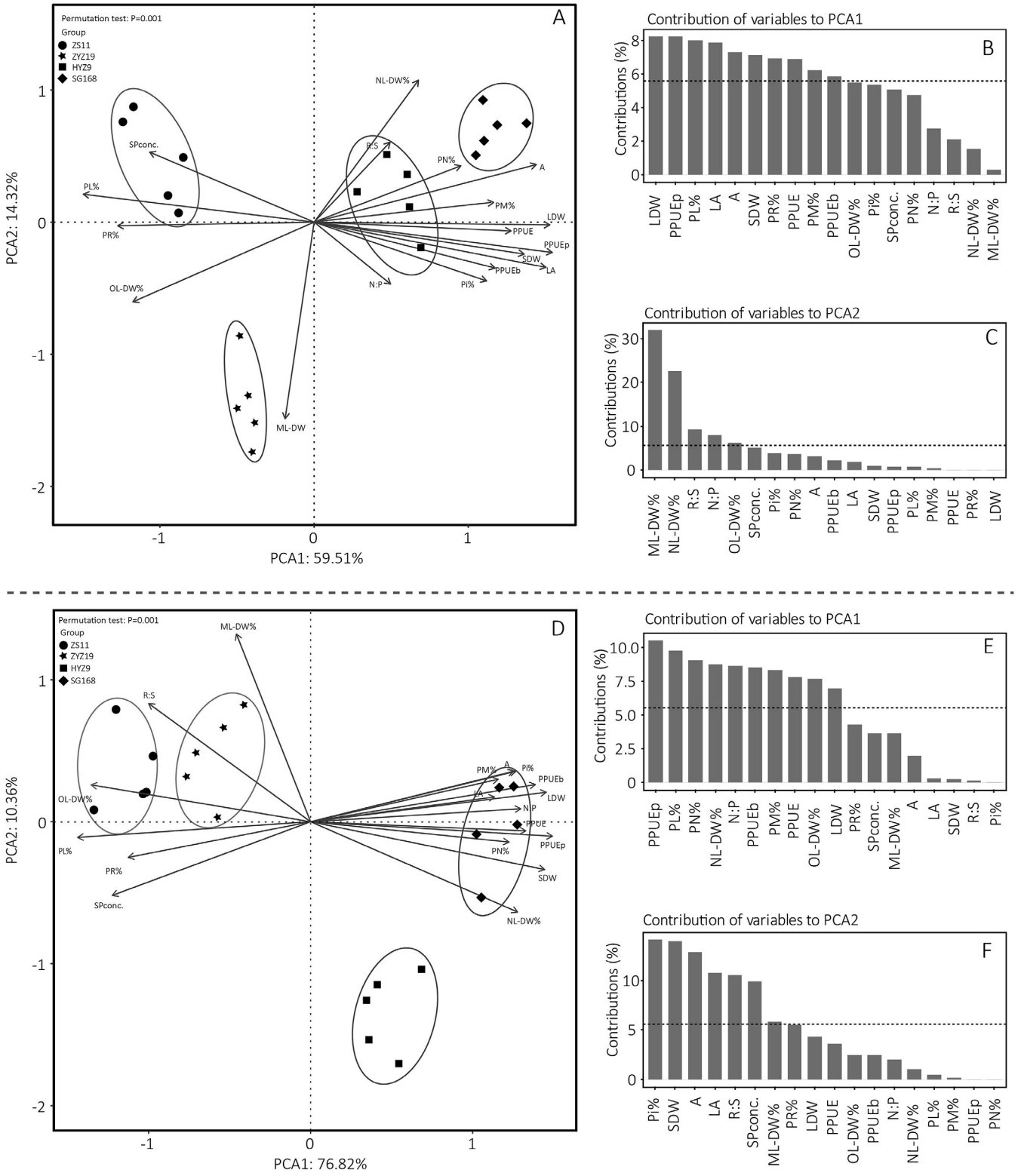

Fig. 3. Scatter plot of principal component analysis (PCA) of the physiological traits and photosynthesis parameters of mature leaves, and plant growth and nutrients uptake related traits at normal-P (A) and low-P (B) treatments, and the contribution of these traits to PCA1 (B, E) and PCA2 (C, F) at normal-P (B, C) and low-P (E, F) treatments. The traits for PCA included P fraction allocation of nucleic acid-P (P_N), inorganic-P (Pi), lipid-P (P_L), metabolite-P (P_M), residual-P (P_R) (percentage of total P allocated to each fraction), leaf area (LA), rate of leaf net CO_2 assimilation (A_{area}), photosynthetic P-use efficiency (PPUE), biomass P-use efficiency (PPUEb), physiological P-use efficiency (PPUEp), N: P ratio in leaf (N:P), dry weight allocation of old leaves (OL-DW%), mature leaves (ML-DW%) and new leaves (NL-DW%) (percentage of shoot dry weight allocated to each leaf), shoot dry weight and root: shoot ratio (R:S).

LDW, leaf area, A_{area}, SDW, PPUEp, PPUEb and PPUE were the main contributors to PCA1 at NP. However, all factors except for the allocation of PL and PR were lower in P-inefficient cultivars than in P-efficient cultivars (Figs. 2D, 3B; Table S4). The second principal component (PCA2) allows for further segregation of genotypes which explained 10.4% and 14.3% at LP and NP, respectively (Fig. 3A, D). The SDW, A_{area}, leaf area, shoot P concentration and root:shoot ratio were primary contributions to PCA2 at LP (Fig. 3F). However, the dry weight allocation of mature leaves (ML-DW%) and new leaves (NL-DW%) contributed to PCA2 at NP (Fig. 3C). All the P fractions contributed 31.0% and 31.8% of the genotypic variations at LP and NP, respectively (Fig. 3B, C, E, F).

The dry weight of the mature leaves of rapeseed were associated with genotypes and their P fractions, N:P ratio, leaf area, A_{area} and PPUE at both LP and NP (Fig. S3). A random forest model indicated that genotypes, the allocation of P_N and P_L, and N:P ratio in mature leaves explained the majority of the effect of Pi supply on LDW at LP, while genotypes explained the most at NP (Fig. S2B, D). The greater dry weight of mature leaves in P-efficient cultivars compared to P-inefficient cultivars at LP condition can be attributed to the allocation of Pi to other P fractions, particularly the balance between P_N (which is higher in P-efficient cultivars) and P_L (which is lower in P-efficient cultivars) at the seedling stage.

3.4. Root morphological adaptations are positively associated with P-efficient cultivars and contribute to greater P uptake

There were significant increases in the root morphological traits of all cultivars, such as total root length (TRL), root surface area (RSA), total root volume (RV), and specific root length (SRL), at LP compared to at NP, respectively (Table S5). Moreover, the above traits of the P-efficient cultivar SG168 were significantly higher than those of the two P-inefficient cultivars at both NP and LP, while they were slightly, but not significantly, higher in the P-efficient cultivar HYZ9 than in the two inefficient cultivars (Table S5). P efficiency (PE) was significantly decreased at LP, whereas those traits were higher in the P-efficient cultivar SG168 than in the two P-inefficient cultivars (Table S5). The secretion

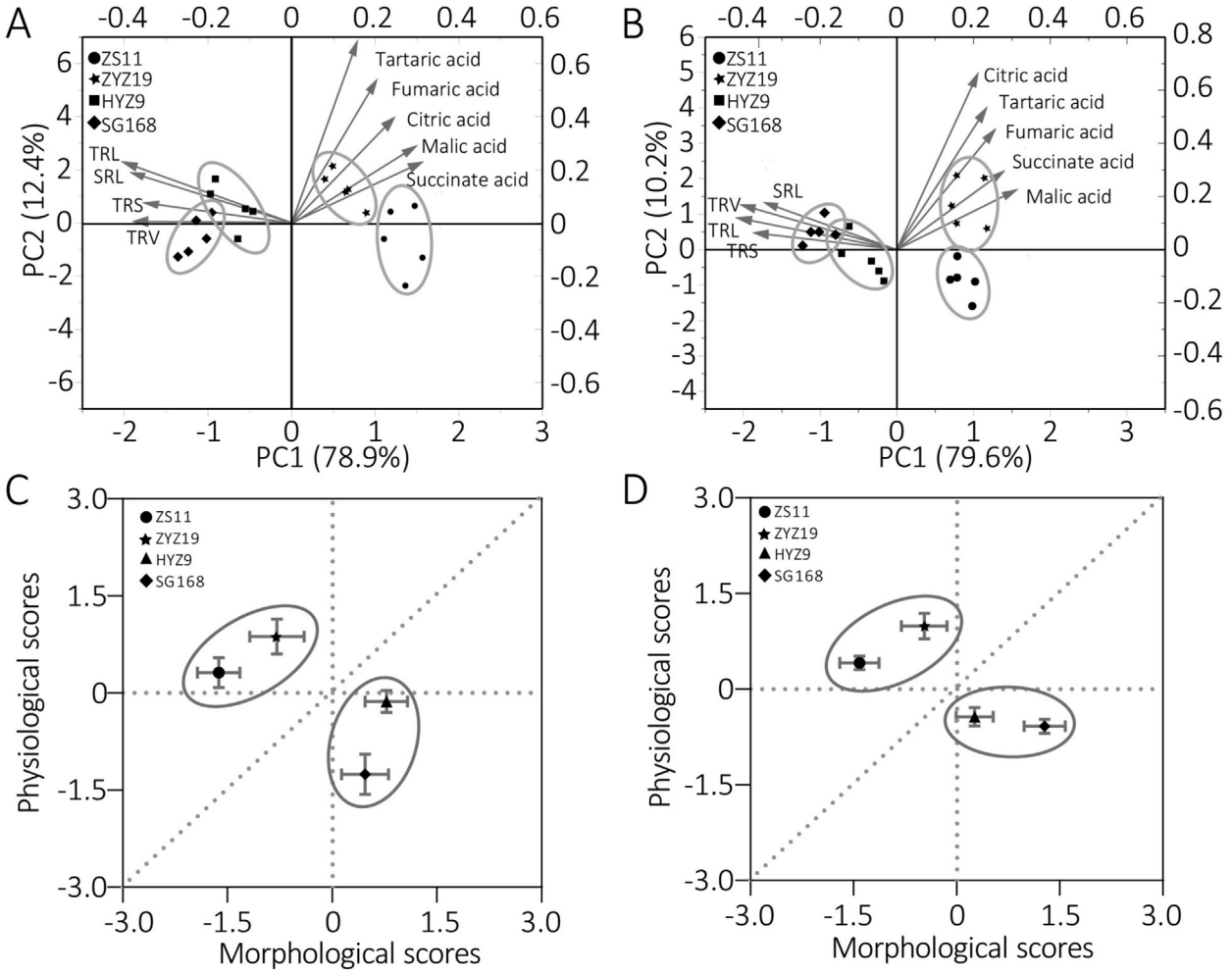

Fig. 4. Principal component analysis (PCA) (A, B) and factor scores (C, D) of four root morphological traits and five root physiological traits of rapeseed cultivars with contrasting P use efficiencies in biplots under normal-P (A, C) and low-P (B, D) treatments. These root traits included four root morphological traits and five root physiological traits. Other traits included P fraction allocations (P_N%, nucleic acid-P; Pi%, inorganic-P; P_L%, lipid-P; P_M%, metabolite-P; P_R%, residual-P), SDW (shoot dry weight), LDW (leaf dry weight), LA (leaf area), N:P (N: P ratio in leaf), A_{area} (rate of leaf net CO_2 assimilation), PPUE (photosynthetic P-use efficiency), PPUEb (biomass P-use efficiency), PPUEp (physiological P-use efficiency), ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168. Low-P, 5 μmol L^{-1} Pi; Normal-P, 250 μmol L^{-1} Pi. Values are means ± SD of five replicates.

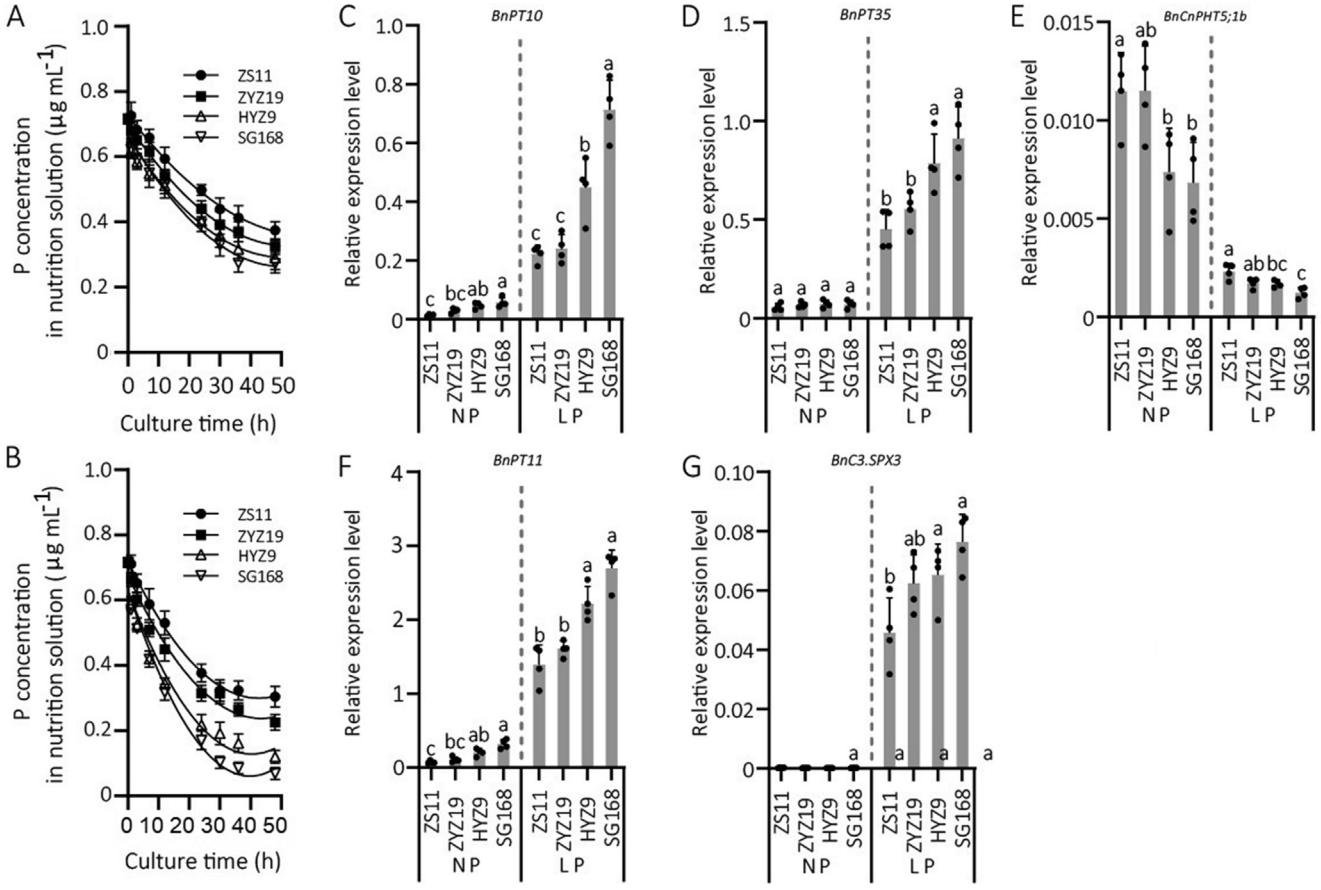

Fig. 5. Phosphate (Pi) uptake dynamic curves and the expression of genes involved in Pi acquisition and transport in roots of rapeseed cultivars with contrasting P use efficiencies grown under normal-P (NP) and low-P (LP) treatments. P uptake dynamics curves under NP (A) and LP (B) treatments, the roots of 23-day-old seedlings from LP and NP were cleaned in a $CaSO_4$ solution (0.1 mol L^{-1}) before being transferred to nutrition solution depleted of phosphate for two days. After that, all seedlings were moved to pots with 40 mL of nutrient solution in 60 μmol L^{-1} Pi (inorganic P). At 0 h, 0.5 h, 3 h, 6 h, 10 h, 20 h, 26 h, and 34 h, measuring Pi concentration in nutrient solution each pot. Values are means ± SD of five replicates. The solid line represents the line of best fit, derived from a polynomial regression model. (C) *BnPT10*; (D) *BnPT35*; (E) *BnCnPHT5;1b*; (F) *BnPT11*; (G) *BnC3.SPX3*; ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168. Low-P, 5 μmol L^{-1} Pi; Normal-P, 250 μmol L^{-1} Pi. Values are means ± SD of four replicates. Different lowercase letters indicate significant differences between cultivars at the same Pi treatment by the Tukey's HSD test ($P < 0.05$).

activity of root exudates (organic acids, such as tartaric acid, fumaric acid, malic acid, citric acid and succinate acid) was enhanced at LP, and they were significantly lower in P-efficient cultivars than in P-inefficient cultivars at both P levels (Table S5). Biplots from PCA analysis clearly showed the different effects of Pi supply on root morphological traits and physiological traits of rapeseed cultivars with contrasting P use efficiencies. P-inefficient cultivars and P-efficient cultivars were clearly distinguished in PCA1 that explained the 79.6% (LP) and 78.9% (NP) of genotypic variations (Fig. 4A, B). P-efficient cultivars primarily exhibited root morphological adaptations, while P-inefficient cultivars were more associated with root physiological adaptations (Fig. 4C, D).

To understand the impact of these root morphological and physiological differences on the P acquisition ability of different cultivars, P uptake by roots of all cultivars was quantified (Fig. 5A, B). P depletion in the nutrient solution was more rapid in P-efficient cultivars than in P-inefficient cultivars at both P levels, especially at LP (Fig. 5A, B). At LP, C_{min} and K_m were reduced, and I_{max} was increased in all cultivars. Compared to P-inefficient cultivars, P-efficient cultivars had lower C_{min} and K_m, but higher I_{max}, both at LP and NP (Table S6). The relative abundance of genes of *BnPT10*, *BnPT11*, *BnPT35* and *BnC3.SPX3* in all rapeseed cultivars were strongly induced, while that of *BnCnPHT5;1b* was strongly inhibited at LP (Fig. 5C–G). Compared to P-inefficient cultivars, the expression levels of *BnPT10*, *BnPT11*, *BnPT35* and *BnC3.SPX3* were significantly higher, while the expression of *BnCnPHT5;1b* was lower in P-efficient cultivars, at both P levels (Fig. 5C–G).

3.5. Difference in the direct and indirect effects of P uptake on shoot dry weight between P-efficient cultivars and P-inefficient cultivars

Root morphological traits, root exudate, P uptake, leaf photosynthesis and PUE had significant direct and indirect effects on SDW at LP and NP (Fig. 6A, C). The variation of SDW was explained 97% (LP) and 99% (NP) by these variables in the SEM, respectively (Fig. 6A, C). Among them, the P uptake had the highest effect size on SDW (0.95 and 0.88) at LP and HP, respectively (Fig. 6B, D). The effects of root morphological traits, P uptake, the leaf photosynthesis and P use efficiency on SDW were positive, while root exudate had a primarily negative effect on SDW at both LP and NP (Fig. 6B, D). These results confirmed that the increase in SDW in rapeseed seedling was primarily driven by the enhanced P uptake, which was largely dependent on the improvements in root morphological traits. P uptake could also be the best explained by BnC3.SPX3, BnPT10, and RSA

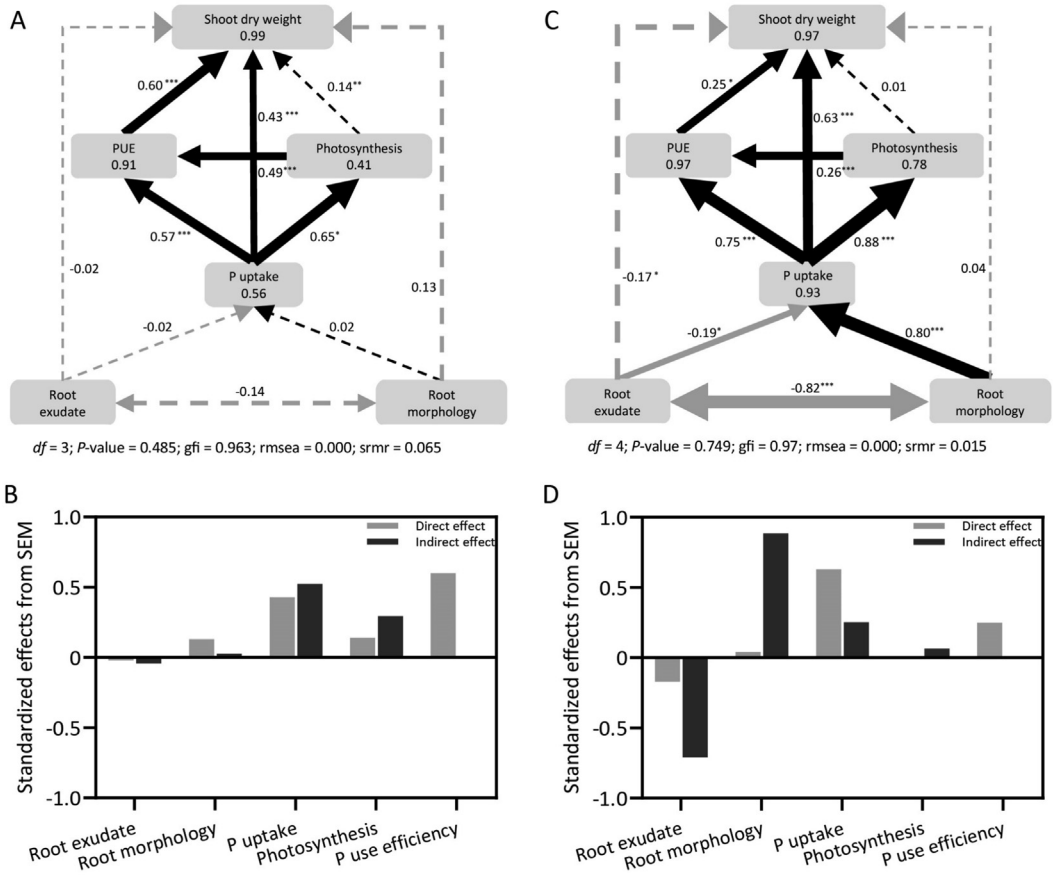

Fig. 6. Structural equation model (SEM) showing the direct and indirect effects of root morphology, root exudates, P uptake, photosynthesis and P use efficiency (PUE) on shoot dry weight (SDW) at the normal-P (A) and low-P (C) treatments, and the standardized direct and indirect effects derived from the SEM at the normal-P (B) and low-P (D) treatments. Low-P, 5 μmol L^{-1} Pi; Normal-P, 250 μmol L^{-1} Pi. The numbers in boxes show the explained percentages of the variance by the predictor variables. The solid and dashed arrows indicate significant and non-significant relationships, respectively. The black and grey arrows indicate positive and negative relationships, respectively. The numbers above the arrows denote the standardized path coefficients (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$), the magnitudes of which are proportional to the thickness of the arrows.

at both P levels (Fig. S4). P-efficient cultivars could effectively balance root morphological and physiological traits, increase P uptake activity, promote P uptake, thus improve PUE and SDW to adapt to P deficiency at the seedling stage.

4. Discussion

4.1. The correlation between the root morphological and physiological adaptations and P uptake capacity

The root morphological and biochemical traits are important for P acquisition, plant growth, and crop yield [56–59]. These traits show a high plasticity in the adaptive response of rapeseed to P deficiency [6,60,61]. In this study, the root morphological traits and root exudates of rapeseed were increased at LP (Table S2). P-efficient cultivars had a 10.8% higher P acquisition efficiency dominated by effective balance of root morphological and physiological adaptations compared with P-inefficient cultivars (Figs. 1G, 4C, D). P-inefficient cultivars seedlings had the lower values in root morphological traits including TRL, RSA, RV, SRL and PE and higher values in root exudates including tartaric acid, fumaric acid, malic acid, citric acid and succinate acid, which are typical genotypes with the root physiological adaptations, while P-efficient cultivars seedlings had the higher values in above root morphological traits and lower values in above root exudates and were dominated as the root morphological adaptations in solution culture experiments (Table S2; Fig. 4C, D). Wen et al. [62] also reports that different herbaceous plants have different functional characteristics of P uptake.

Field trials indicated that seed yield and yield-related traits of rapeseed cultivars were significantly positively correlated with their root morphology traits including TRL, RSA, TRV and RAD at the growth stage, particularly during the seedling stage [11]. Our previous field trials also showed that seed yield and P uptake of rapeseed were significantly and positively correlated with root morphological adaptation traits, and weakly correlated with root exudates at the seedling stage [6]. These are consistent with the results of this study. The reason is that the distance the root exudates affected in soil range in less than a few millimeters from the root surface [60,63]. P is very difficult to move in the soil, with the extremely slow diffusion and less than 2 mm of diffusion distance in the soil, with P uptake by the root hair is less than 4 mm distance from root surface [64]. In our previous study, the P-efficient cultivar had lower K_m and C_{min} values and higher I_{max} and developed longer and denser lateral root hair with greater number of root tips and branches under low-P stress, which resulted in a better developed root system and more efficient uptake of P [65]. Thus, in this study, we did not collect the root hair data. Therefore, root system architecture has a key role in P uptake whether in solution culture or in the field trials [66].

The improved P acquisition efficiency in P-efficient cultivar seedlings at LP was primarily not only driven by their enhanced root morphological traits, but also the upregulation of Pi trans-

porter genes (Table S2; Figs. 1G, 5C–G). The expression of *BnPT10*, *BnPT11* and *BnPT35* were induced more in P-efficient cultivars than P-inefficient cultivars (Fig. 5C, D, F). It has been reported that *BnPT10*, *BnPT11* and *BnPT35* are induced by P deficiency, and positively regulate P uptake [24,54]. The expression of *BnC3.SPX3* was also induced more in P-efficient cultivars than in P-inefficient cultivars at LP (Fig. 5G). The expression of *BnSPX3* was significantly induced by P deficiency to adapt to subsequent fluctuations in external environmental Pi to maintain Pi homeostasis in plants, and to prevent from P toxicity on plants when external environmental P is suddenly increased [26]. We speculate that P-efficient cultivars seedlings are more capable of maintaining plant Pi homeostasis than P-inefficient cultivars in response to external Pi fluctuations. The expression of *BnPHT5;1b* was lower and Pi concentrations was higher in P-efficient cultivars than P-inefficient cultivars (Figs. 2, 5E). Genotypes with lower expression of *BnPHT5;1b* under P deficiency can maintain higher Pi concentrations in the cytoplasm [67].

4.2. Phospholipid remobilization to improve P use efficiency

In this study, compared to P-inefficient cultivars, P-efficient cultivars had higher investments in P_N, Pi, and P_M, lower investments in P_L, P_R, which significantly increased the N:P ratio and ultimately maintained relatively higher photosynthetic rates to improve PUE at the seedling stage (Fig. 2D; Table S4). Lipid remobilization involves the replacement of P_L with glycolipids that do not contain P, which makes total P distributed more to other P fractions involved in the plant physiological and metabolic processes, which is important in improving the tolerance to low P stress [32,37,68]. Lipid remobilization might also play a major role in reducing the P_L fraction allocation in rapeseed under severe P deficiency. In this study, there were significant correlations between PPUE and the concentration and allocation ratio of all P fractions in rapeseed (Fig. S2A, C). Among them, there was a significant negative correlation between PPUE and the concentration and allocation ratio of P_L and P_R (Fig. S2A, C). Additionally, the concentration and allocation ratio of P_L and P_R were lower in P-efficient cultivars compared with P-inefficient cultivars, and the A_{area} and PPUE of P-efficient cultivars was higher than that of P-inefficient cultivars (Fig. 2B–D; Table S4). BnNPC4 has been reported to involve in phosphosphingolipid hydrolysis and remobilization in rapeseed during Pi starvation [36]. In this study, the expression of *BnNPC4* was induced in mature leaves at LP, and that was higher in P-efficient cultivars than in P-inefficient cultivars (Fig. 2H).

The investment of P_L fraction was decreased to improve the investments of P_N and P_M fractions at LP, which resulted in enhancing photosynthetic rates and PUE in P-efficient cultivars than P-inefficient cultivars (Fig. 2D; Table S4). Most lipids on membranes in chloroplasts do not contain P (e.g., galactosyl diacylglycerol, dilauryl diacylglycerol, etc.), although a little P-containing substances (e.g., phosphatidylglycerol) are essential for electron transport in photosystem II [68,69]. Thus, the P_L on membranes outside chloroplasts can be at least partially replaced by the non-P-containing glycolipids. It has also been shown that replacing the P_L with P-free glycolipids does not impair photosynthetic capacity [33,37]. However, if the P_L concentration in chloroplast is significantly reduced, photosynthesis will be theoretically affected [70]. This is consistent with the results of the present study that the reducing P_L investment enhances the photosynthetic capacity of P-efficient cultivars more than P-inefficient cultivars in response to the low P stress (Fig. 2D; Table S4).

The P_N is mainly derived from RNA, which is not replaced by P-free material [71]. Decreasing the P_N allocation ratio reduces the protein synthesis and turnover, significantly inhibiting photosynthesis [34,37]. In this study, increasing the P_N allocation ratio effec-

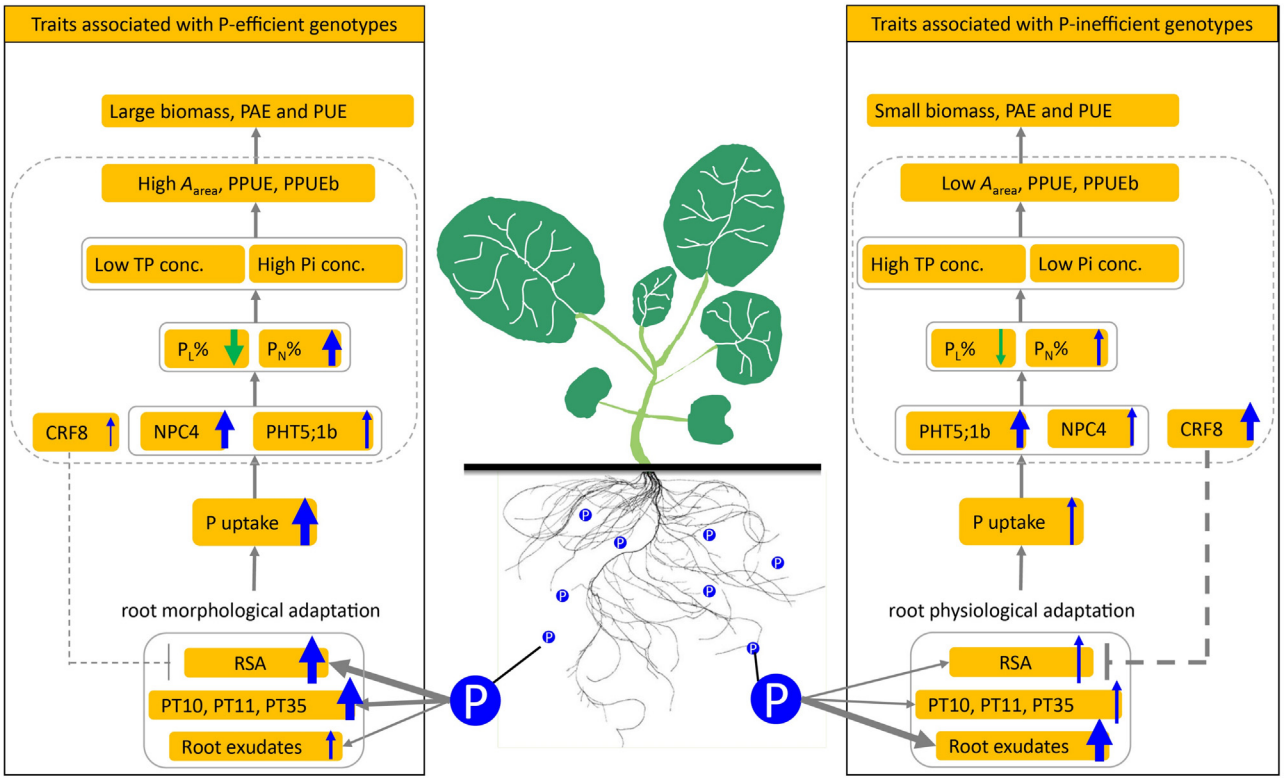

Fig. 7. The schematic illustrating key findings of this study. The box shows the key responses in leaf and root traits of P-efficient and P-inefficient cultivars under low-P treatment. The blue arrows indicate the phenotype enhanced at low-P treatment. The green arrows represent the phenotype reduced at low-P treatment. The thickness of arrows represents the strength of the trait of rapeseed in response to low P stress.

tively enhanced the photosynthesis, and the P_N allocation ratio was higher in P-efficient cultivars than P-inefficient cultivars at both LP and NP (Fig. 2D; Table S4). Under the low P conditions, the Pi concentration is rapidly reduced in cells of most plant, negatively affecting photosynthesis [28]. BnPHT5;1b and BnSPX3 play important roles in the cellular Pi homeostasis in rapeseed [54,67]. The present study found that the decrease of Pi concentration significantly inhibited photosynthesis at LP, while the Pi concentration of P-efficient cultivars was higher than that of P-inefficient cultivars (Fig. 2B, C). Furthermore, the concentrations of P_M and P_R in the cell were relatively stable and were rarely affected by fluctuation in P concentration in the external environment (Fig. 2B, C). Similarly, the allocations of P_M and P_R were significantly negatively correlated with A_{area} and PPUE (Fig. S2A, C). Altogether, reducing Pi, P_M and P_R severely inhibit photosynthesis [37]. Therefore, reducing P_L allocation is an effective way to improve the P use efficiency while maintaining rapid photosynthesis and promoting rapeseed seedling growth at LP.

P-efficient rapeseed cultivars enhanced P acquisition efficiency under LP conditions by effectively balancing root morphological and physiological adaptations, and inducing the expression of Pi transporter, compared to P-inefficient cultivars at the seedling stage (Fig. 7). The reduction of P_L allocation not only increased P_N allocation but also maintained the rapid photosynthetic rates of rapeseed, and effectively improved P use efficiency in response to low P stress (Fig. 7).

CRediT authorship contribution statement

Bingbing Zhang: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Xinxin Zhu:** Writing – review & editing, Investigation. **Pan Yuan:** Writing – review & editing. **Bei Han:** Writing – review & editing. **Tao Wu:** Investigation. **Ismail Din:** Writing – review & editing, Formal analysis. **Chuang Wang:** Writing – review & editing. **John P. Hammond:** Writing – review & editing. **Sheliang Wang:** Writing – review & editing. **Guangda Ding:** Writing – review & editing. **Hongmei Cai:** Writing – review & editing. **Zhuqing Zhao:** Writing – review & editing. **Fangshen Xu:** Writing – review & editing. **Lei Shi:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2024.12.022.

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