

*Trade-offs between root-secreted acid phosphatase and root morphology traits, and their contribution to phosphorus acquisition in Brassica napus*

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Accepted Version

Li, H., Wang, C. ORCID: <https://orcid.org/0000-0002-7663-7433>, Zhang, B., Liu, H., Hammond, J. P. ORCID: <https://orcid.org/0000-0002-6241-3551>, Wang, X., Ding, G., Cai, H. ORCID: <https://orcid.org/0000-0002-1430-5126>, Wang, S., Xu, F. ORCID: <https://orcid.org/0000-0003-3564-1644> and Shi, L. ORCID: <https://orcid.org/0000-0002-5312-8521> (2024) Trade-offs between root-secreted acid phosphatase and root morphology traits, and their contribution to phosphorus acquisition in Brassica napus. Physiologia Plantarum, 176 (2). e14247. ISSN 0031-9317 doi: 10.1111/ppl.14247 Available at <https://centaur.reading.ac.uk/115959/>

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Published version at: <http://dx.doi.org/10.1111/ppl.14247>

To link to this article DOI: <http://dx.doi.org/10.1111/ppl.14247>

Publisher: Wiley

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**Trade-offs between root-secreted acid phosphatase and root morphology traits, and their contribution to phosphorus acquisition in *Brassica napus***

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Edited by K-J Dietz

**Abstract**

Oilseed rape (*Brassica napus*) is one of the most important oil crops in the world and shows sensitivity to low phosphorus (P) availability. In many soils, organic P (Po) is the main component of the soil P pool. Po must be mineralised to Pi through phosphatases, and then taken up by plants. However, the relationship between root-secreted acid phosphatases (APase) and root morphology traits, two important P-acquisition strategies in response to P deficiency, is unclear among *B. napus* genotypes. This study aimed to understand their relationship and how they affect P acquisition, which is crucial for the sustainable utilisation of agricultural P resources. This study showed significant genotypic variations in root-secreted APase activity per unit root fresh weight (SAP) and total root-secreted APase activity per plant (total SAP) among 350 *B. napus* genotypes. Seed yield was positively correlated with total SAP but not significantly correlated with SAP. Six root traits of 18 *B. napus* genotypes with contrasting root biomass were compared under normal Pi, low Pi and Po. Genotypes with longer total root length (TRL) reduced SAP, but those with shorter TRL increased SAP under P deficiency. Additionally, TRL was important in P-acquisition under three P treatments, and

total SAP was also important in P-acquisition under Po treatment. In conclusion, trade-offs existed between the two P-acquisition strategies among *B. napus* genotypes under P-deficient conditions. Total SAP was an important root trait under Po conditions. These results might help to breed *B. napus* with greater P-acquisition ability under low P availability conditions.

## **1-Introduction**

Phosphorus (P) is the second most essential macronutrient for plant growth, accounting for about 0.2% of a plant's dry weight (Schachtman et al., 1998). Plants acquire P from the soil in the form of inorganic P (Pi, phosphate). However, Pi can form strong ionic interactions with metal cations (e.g.,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$  and  $\text{Fe}^{3+}$ ) present in the soil, resulting in the formation of unavailable forms (Chen & Liao, 2016). In comparison, organic P (Po) is relatively abundant and around 35-65% of total P in soil, sometimes even as high as 90% in organically managed agricultural soils (Shen et al., 2011; Zhang et al., 2023). However, Po must be mineralised to Pi through the phosphatases (e.g., phosphatases, diesterases, phytases), and then taken up by plants (Richardson et al., 2000; Tarafdar & Claassen, 1988; Zhang et al., 2023). Therefore, the amount of available Pi in the soil is often inadequate to meet plant requirements. In many natural ecosystems and arable soils, the amount of available Pi is below the concentration required for optimal growth of plants, which greatly restrains agricultural production worldwide (Neset & Cordell, 2012; Vance, 2003).

In response to P deficiency, plant roots have evolved two main strategies to enhance soil P acquisition (Wang et al., 2018). One is to enhance Pi uptake by exploiting a greater volume of soil through changing root system architecture and/or fostering symbiotic relationships with arbuscular mycorrhizal fungi (AMF) (Liu, 2021; Lynch, 2019; Poirier et al., 2022; Sawers et al., 2017; Smith et al., 2011; White et al., 2013). The other is to increase the mobilization of both Po and Pi components from the soil by increasing the release of root exudates into the rhizosphere, including hydrolytic enzymes (e.g., APase, RNase and phytase), carboxylates and protons/hydroxides (Deng et al., 2020; Poirier et al., 2022; Wang et al., 2018; Wang & Lambers, 2019; Wen et al., 2021). All these adjustments in root functional traits could contribute to more plant P acquisition, but the costs of energy and resources may limit a plant's ability to enhance all these strategies simultaneously. Because root functional traits are able to develop by investing carbon (Nguyen, 2003; Jones et al., 2009), and based on the concept of the trait economics spectrum, there is always an equilibrium between acquiring and utilizing resources, with a specific focus on the trade-offs between features such as root functional traits (Honvault et al., 2020; Reich & Cornelissen, 2014; Wen et al., 2019). Consequently, different plant species and even different genotypes of a particular species can exhibit contrasting abilities in root functional properties.

Large interspecific variations in root functional traits associated with P acquisition have been demonstrated in pasture legumes, grass species, crops, and tree species (Becquer et al., 2021; Honvault et al., 2020; Kidd et al., 2015; Lyu et al., 2016; Ushio et al., 2015; Waddell et al., 2016; Wen et al., 2019). Species with thinner roots depend more on their root morphology to enhance P acquisition by increasing soil exploration, suggesting a resource-acquisitive strategy; conversely, species with thicker roots tend to rely on greater colonization by AMF, which compensates for a low root absorptive surface or depend more root exudates (e.g., carboxylate exudation) to mine sparingly soluble P in the rhizosphere (Kidd et al., 2015; Wen et al., 2019; Wen et al., 2021). However, there was a negative correlation between root diameter and root APase activity among tree species (Han et al., 2021; Ushio et al., 2015). Genotype variations in root functional traits are also reported, but there is a different relationship between root morphology traits and rhizosphere exudates in response to low P stress among different species (Wang et al., 2021; When et al., 2020). The rhizosphere APase activity is negatively correlated with root length but positively correlated with root diameter among wheat (*Triticum aestivum*) genotypes (Wang et al., 2021). However, root secrete carboxylate is negatively correlated with root diameter but positively correlated with root length among chickpea (*Cicer arietinum*) genotypes (When et al., 2020). These indicate that different species or different genotypes in the same species have different interactions between root functional traits (e.g., root morphology, root exudates, AMF). Much progress has been made in interactions of root morphology and mycorrhizal symbioses, but the roles of root exudates, especially root-secreted APases have been largely ignored (Wen et al., 2019). APases (E.C. 3.1.3.2) catalyse the cleavage of a broad array of phosphomonoesters (Po sources) with optimal activity below pH 7.0 to release Pi (Bhadouria & Giri, 2022; Vincent et al., 1992; Wang & Liu, 2018). It is a common response to low soil P availability to increase the activity of root-secreted APases in most crops, such as oilseed rape (*Brassica napus*), wheat, rice (*Oryza sativa*), soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) (Bhadouria et al., 2023; Ciereszko et al., 2011; Du et al., 2022; Duan et al., 2020; Liang et al., 2010; Wu et al., 2018). Pi is released from Po by root-secreted APases in the rhizosphere, which is closely related to the efficiency of P acquisition in crops (Deng et al., 2020; Duff et al., 1994; Lu et al., 2016; Mehra et al., 2017).

*Brassica napus* is one of the most important and profitable oil crops in the world (Angelovič et al., 2013), but it is sensitive to low soil P availability (Ding et al., 2012; Duan et al., 2020; Shi et al., 2013; Yuan et al., 2016). Root morphology traits of *B. napus* in response to low P availability have been well studied (Duan et al., 2021; Li et al., 2022; Wang et al., 2017; Xu et al., 2022; Xu et al., 2023; Yang et al., 2010), and wide genotype variations in root morphology traits and seed yield (SY) are reported among a large association panel of *B. napus* with diverse genetic backgrounds under P-deficient conditions (Wang et al., 2017; Liu et al., 2023). Recently, Duan et al. (2020) report that rhizosphere APase activity of *B. napus* increased from the leaf development stage to pod development in soils with low P availability, especially during the leaf development stage. This suggests that the root-secreted APase of *B. napus*

should also be important for adaptation to low soil P availability. However, the genotype variations of root-secreted APase in response to low P availability and their effect on seed yield in *B. napus* are still unknown. Additionally, *B. napus* is a nonmycorrhizal species with a thinner root; whether there is a coordinated or trade-offs in P acquisition between root morphology and secreted APase under different P conditions is still unclear.

In this study, the root biomass, root-secreted APase activity per unit root fresh weight (SAP) and total root-secreted APase activity per plant (total SAP) of 350 *B. napus* genotypes with various genetic backgrounds under P-deficient conditions were quantified, and it was found that these root traits have wide genotype variations and different effects on SY. Subsequently, 18 *B. napus* genotypes with contrasting root biomass and SAP were selected from the above 350 *B. napus* genotypes to explore the effect of SAP and root morphology on P acquisition, and results showed that there were trade-offs between the two P-acquisition strategies among *B. napus* under P-deficient conditions. Total SAP was an important root trait under Po conditions. Understanding the interactions of root morphology and root-secreted APase will provide valuable insights into how to explore root functional traits for sustainably managed systems and be helpful for breeding *B. napus* with greater P acquisition ability with limited P availability.

## **2-Materials and methods**

### **2.1-Plant materials and growth conditions**

#### **Experiment 1**

A total of 350 genotypes from an association panel of oilseed rape (*Brassica napus*) with diverse genetic backgrounds, including 303 semi-winter, 41 spring, 4 winter and 2 unknown types collected worldwide, were used in this study (Table S1). The flowering time of them grown in Wuhan ranged from 146 to 174 days (Han et al., 2022). The seeds were washed three times with pure water and then placed at 4°C for overnight soaking. The imbibed seeds were germinated on a piece of gauze moistened with pure water for six days, and then transferred to Hoagland nutrient solution (Hoagland & Arnon, 1950) without phosphate at pH 5.6 for five days. Then, the plants were used for the measurement of the fresh root weight (RFW), root-secreted APase activity per unit RFW (SAP) and total root-secreted APase activity per plant (total SAP). A total of 54 plants were grown in each pot containing 11 L nutrient solution and all the plants were cultivated in a greenhouse with a 16 h / 8 h (light/dark) at 22°C. RFW, SAP and total SAP were used to cluster genotypes into three different classes: (1) high RFW but low SAP, (2) high SAP but low RFW, and (3) low RFW and SAP (Fig. 1A). In addition, the differences in seed yield under P-deficient soil among the three classes were compared according to the data provided by Liu et al. (2023).

#### **Experiment 2**

Nine Class 1 genotypes, which had high root biomass but low SAP, and nine Class 2 genotypes, which had high SAP but low root biomass, were selected from the above association panel to explore the effect of different root functional strategies on P acquisition. The seeds of these 18 *B. napus* genotypes were surface-sterilized for 10 min using 1% NaClO, then washed five times with pure water, and then placed at 4°C for overnight soaking. The imbibed seeds were germinated on a piece of gauze moistened with pure water for six days, and uniformly sized seedlings were transferred to a Hoagland nutrient solution (Hoagland & Arnon, 1950) for four days. The seedlings were then transferred to Hoagland nutrient solution with different P treatments: NP (normal Pi, 500  $\mu$ M  $\text{KH}_2\text{PO}_4$ ), LP (low Pi, 5  $\mu$ M  $\text{KH}_2\text{PO}_4$ ) and Po (100  $\mu$ M ATP as a P source; Gao et al., 2017; Deng 2020). After 10 days, four replicate plants of each genotype were used for the measurement of root functional traits, and after 15 days, four replicate plants of each genotype were used for the measurement of P content traits and shoot biomass. There was no observed difference in the shoot growth among *B. napus* plants grown under normal Pi, low Pi and Po after 10 days. However, after 15 days, the difference in shoot growth among different P treatments could be observed. It was the reason why the plants were sampled for the measurement of P content traits and shoot biomass five days later. The plants were cultivated in a greenhouse at 22°C with a 16 h / 8 h (light/dark). The nutrient solutions were renewed every 4 days, and the pH was adjusted to 5.6.

## 2.2-Root secreted APase activity

The plant roots were rinsed in distilled water and transferred to centrifuge tubes containing a culture medium with 5 mM p-NPP (pH 5.5). The seedlings were then placed in an incubator at 35°C for 30 min. The reaction was stopped with 1 M NaOH and the absorbance was determined at 410 nm using a microplate assay (Spark; Tecan). Root-secreted APase activity was expressed as the amount of p-NP generated by hydrolysis of p-NPP per unit time, including SAP and total SAP.

## 2.3-Root morphology traits

After measurement of root-secreted APase activity, the plant roots were washed, and then the entire root was spread out in the water of a transparent plastic tray and scanned at 300 dpi using a flatbed scanner (Epson Perfection V700). Root images were analysed with Win-RHIZO 2009 software (Regent Instruments Inc.) to obtain total root length (TRL), average root diameter (ARD) and total root surface area (TRA). Finally, the plant roots were weighed to obtain RFW after removing the water with absorbent paper, and then the roots were dried and weighed to obtain root dry weight (RDW).

## 2.4-P content traits

The plant samples were divided into shoot and root and dried under 80°C to a constant weight for determination of the dry weight. A total of 0.05 g of dried samples were pre-digested in glass tubes with H<sub>2</sub>SO<sub>4</sub> overnight. The tubes were then heated to 120°C for 1 h and five drops of 30% H<sub>2</sub>O<sub>2</sub> were added every 30 min until the solution turned colourless and transparent. The digestion was continued for an additional 30 min. The P concentration was determined by the molybdenum blue colorimetry method (Chen et al., 2007) at 700 nm by a microplate assay (Spark; TECAN). The total P acquisition per plant (plant P content) was defined as the sum of shoot P content and root P content.

## 2.5-Statistical analyses

Two-way ANOVA with a randomized block was used to examine the effects of P treatments, genotypes, and their interaction on root functional traits. Cluster analysis and visualisation for total SAP, SAP and RFW of 350 *B. napus* genotypes were performed with the R package 'pheatmap'. Principal component analysis (PCA) was performed with the R package 'FactoMineR'. Pearson's correlation analysis was performed with the R package 'PerformanceAnalytics', and a heatmap of the correlation coefficient matrix was generated by using the R package 'corrplot' package. To examine the importance of different root traits on P acquisition, we conducted redundancy analysis (RDA) using the R package 'vegan' package, and random forest analysis by using the R package 'rfPermute' package. Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of these predictors (Breiman, 2001). All R packages were used in R (version 4.2.1). To characterize the plasticity of root traits in response to low P availability among the *B. napus* genotypes, the response ratio (RR) of six traits was calculated. The RR was defined as the extent of plant traits in response to low P or Po, and calculated as  $RR = \sum [(R'_{ij'} - R_{ij}) / (R'_{ij'} + R_{ij})] / n$ , where  $R'_{ij'}$  and  $R_{ij}$  are root traits for a given genotype at LP/Po ( $i'$ ) and NP ( $i$ ), with  $j'$  and  $j$  being two randomly selected individuals from four replicates of the same genotype at LP/Po and NP, respectively, and  $n$  is the number of  $R'_{ij'} - R_{ij}$  values (Wen et al., 2020).



### 3-Results

#### 3.1-Root-secreted APase activity and root fresh weight vary significantly in an association panel of *B. napus* under P deficiency

To identify genotypic differences in root-secreted APase activity, 350 diverse *B. napus* genotypes were used in this study. Wide phenotypic variations were observed for root fresh weight (RFW), root-secreted APase activity per unit RFW (SAP), total root-secreted APase activity per plant (total SAP) and seed yield (SY) among the association panel of *B. napus*, with a 3.3-fold variation in total SAP between the extremes, a 2.9-fold variation in SAP, a 4.5-fold variation in RFW, a 31.9-fold variation in SY (Table 1). Interestingly, the cluster analyses divided the association panel of *B. napus* into three main classes (Fig. 1A). Among them, Class 1 (high RFW, low SAP), Class 2 (low RFW, high SAP) and Class 3 (low RFW, low SAP) included 107, 81, and 162 *B. napus* genotypes, respectively (Fig. 1A).

Mean total SAP had a small difference (8.2%) between the Class 1 and Class 2 genotypes, but mean SAP and RFW had a relatively large difference between them (Fig. 1B-D). Specifically, Class 1 genotypes were 38.1% higher in mean SAP (Fig. 1C) but 33.3% lower in mean RFW than Class 2 genotypes (Fig. 1D). The Class 3 genotypes had a relative smaller value in the above three traits as compared with Class 1 and Class 2 genotypes (Fig. 1B-D). Additionally, the Class 1 genotypes were also 30.3% higher than Class 2 genotypes and 42.5% higher than Class 3 genotypes in SY (Fig. 1E). As expected, total SAP had a positive correlation with SAP and RFW ( $P < 0.001$ ,  $r = 0.26$  and  $0.65$ ), and there was a negative correlation between SAP and RFW in the association panel ( $P < 0.001$ ,  $r = -0.49$ ; Fig. 1F). In addition, the SY was positively correlated with total SAP and RFW ( $P < 0.001$ ,  $r = 0.2$  and  $0.22$ ) but weakly correlated with SAP ( $P = \text{ns}$ ,  $r = -0.05$ ) under P deficiency (Fig. 1F). Principal component analysis (PCA) showed that the first two components (PC1 and PC2) accounted for 56.7% and 41.6% of the total variation, respectively (Fig. 1G). The distributions of Class 1 genotypes were significantly different from those of Class 2 and Class 3, and there was no significant difference between Class 2 and Class 3 genotypes in PC1 (Fig. 1G, H). The distributions of the three classes genotypes were significantly different in PC2 ( $P < 0.0001$ ; Fig. 1G, H). These showed that root-secreted APase and root morphology traits, as different P acquisition strategies, perhaps existed in a wide range of *B. napus* adapted to low P availabilities.

### 3.2-Differences in root functional traits and P-efficient traits between Class 1 and Class 2 *B. napus* genotypes

Nine Class 1 genotypes with high root biomass but low SAP and nine Class 2 genotypes with high SAP but low root biomass were randomly selected from the association panel of 350 *B. napus* to evaluate the effect of different root traits on P acquisition and plant growth. Six root functional traits, including total SAP, SAP, root dry weight (RDW), total root length (TRL), total root surface area (TRA) and average root diameter (ARD) of the 18 *B. napus* genotypes were measured under three P treatments. Similar to the association panel of 350 *B. napus* genotypes, all root traits showed substantial genotypic variation among 18 *B. napus* genotypes (Fig. S1; Tables 2 and S2). All root traits except for ARD were significantly influenced by the P treatment and the interaction between P treatments and genotypes (Table 2), and they were significantly increased under the LP conditions (Figs 2 and S1). More specifically, Class 1 genotypes showed significantly higher RDW, TRL and TRA than Class 2 genotypes under both NP and LP conditions (Fig. 2A, D, E). In contrast, Class 2 genotypes had significantly higher SAP than Class 1 genotypes under LP conditions, and there was a similar tendency under NP conditions (Fig. 2B). These differences between Class 1 and Class 2 genotypes resulted in a similar total SAP under both NP and LP conditions (Fig. 2C). Interestingly, under Po treatment, Class 1 genotypes also had significantly lower SAP than Class 2 genotypes, but there were no significant differences in other root traits between the two classes of genotypes, even though Class 1 genotypes exhibited higher mean values in RDW and TRL than Class 2 genotypes (Fig. 2).

Five P content traits and shoot biomass within the 18 *B. napus* genotypes, including shoot P content, root P content, plant P content, shoot P concentration, root P concentration and shoot dry weight, exhibited substantial genotypic variation across the three P treatments (Fig. S2; Tables 2 and S2). All of these traits were significantly influenced by the P treatment (Figs 3 and S2; Table 2), and all of these traits, except for root P concentration, were significantly influenced by the interaction between P treatments and genotypes (Table 2). Although there was no significant difference in shoot P concentration and root P concentration between Class 1 and Class 2 genotypes under the three P treatments (Fig. 3A, B), Class 1 genotypes showed significantly higher values in shoot dry weight and plant tissue P content than Class 2 genotypes under both NP and LP conditions ( $P < 0.05$  to  $P < 0.001$ ; Fig. 3C, D, E, F). Interestingly, there were no significant differences in the shoot biomass and plant P content between Class 1 and Class 2 genotypes under Po treatment (Fig. 3C- F).

### 3.3-Trade-offs among root functional traits in response to P deficiency

Principal component analysis (PCA) based on six root traits of 18 *B. napus* genotypes showed that the Class 1 and Class 2 genotypes were clustered and the distributions of Class 1 and Class 2 genotypes were significantly different in PC1 under NP and LP treatments but were not significantly different under the Po treatment (Fig. 4). Under NP treatment, the first two components (PC1 and PC2) accounted for 52.3% and 31.2% of the total variation, respectively (Fig. 4A). The TRL, RDW and total SAP scored high in the PC1, while ARD, TRA and SAP scored high in the PC2 (Fig. 4A; Table S3). The distributions of genotypes in the two classes were significantly different in the PC1 ( $P < 0.01$ ; Fig. 4D). Under LP treatment, the PC1 and PC2 accounted for 55% and 25.2% of the total variation, respectively (Fig. 4B), the PC1 primarily comprised SAP (negative direction), TRL and RDW (positive direction), and the PC2 primarily comprised ARD, TRA and total SAP (Fig. 4B; Table S3). Additionally, the distributions of genotypes in the two classes were significantly different in the PC1 ( $P < 0.0001$ ; Fig. 5E); the Class 1 genotypes were mainly clustered in the direction of TRL and RDW parameters, whereas the Class 2 genotypes tended to cluster in the direction of SAP (Fig. 4B). Under Po treatment, the first two components accounted for 53.1% and 26.4% of the total variation, respectively (Fig. 4C). The TRL, RDW and total SAP had high scores in PC1, and the SAP, ARD and TRA had high scores in PC2 (Fig. 4C; Table S3).

There was a positive correlation between root morphology traits under three P treatments (Fig. 5). TRL and RDW were significantly positively correlated with total SAP ( $r = 0.79$ ), but not significantly correlated with SAP under NP treatment (Fig. 5A). Under LP conditions, the total SAP was significantly positively correlated with RDW ( $P < 0.05$ ,  $r = 0.60$ ) and not significantly correlated with root morphology traits, but SAP was significantly negatively correlated with root morphology traits (TRL and TRA,  $r = -0.78$  and  $-0.66$ , respectively) and RDW ( $r = 0.74$ ) (Fig. 5B). Under Po treatment, the TRL, TRA and RDW were positively correlated with total SAP ( $r = 0.66$  to  $0.82$ ) but weakly negative correlated with SAP ( $r = -0.24$  and  $-0.25$ ; Fig. 5C). Overall, these results revealed that trade-offs occurred between SAP and root morphology traits (i.e., TRL and TRA) of *B. napus* in response to Pi deficiency. Although Class 1 and Class 2 genotypes both arrived at the same total SAP: the Class 1 genotypes with bigger root morphology but lower SAP, whereas Class 2 genotypes had smaller root morphology but higher SAP. Therefore, there was genotypic variation between these groups in terms of carbon allocation.

### 3.4-Contribution of root traits to P acquisition under different P treatments

Almost all root traits across 18 *B. napus* genotypes showed a positive response ratio in response to low P (Fig. S3A; except for ARD) and Po treatments (Fig. S3B). These traits showed different correlations with the P content traits and shoot biomass under different P treatments (Fig. 5). At NP conditions, plant tissue P content traits and shoot dry weight were significantly positively correlated with the TRL and RDW ( $r = 0.67$  to  $0.86$ ), but not significantly correlated with SAP, TRA and ARD, and only shoot P content and shoot dry weight were slightly positively correlated with total SAP (Figs 5A, 6A-D and S4A, B). All root traits were not significantly correlated with shoot P concentration and root P concentration (Fig. 5A). At LP conditions, the root morphology traits (i.e., TRL and TRA) and RDW were significantly positively correlated with the shoot P content and dry weight ( $r = 0.56$  to  $0.88$ ; Figs 5B, 6G, H, and S4C). However, the shoot P content and dry weight were significantly negatively correlated with SAP ( $r = -0.64$  and  $-0.66$ ) and were not significantly correlated with total SAP and ARD (Figs 5B, 6E, F, and S4D). All root traits except for total SAP were not significantly correlated with shoot and root P concentration (Fig. 5B). The total SAP was significantly negatively correlated with shoot P concentration ( $r = -0.58$ ; Fig. 5B). At Po conditions, plant tissue P content and shoot dry weight were strongly positively correlated with total SAP, TRL and RDW ( $r = 0.80$  to  $0.93$ ), but they were not significantly correlated with SAP and TRA (Figs 5C, 6I-L, and S4E). However, the plant P content was significantly negatively correlated with ARD ( $r = -0.48$ ; Figs 5C and S4F). The root P concentration was significantly negatively correlated with total SAP but was not significantly correlated with other root traits (Fig. 5C).

Redundancy analysis (RDA) was used to explore the importance of root traits on P acquisition. All three P content traits had high scores in the positive direction of RDA1 under three P treatments (Fig. 7A-C; Table S4). Under NP treatment, all six root traits on RDA1 and RDA2 could explain 67.4% and 1.5% of the total variation of the three P-content traits, respectively (Fig. 7A). Among these root traits, RDW and TRL were the most important factors in promoting P uptake, which had high scores in the positive direction of RDA1 (Fig. 7A; Table S4). Under LP treatment, all root traits in the RDA1 represented 40.3% and RDA2 represented 2.1% of the total variation of the three P-content traits, respectively (Fig. 7B). The TRL and RDW had high scores in the positive direction of RDA1, which were important factors in promoting P accumulation; however, the SAP had high score in the negative direction of RDA1, which decreased the plant P accumulation (Fig. 7B; Table S4). Under Po treatment, the explained variations of root traits on the three P-content traits in RDA1 and RDA2 were 84.9% and 6.4%, respectively (Fig. 7C). The TRL, RDW and total SAP had high scores in the positive direction of RDA1, which were important factors in promoting P accumulation (Fig. 7C; Table S4). The percentage of increase of mean square error [Increase in MSE (%)] was used to

estimate the importance degree of root functional traits to P accumulation. The TRL and RDW had high values of increase in MSE (%) in three P treatments. The total SAP had a high value of increase in MSE (%) but SAP had a low value of increase in MSE (%) under Po treatment (Fig. 7D, E, F).

#### 4-Discussion

### 4.1-Trade-offs between root-secreted acid phosphatase and root morphology traits

Root-secreted APases and root morphology traits have been widely studied as two distinct strategies for P acquisition (Lyu et al., 2016; Ushio et al., 2015; Zhou et al., 2016). Root-secreted APases are generally thought to promote P availability by hydrolyzing Po, and root morphology traits are changed to improve P uptake through a great exploration of the soil volume (Duff et al., 1994; Tarafdar & Claassen, 1988; Wang et al., 2018). Large phenotypic variations in root morphology traits were observed in response to P deficiency among *B. napus* genotypes with diverse genetic backgrounds (Wang et al., 2017), but root-secreted APase activity was not investigated. In this study, large phenotypic variations in root-secreted APase activity (i.e., total SAP and SAP) were also observed among an association panel of *B. napus* under the P-deficient condition (Table 1; Table S1). Genotypes with large root biomass had reduced SAP (Class 1 genotypes); conversely, genotypes with smaller root biomass had enhanced SAP (Class 2 genotypes) (Fig. 1A, C, D; Table S1). This indicated that perhaps there existed a significant difference in P acquisition strategies within the association panel of *B. napus*.

SAP and root morphology (i.e., TRL, TRA) of 18 *B. napus* genotypes were significantly increased by P deficiency (Figs 2B, D, E and S1B, D, E). Class 1 genotypes had larger root system than Class 2 genotypes, but Class 2 genotypes had higher SAP than Class 1 genotypes under P-deficient conditions (Figs 2B, D, E and S1B, D, E). Furthermore, SAP was negatively correlated with TRL and TRA, especially under P-deficient conditions ( $r = -0.78$  and  $-0.66$ ; Figs 4B and 5B). Very similar results were also reported among wheat and oilseed rape genotypes under P-deficient soil (Duan et al., 2020; Wang et al., 2021). Both root development and root exudates require the consumption of carbon sources; therefore, the input of carbon resources determines the relationship between the two P acquisition strategies. Unlike oilseed rape and wheat, the root exudates (e.g., root-secreted APase and carboxylate) were negatively correlated with root diameter in maize and chickpea genotypes, even among tree species (Han et al., 2021; Ushio et al., 2015; Wen et al., 2020; Yang et al., 2022). These indicated that there were a series of different trade-offs among the root functional traits of P acquisition in response to P deficiency in both interspecific plants and

intraspecific plants.

Recently, root exudation was incorporated into the root economics space for a holistic understanding of soil nutrient acquisition (Wen et al., 2021). According to the “resource economy hypothesis”, the cost (i.e., energy and resource input) of root functional traits regulation may limit the plant's ability to regulate these strategies simultaneously and efficiently (Lambers et al., 2006; Raven et al., 2018; Ryan et al., 2012). In this study, under LP treatment, Class 1 genotypes relied more on root morphology and increased the interception of P, suggesting a preference for “resource-acquisition” strategies. In contrast, Class 2 genotypes had a strong ability to secrete APase from their roots and contributed to the hydrolysis of Po to improve the P availability, suggesting a typical “resource-conservative” strategy (Figs 2 and 4B). It is worth noting that the SAP, TRL and TRA were significantly induced by Pi deficiency in both Class 1 and 2 genotypes (Figs 2B, D, E and S1B, D, E), indicating *B. napus* evolved different strategies to adapt to the Pi limitation conditions. The molecular mechanisms that control the trade-offs between SAP and root morphology (i.e., TRL and TRA) under P-deficient conditions need further research in the future.

## 4.2-Contribution of root functional traits to P acquisition

The root morphology traits had high plasticity in response to P deficiency stress among most crops (Liu, 2021; Lambers, 2022; Poirier et al., 2022). In this study, the root morphology traits of *B. napus* were increased by low P availability, and there was a great variation in the above traits among 18 *B. napus* genotypes (Figs 2D, E, F, and S1D, E, F; Table S2). Although there was no significant difference in plant tissue P concentration between Class 1 and Class 2 genotypes, the Class 1 genotypes were significantly higher in plant P accumulation than Class 2 genotypes under both NP and LP treatments because the root morphology and tissue biomass of the former was more than the latter (Fig. 3). Plant P acquisition is positively correlated with root extension, and species with high P efficiency tend to rely on root morphology to improve the exploration range of soil (Becquer et al., 2021; Cornish et al., 1984; Haling et al., 2016; Lambers et al., 2006). In the present study, the plant P content was significantly positively correlated with TRL and RDW under three P treatments (Figs 5 and 6C, G, K, D, H, L); besides, the root biomass was positively correlated with SY among 350 *B. napus* under P-deficient conditions (Fig. 1F). Redundancy and random forest analysis also showed that TRL and RDW were very important factors for P acquisition under three P treatments (Fig. 7). Additionally, a recent report also showed SY is positively correlated with TRL under P-sufficient or P-deficient conditions in *B. napus* (Duan et al., 2020). Therefore, TRL and RDW were significant traits for P acquisition and should be the first root trait to be considered in P-efficient breeding in *B. napus*.

Root-secreted APases enhance the P availability by mineralisation of Po as part of the P acquisition strategy (Tarafdar & Claassen, 1988). However, the efficiency of secrete APases is

different among different plant species (Lyu et al., 2016; Razavi et al., 2016). For example, root-secreted APase activity in legumes reaches the highest rate 12 d after germination (significantly higher than that of cereals and oilseed species), but then gradually decreases, while the total SAP of oilseed species increases linearly with the age of the plants (Yadav & Tarafdar, 2001). Species with high root-secreted APase activity usually have high P acquisition ability in a Po environment (Bhadouria et al., 2023; Deng et al., 2020; Liang et al., 2010; Liu et al., 2016; Liu et al., 2018; Mehra, et al., 2017; Zhu et al., 2020). In this study, Class 2 genotypes had higher SAP but lower tissue P content and shoot biomass than Class 1 genotypes (Figs 2B and 3C-F). The tissue P content was significantly negatively correlated with SAP under LP conditions (Figs 5B and 6F). This is likely due to the lack of Po in the growing media and the consumed resources of the root-secreted APases under LP conditions in this study. However, under Po conditions, the shoot biomass and tissue P content were not significantly different between Class 1 and Class 2 genotypes (Fig. 3C-F). The plant tissue P content and shoot biomass were significantly positively correlated with total SAP ( $r = 0.80$  and  $0.81$ ), like root systems size (i.e., RDW and TRL; Fig. 5C). Overall, these results suggested that the Class 1 genotypes with larger root system were favourable genotypes to adapt to different P availability conditions, and the Class 2 genotypes with high SAP were suitable for rich Po conditions. Since total SAP scales with root system size (RDW/TRL), it is difficult to separate these results, but it does highlight the trade-offs between root system size and SAP, with both competing for carbon resources. Some genotypes have evolved preferences for carbon allocation to root system size (Class 1 genotypes), whilst others have directed these resources to SAP (Class 2 genotypes). Both traits remain important factors in the acquisition of Po.

Previous studies have shown that root-secreted APase activity was increased under P deficiency in *B. napus* (Duan et al., 2020; Zhang et al., 2010), indicating that root-secreted APase was also an important factor in response to low P stress in *B. napus*. Although there were huge differences in SAP, RDW and TRL, there was no significant difference in the total SAP between the Class 1 and Class 2 genotypes under LP conditions (Fig. 2). Although the SAP had higher response ratio than total SAP in response to Po, the total SAP had the highest response ratio in response to P deficiency compared to other root traits (Fig. S3). RDA and random forest analysis further demonstrated that total SAP was as important for P acquisition as RDW and TRL under Po conditions (Fig. 7C, F). However, in previous studies, root-secreted APases promoted rhizosphere Po utilization in many crops, but these studies were only confined to SAP and did not investigate total SAP (Deng et al., 2020; Liu et al., 2016; Liu et al., 2018; Lu et al., 2016; Zhu et al., 2020). Therefore, total SAP should receive the same attention when evaluating the Po utilization efficiency in crops.

In conclusion, the present study showed a significant genotypic variation of root-secreted APase activity (i.e., SAP and total SAP) in an association panel of *B. napus*, and root system size and SAP resulted in different P acquisition strategies across a wide range of *B. napus* genotypes. Class 1 genotypes had higher root biomass and better root morphology (i.e., TRL and TRA) than Class 2 genotypes, which increased the interception of P in the “resource-

acquisition" strategy under P-deficiency. Conversely, Class 2 genotypes had smaller root system but higher SAP than Class 1 genotypes, which increased P-activation in the "resource-conservation" strategy under P-deficiency. Root morphology was important in P-acquisition under different P-availability conditions, and total SAP was also an important factor for P-acquisition under Po condition. These are highly valuable for breeding and selecting genotypes with high P-acquisition efficiency.

### **Author contributions**

Hao Li and Lei Shi contributed to the study conception and design. Material preparation and data collection were performed by Hao Li, Bingbing Zhang, Haijiang Liu, Xiaohua Wang, Guangda Ding, Hongmei Cai, Sheliang Wang and Fangshen Xu. Data analysis was performed by Hao Li and Chuang Wang. The first draft of the manuscript was written by Hao Li, Lei Shi, Chuang Wang and John P. Hammond and all authors commented on previous versions of the manuscript. All authors read carefully and approved the final manuscript.

### **Acknowledgments**

This work was supported by the National Key Research and Development Program of China (Grants No.2023YFD1700204) and National Nature Science Foundation of China (Grants No. 32172662 and 32201868).

### **Competing Interests**

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.

### **Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Figures

Fig. 1 Differences in total root-secreted APase activity per plant (total SAP), root-secreted APase activity per unit root fresh weight (SAP) and root fresh weight (RFW) among an association panel of 350 *B. napus* genotypes under P deficiency. (A-E) Cluster analysis of total SAP, SAP and RFW (A). The box plots show the difference in total SAP (B), SAP (C), RFW (D)

and seed yield (SY) (E) among the three Classes of genotypes. The number above the box represents the mean of the investigated traits of each class of genotypes. (F) Pearson correlation matrix of total SAP, SAP, RFW and SY. (G) Principal component analysis (PCA) of total SAP, SAP and RFW. (H) PC scores of three classes of genotypes in the first two principal components. Class 1, Class 2 and Class 3 included 107, 81, and 162 *B. napus* genotypes, respectively. Seeds were germinated for six days and then transferred to a nutrient solution without Pi for five days. The SY data were provided by Liu et al. (2023). Significant difference based on Duncan's post-hoc analysis at  $P < 0.05$ .

Fig. 2 Box plots showing the differences of root functional traits among 18 *B. napus* genotypes between Class 1 (blue) *B. napus* genotypes and Class 2 (red) genotypes. (A) RDW, root dry weight; (B) SAP, root-secreted APase activity per unit root fresh weight; (C) Total SAP, total root-secreted APase activity per plant; (D) TRL, total root length; (E) TRA, total root surface area; (F) ARD, average root diameter. Student's t-test was used for comparisons between two classes of genotypes (ns, not significance,  $*P\text{-value} < 0.05$ ,  $**P\text{-value} < 0.01$ ,  $***P\text{-value} < 0.001$ ). The points in the box represented the means of four biological replicates of each genotype. Boxes with different upper-case letters and lower-case letters are significantly different at  $P < 0.05$  in the investigated traits of Class 1 genotypes between three P treatments (upper-case), and Class 2 genotypes between three P treatments (lower-case). NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); Po (100  $\mu\text{M}$  ATP).

Fig. 3 Box plots showing the differences of P accumulation traits and shoot biomass among 18 *B. napus* genotypes between Class 1 (blue) *B. napus* genotypes and Class 2 (red) genotypes. (A) Shoot P concentration, (B) root P concentration, (C) shoot dry weight, (D) shoot P content, (E) root P content and (F) plant P content. Student's t-test was used for comparisons between two classes of genotypes (ns, not significance,  $*P\text{-value} < 0.05$ ,  $**P\text{-value} < 0.01$ ,  $***P\text{-value} < 0.001$ ). The points in the box represented the means of four biological replicates of each genotype. Boxes with different upper-case letters and lower-case letters are significantly different at  $P < 0.05$  in the investigated traits of Class 1 genotypes between three P treatments (upper-case), and Class 2 genotypes between three P treatments (lower-case). NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); Po (100  $\mu\text{M}$  ATP).

Fig. 4 (A-C) Principal component analysis (PCA) of six root traits among 18 *B. napus* genotypes from Class 1 (blue) and Class 2 (red). NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ) (A); LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ) (B) and Po (100  $\mu\text{M}$  ATP) (C). (D-F) The box plots showed the differences of PC score in the first two principal components of Class 1 and Class 2 genotypes under NP (D), LP (E) and Po (F). Student's t-test was used for comparisons between two classes of genotypes (ns, not significance,  $*P\text{-value} < 0.05$ ,  $**P\text{-value} < 0.01$ ,  $***P\text{-value} < 0.001$ ). Total SAP, total root-secreted APase activity per plant; SAP, root-secreted APase activity per unit root fresh weight; RDW, root dry weight; TRL, total root length; TRA, root surface area; ARD, average

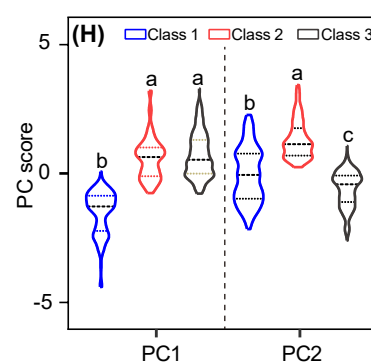
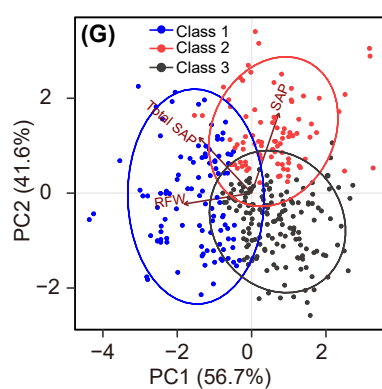
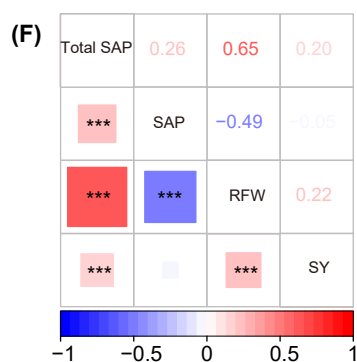
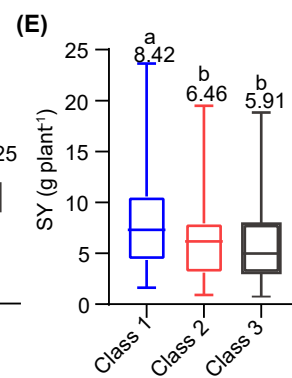
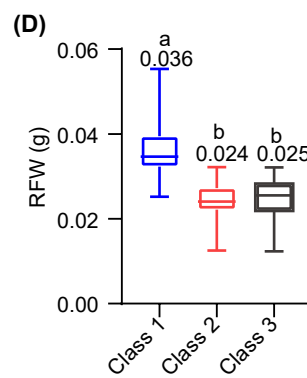
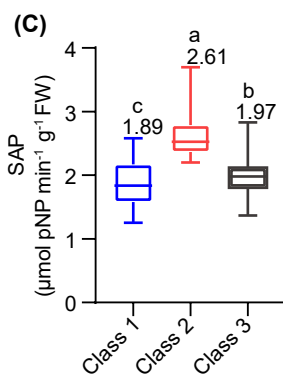
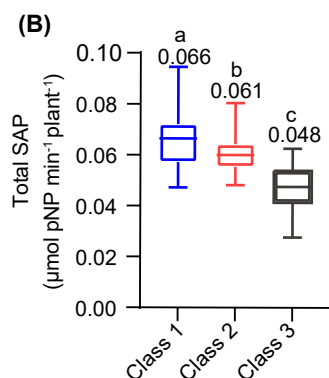
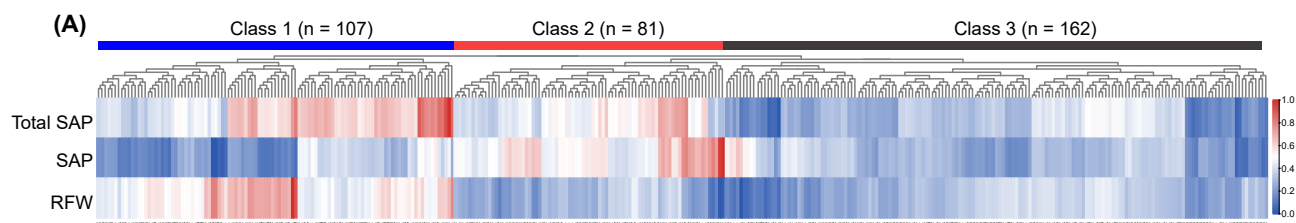
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root diameter.

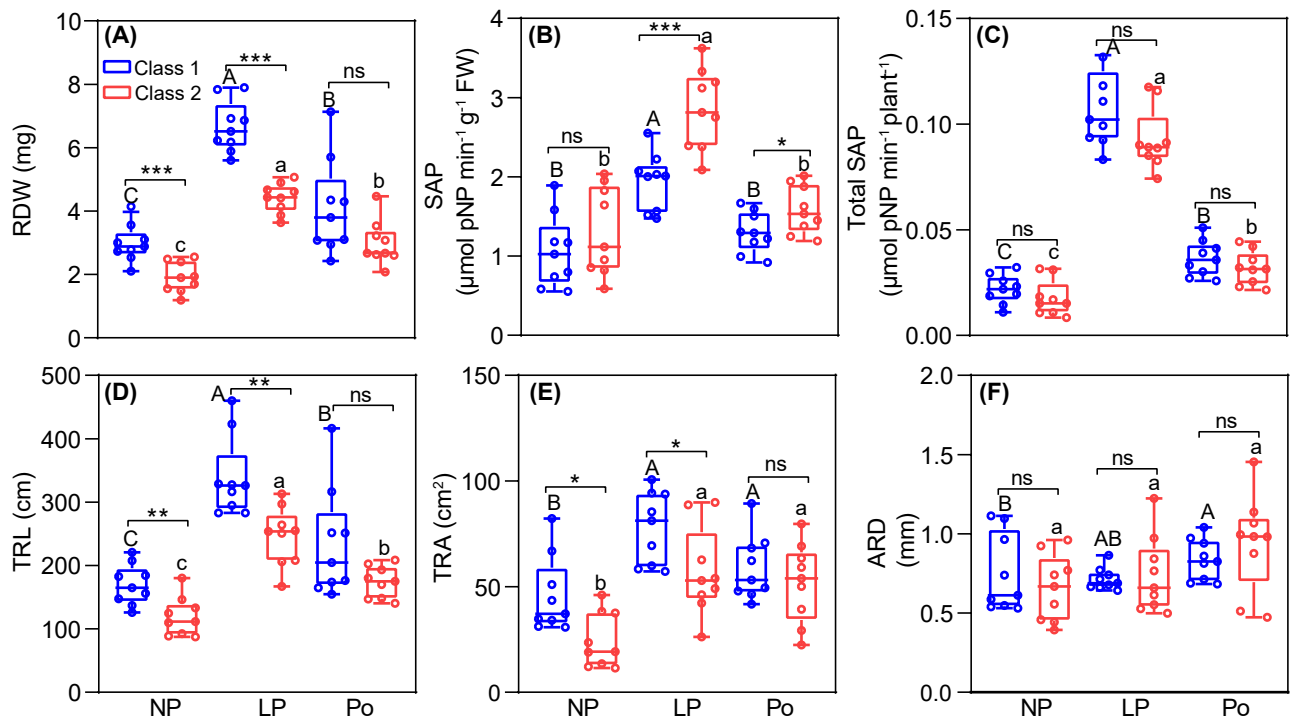
**Fig. 5 Pearson correlation matrix showing the relationships between the parameters among 12 traits for 18 *B. napus* genotypes.** (A) NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); (B) LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ) and (C) Po (100  $\mu\text{M}$  ATP). The sizes of the squares are proportional to the correlation coefficients, and the asterisks indicate that the trait–trait correlations are significant (\**P*-value < 0.05, \*\**P*-value < 0.01, \*\*\**P*-value < 0.001). Total SAP, total root-secreted APase activity per plant; SAP, root-secreted APase activity per unit root fresh weight; RDW, root dry weight; TRL, total root length; ARD, average root diameter; TRA, root surface area; RPC, root P content; SPC, shoot P content; PPC, plant P content; SDW, shoot dry weight; R [P], root P concentration; S [P], shoot P concentration.

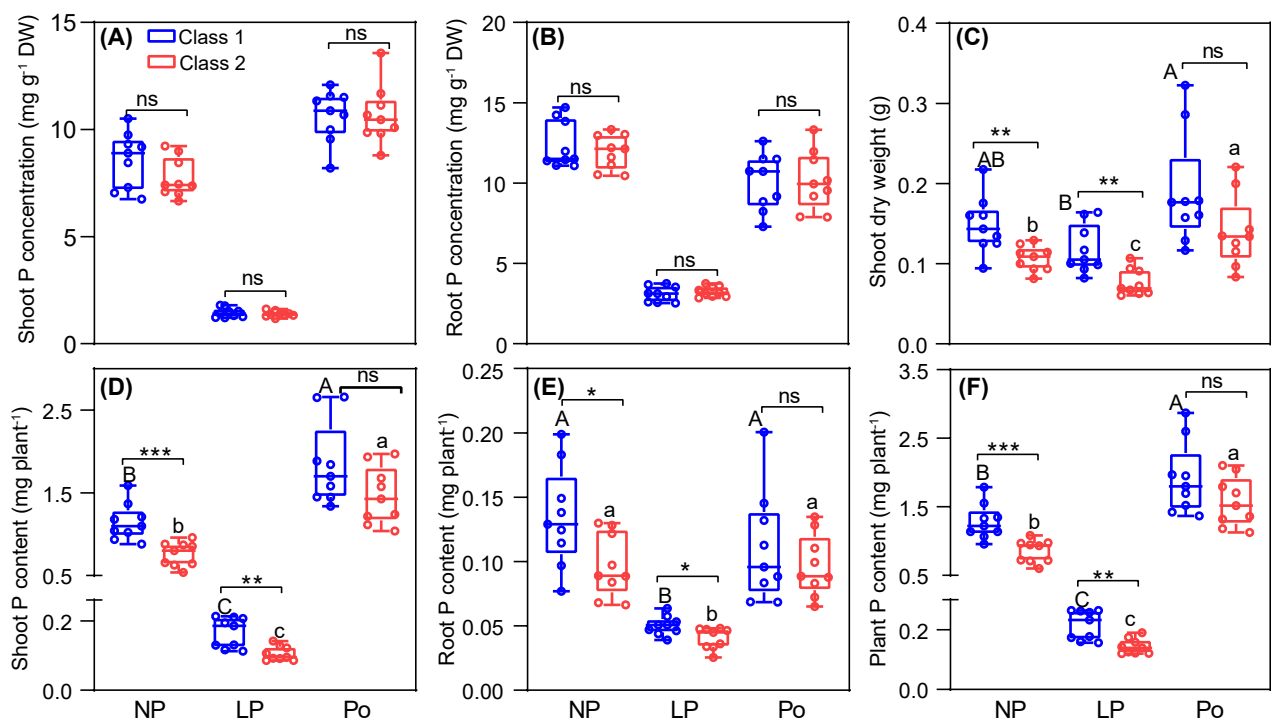
**Fig. 6 The linear relationships between plant P content and root traits among 18 *B. napus* genotypes.** (A–D) NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); (E–H) LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ) and (I–L) Po (100  $\mu\text{M}$  ATP). The shaded area around the regression line represents the 95% confidence interval. PPC, plant P content; Total SAP, total root-secreted APase activity per plant; SAP, root-secreted APase activity per unit root fresh weight; RDW, root dry weight; TRL, total root length.

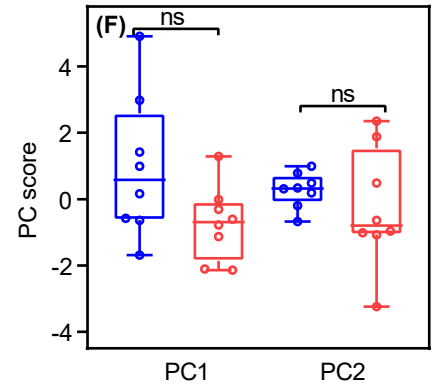
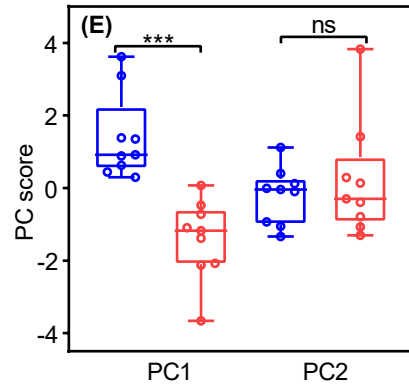
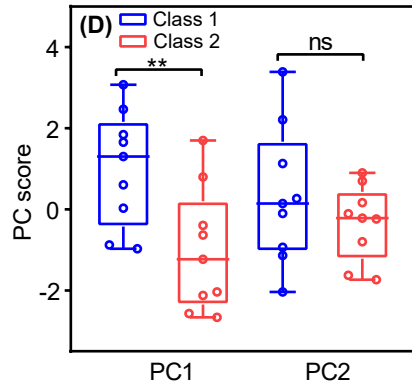
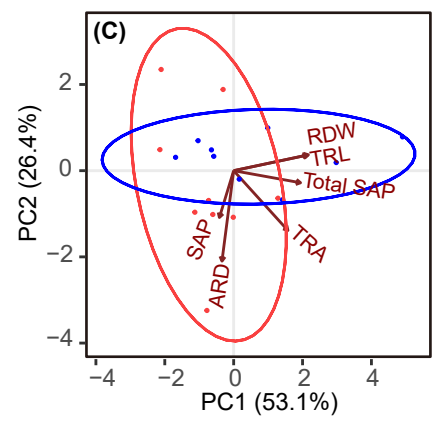
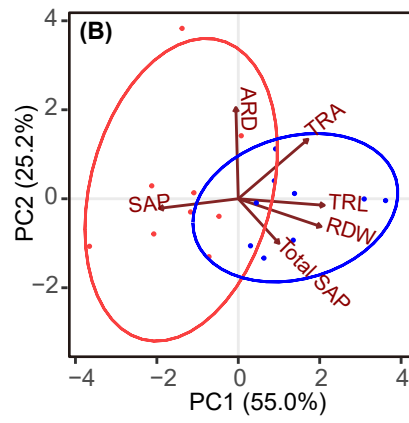
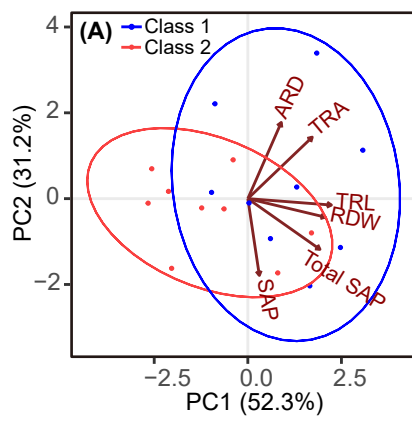
**Fig. 7 Difference in the importance of root traits for phosphorus acquisition in *B. napus*.** (A–C) Redundancy analysis of P content in relation to root functional traits under NP (A), LP (B) and Po (C). (D–F) Random forest analysis of plant P content in relation to root functional traits under NP (D), LP (E), and Po (F). Significance levels are as follows: \**P*-value < 0.05, \*\**P*-value < 0.01. NP, normal P (500  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); LP, low P (5  $\mu\text{M}$   $\text{KH}_2\text{PO}_4$ ); Po (100  $\mu\text{M}$  ATP); Total SAP, total root-secreted APases activity per plant; SAP, root-secreted APases activity per unit root fresh weight; RDW, root dry weight; TRL, total root length; ARD, average root diameter; TRA, root surface area; RPC, root P content; SPC, shoot P content; PPC, Plant P content; MSE, mean squared error.



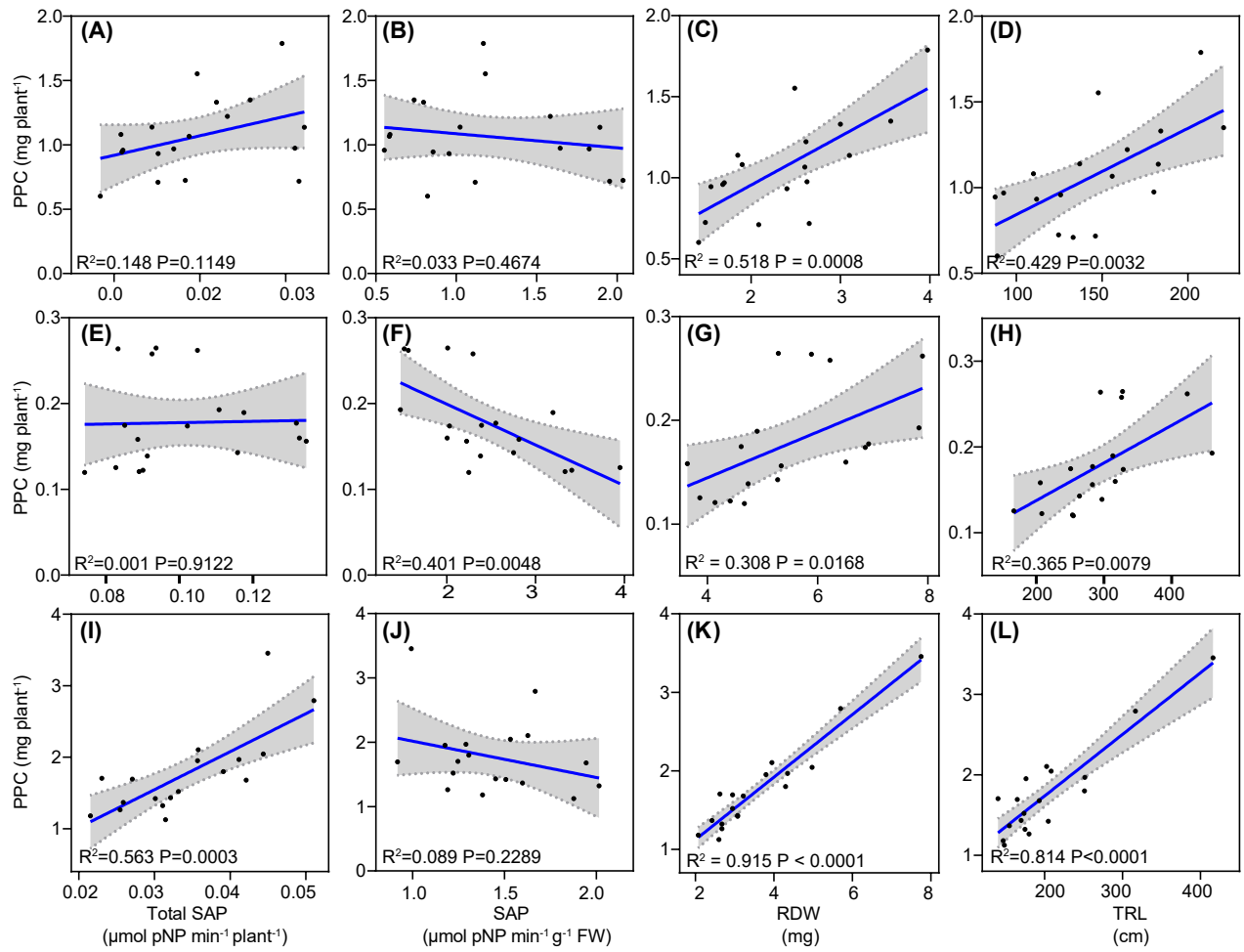


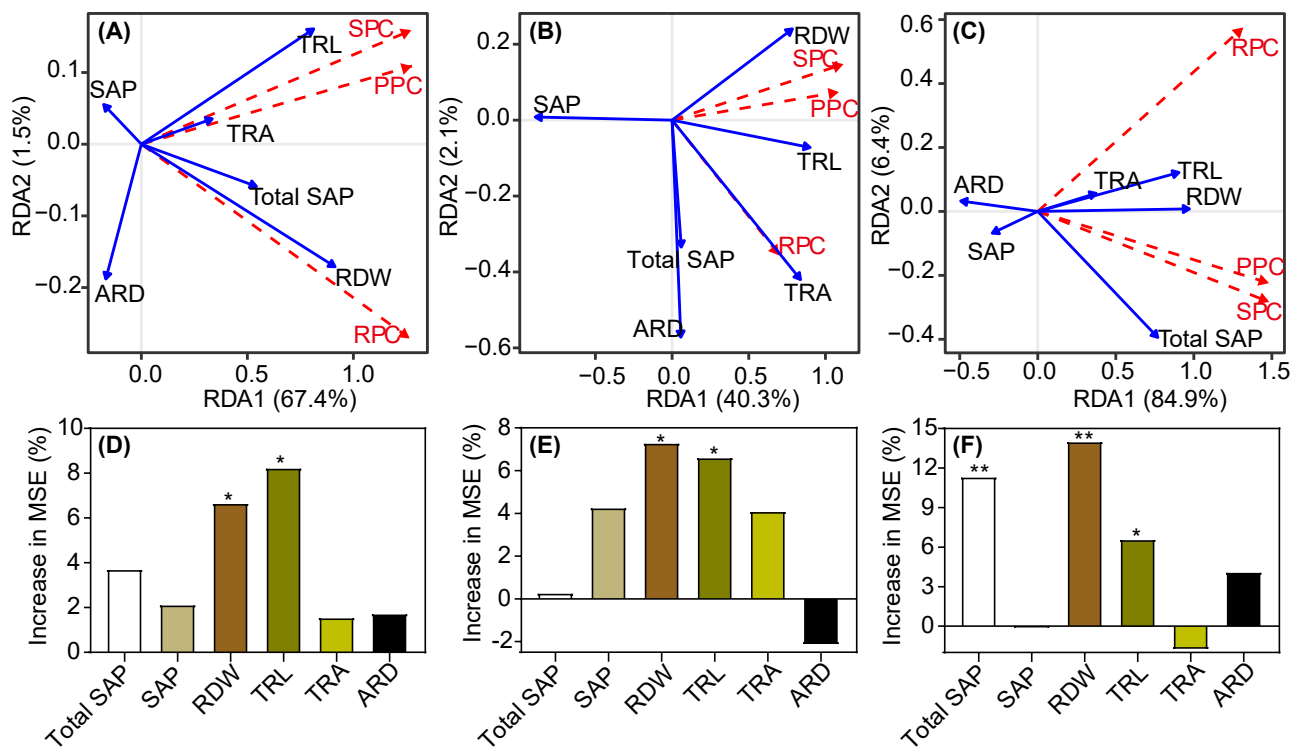










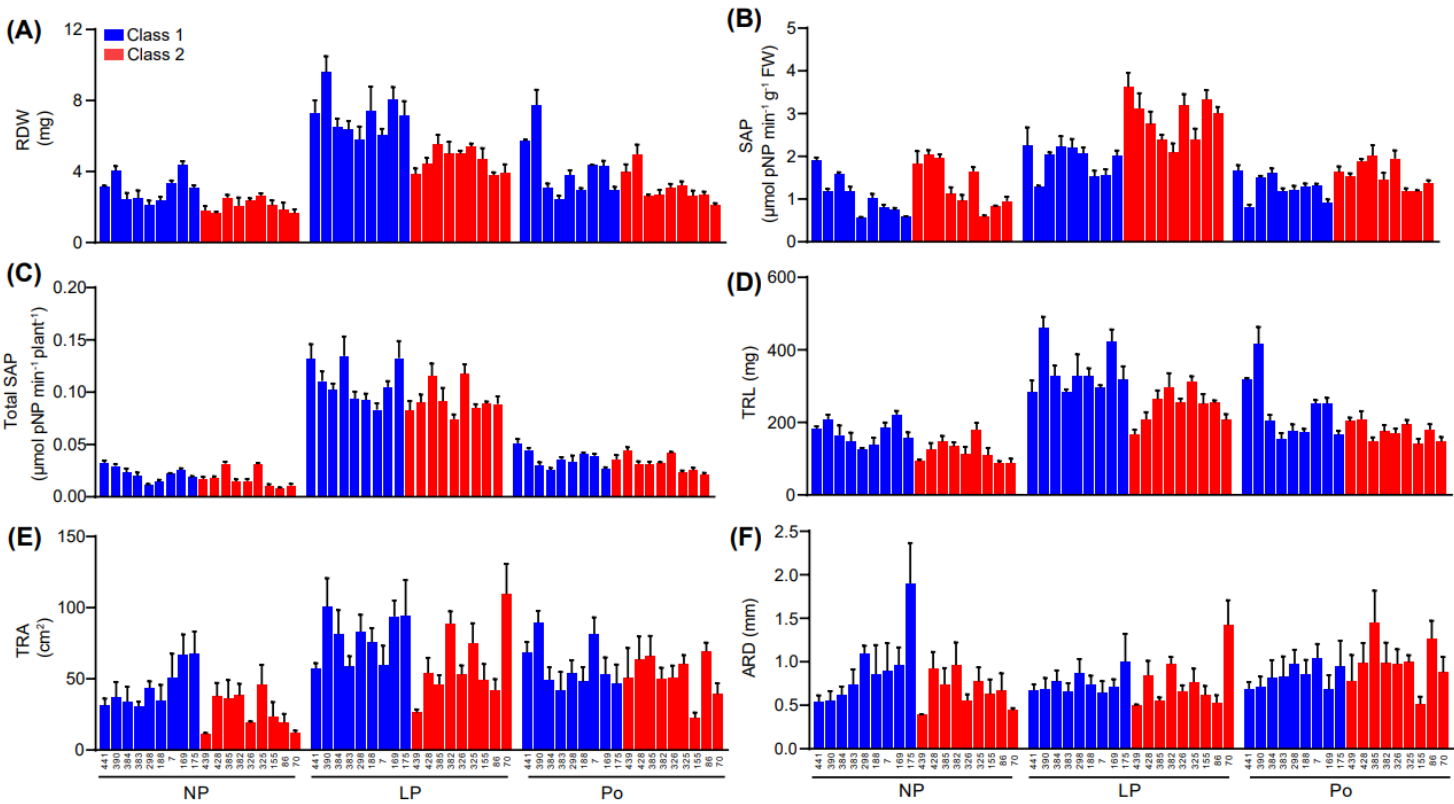


*Brassica napus*

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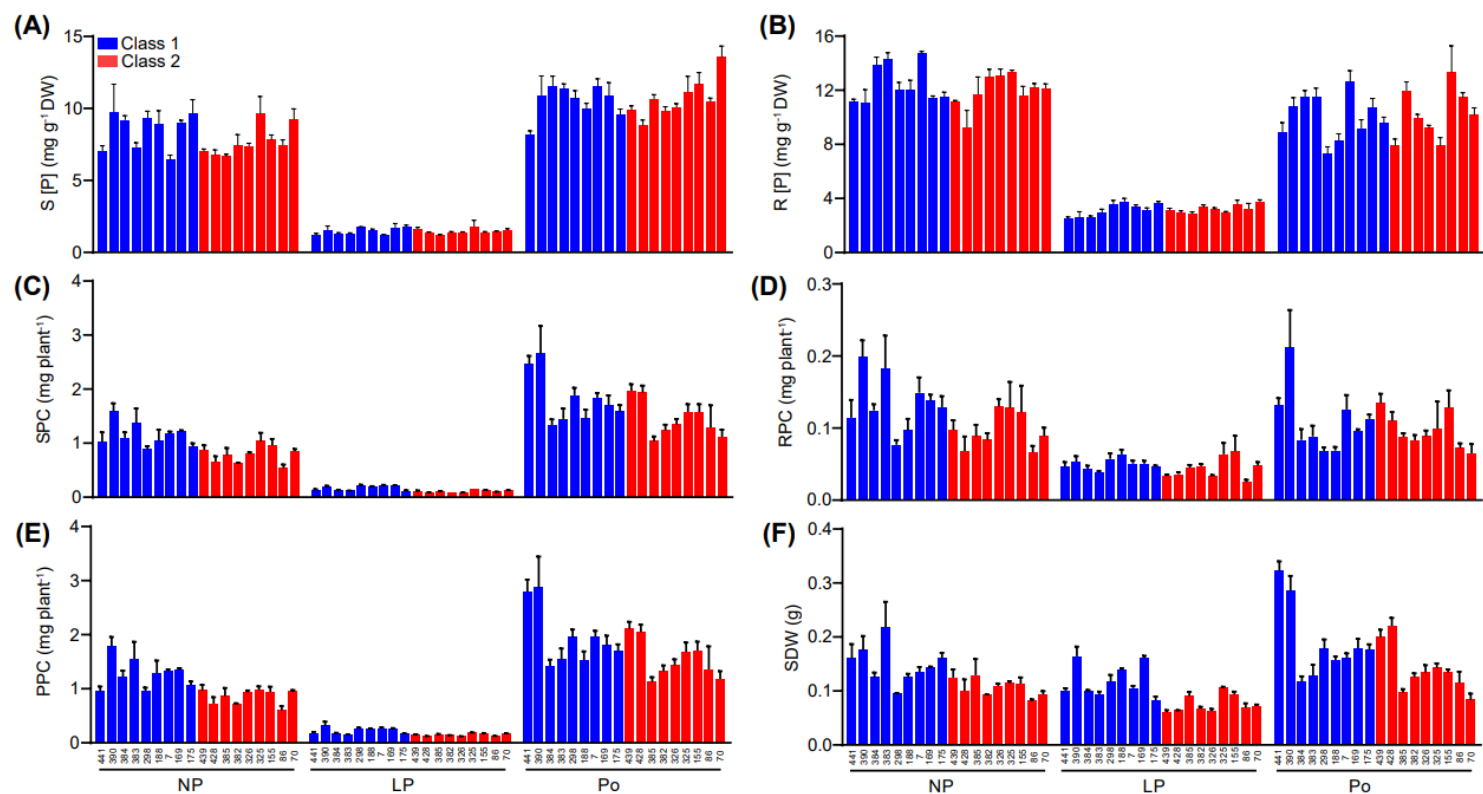
Hao Li<sup>1,2</sup>, Chuang Wang<sup>2</sup>, Bingbing Zhang<sup>1,2</sup>, Haijiang Liu<sup>1,2</sup>, John P. Hammond<sup>3</sup>, Xiaohua Wang<sup>4</sup>, Guangda Ding<sup>2</sup>, Hongmei Cai<sup>2</sup>, Sheliang Wang<sup>2</sup>, Fangsen Xu<sup>1,2</sup> and Lei Shi<sup>1,2\*</sup>

Fig. S1



**Fig. S1. Difference in root functional traits among 18 *B. napus* genotypes from Class 1 (blue) and Class 2 (red) under three P treatments.** (A) RDW, root dry weight; (B) SAP, root secreted APase activity per unit root fresh weight; (C) Total SAP, total root secreted APase activity per plant; (D) TRL, total root length; (E) TRA, total root surface area; (F) ARD, average root diameter. NP, normal P (500  $\mu\text{M KH}_2\text{PO}_4$ ); LP, low P (5  $\mu\text{M KH}_2\text{PO}_4$ ); Po (100  $\mu\text{M ATP}$ ). Data were means of four biological replicates with error bars indicating SD.

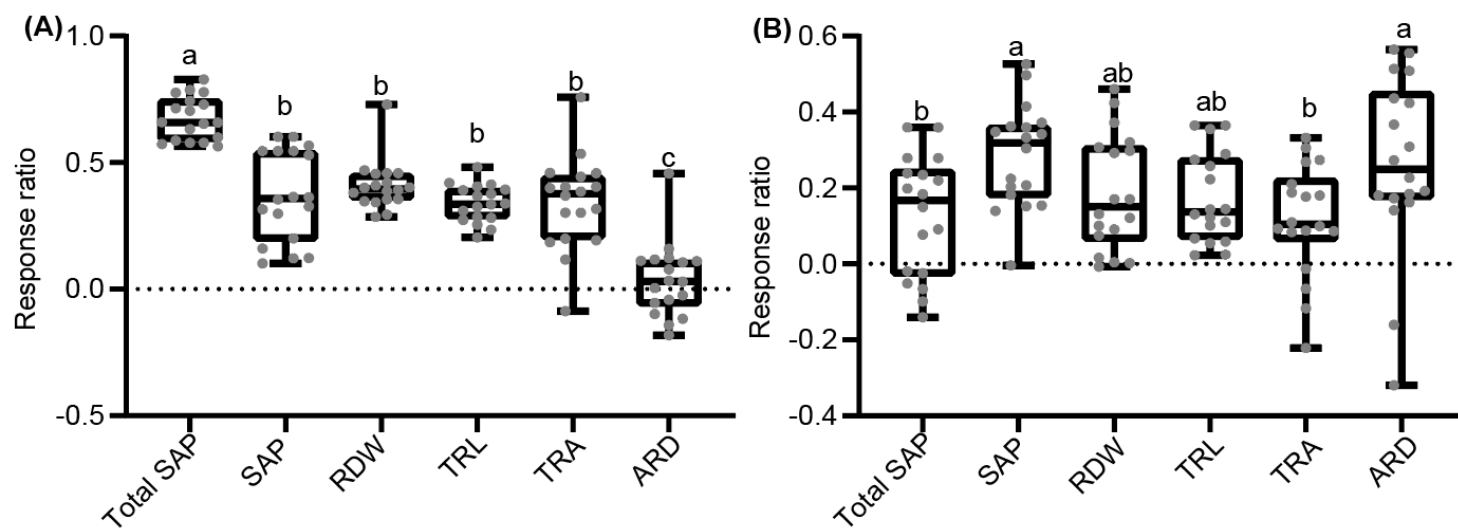
Fig. S2



**Fig. S2. Difference in P accumulation traits and shoot biomass among 18 *B. napus* genotypes from Class 1 (blue) and Class 2 (red) under three P treatments.** (A) S [P], shoot P concentration; (B) R [P], root P concentration; (C) SPC, shoot P content; (D) RPC, root P content; (E) PPC, plant P content; (F) SDW, shoot dry weight. NP, normal P (500  $\mu$ M  $\text{KH}_2\text{PO}_4$ ); LP, low P (5  $\mu$ M  $\text{KH}_2\text{PO}_4$ ); Po (100  $\mu$ M ATP). Data were means of four biological replicates with error bars indicating SD.

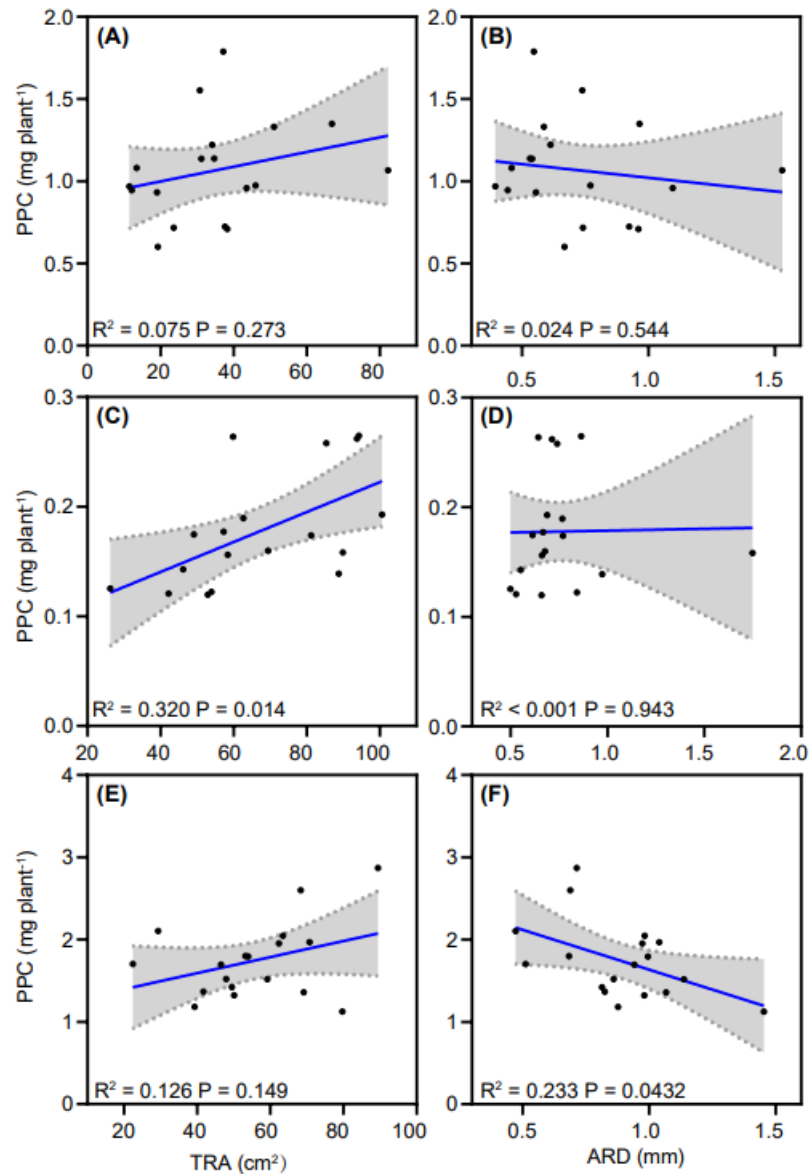


**Fig. S3**



**Fig. S3. Difference in response ratio of six root functional traits among 18 *B. napus* genotypes in response to (A) LP and (B) Po treatments.** Total SAP, total root secreted APase activity per plant; SAP, root secreted APase activity per unit root fresh weight; RDW, root dry weight; TRL, total root length; TRA, total root surface area; ARD, average root diameter. Significant difference at  $P < 0.05$  in the investigated traits.

**Fig. S4**



**Fig. S4. The linear relationships between plant P content and TRA and ARD.** (A-B) NP, normal P (500  $\mu$ M  $\text{KH}_2\text{PO}_4$ ), (C-D) LP, low P (5  $\mu$ M  $\text{KH}_2\text{PO}_4$ ) and (E-F) Po (100  $\mu$ M ATP). The shaded area around the regression line represents the 95% confidence interval. TRA, root surface area; ARD, average root diameter; PPC, plant P content.