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Highlights

- Seed yield of oilseed rape was significantly correlated with root morphology traits
- SG168 and HYZ9 had better root morphology, and achieved high seed yield under Pi deficiency
- Applications of RA and PSB are two nature-based solutions to reduce Pi fertilizer application rates

Abbreviations: BN, branch number; DW-Pe, dry weight of pericarp; DW-Se, seed yield per plant; DW-St, dry weight of stem; HI, harvest index; P, phosphorus; PE, total P uptake per total root length at leaf development stage; PH, plant height; PN, pod number; PPFP, P partial factor productivity; PRE, P recovery efficiency; RAD, root average diameter; RSA, root surface area; S1, leaf development stage; S2, stem elongation stage; S3, flowering stage; S4, ripening stage; SDW-L, shoot dry weight at leaf development stage; SDW-S, shoot dry weight at stem elongation stage; SDW-F, shoot dry weight at flowering stage; SDW-R, shoot dry weight at ripening stage; SRL, specific root length; SY, seed yield; TP-L, total P content in shoot at leaf development stage; TP-S, total P content in shoot at stem elongation stage; TP-F, total P content in shoot at flowering stage; TP-R, total P content in shoot at ripening stage; TP-Pe, total P content in pericarp; TP-Se, total P content in seed; TP-St, total P content in stem; TRL, total root length; TRV, total root volume; TSW, 1000-seed weight.

Abstract

Oilseed rape (*Brassica napus* L.) is the most important temperate oil crop globally. Maintenance of soil phosphate (Pi) availability, through the application of Pi fertilizers and manures, is needed to maintain seed yield of oilseed rape. Over-application of the Pi fertilizers results in Pi accumulation in agricultural soils and adjacent ecosystems, where it can drive eutrophication in freshwater and coastal systems. In this study, two years of field experiments were conducted to explore the optimal Pi fertilizer application rate for four oilseed rape cultivars and the potential of nature-based solutions including Pi solubilizing bacteria (PSB) and rooting agent (RA) to reduce Pi fertilizer application rates for oilseed rape. The seed yields of cultivars Shengguang 168 (SG168) and Huayouza 9 (HYZ9) were significantly higher than those of cultivars Zhongyouza 19 (ZYZ19) and Zhongshuang 11 (ZS11) across all Pi application rates. In comparison with Farmers' fertilizer practice ($P_{26.2}$, 26.2 kg P ha^{-1}), Pi fertilizers could be reduced by more than 25% for the four cultivars, and be reduced by as much as 50% for SG168. The shoot dry weight and seed yield of ZS11 with the addition of RA at $P_{21.0}$ (21.0 kg P ha^{-1}) in Expt. 2-1 and $P_{15.7}$ (15.7 kg P ha^{-1}) in Expt. 2-2 showed no significant difference to that of $P_{26.2}$ at the ripening stage, but were significantly higher than that of $P_{21.0}$ and that of $P_{15.7}$, respectively. At P_0 (0 kg P ha^{-1}), addition of PSB significantly increased the shoot dry weight and seed yield of ZS11 at the ripening stage. However, at $P_{21.0}$ in Expt. 2-1 or at $P_{15.7}$ in Expt. 2-2, addition of PSB had no effect on shoot dry weight and seed yield of ZS11. These results highlighted the feasibility and potential to reduce the application rate and improve the use efficiency of Pi fertilizers in oilseed rape using nature-based solutions.

Keywords: oilseed rape; phosphate fertilizer; genotypes; rooting agent; phosphate solubilizing bacteria

1. Introduction

Phosphorus (P) is an essential macronutrient for plant development and reproduction (López-Bucio et al., 2000). Due to weathering and mineralization processes, concentrations of inorganic phosphate (orthophosphate; Pi, the only form of P assimilated by plants), across a range of soils are commonly suboptimal for vegetative growth and crop productivity (López-Arredondo et al., 2014). Approximately 70% of global cultivated land, including acidic and alkaline calcareous soils, suffers from low Pi availability (López-Arredondo et al., 2014).

Oilseed rape (*Brassica napus* L.) is not only an important oil crop, but also a potent source of high-quality vegetable proteins as an excellent feedstuff for farm animals, mainly ruminants (cattle, sheep, etc.) (Ramchiary et al., 2017). In China, oilseed rape production corresponds to an annual production of 5 million tons of edible oil (accounting for 55% of total vegetable oil production) and more than 6 million tons of high-quality protein feedstuff (Hu et al., 2017; Friedt et al., 2018). The Yangtze River Basin is the main growing area of oilseed rape in China, which has weathered acid soils (mostly ultisols and oxisols high in aluminum (Al) and iron (Fe) oxides) that have very low availabilities of Pi (Yan et al., 2006). Low Pi supply to oilseed rape causes variation in the color of old leaves, which darken and go purple, inhibits root and shoot growth and decreases plant height (PH) and effective branch number (BN), and finally significantly decreases seed yield (Zhang et al., 2010; Lyu et al., 2016; Yuan et al., 2016; Duan et al., 2020; Liu et al., 2021). Application of Pi fertilizers is therefore necessary to maintain the seed yield of oilseed rape (Nourgholipour et al., 2018; Cathcart, 2015; Van Vuuren et al., 2010).

In China, from 1980 to 2007, the average accumulation of P in soil each year was reported to be 242 kg P ha^{-1}, resulting in the average soil available P (Olsen-P) increasing from 7.4 to 24.7 mg kg^{-1} (Li et al., 2011). The increase in soil Olsen P occurs in all agroecological regions of China, ranging from 17.5 mg kg^{-1} in the middle-lower Yangtze plain to 25.4 mg kg^{-1} in South China (Li et al., 2011; Zhong et al., 2004; MacDonald et al., 2011). Oilseed rape production has been greatly improved by the increase of Pi fertilizer input, however, the overuse of Pi fertilizer not only causes low P use efficiency and the accumulation of P in soil, but increases environmental risk (Zhang et al., 2008 and 2012; Feng et al., 2019). Hence, it is imperative that we develop efficient systems based on genotypic differences and natural processes to increase, or at least maintain, production at minimal environmental cost.

Plants have evolved several strategies to enhance rhizosphere soil Pi availability, Pi uptake, translocation and utilization under P-deficient conditions (Wang et al., 2019). These include the enhancement of soil Pi availability and an increase of Pi uptake capacity. The former is achieved by rhizosphere acidification, secretion of organic acids (OAs) and hydrolytic enzymes (APase, RNase, and phytase) (Lambers et al., 2013; López-Arredondo et al., 2014). The latter is achieved by modifying root system architecture, by increasing the abundance of high-affinity Pi transporters, and by forming associations with arbuscular mycorrhizal (AM) fungi and beneficial rhizosphere microorganisms (Lynch et al., 1995; Niu et al., 2013; Bayle et al., 2011; Zhang et al., 2014, 2016 and 2018). These adaptive strategies provide opportunities for developing new methods to promote the efficient uptake and use of P when Pi is in short supply and, thereby, reduce the input of Pi fertilizer.

Nature-based solutions (NBS) refer to the sustainable management and use of natural features and processes to tackle socio-environmental challenges, which can improve nutrient use-efficiency (NUE), reduce nutrient loss and increase nutrient recapture and recycling, and are seen as a key innovation to deliver sustainable crop production (Keesstra et al., 2018). Root growth regulators (e.g. root agents) greatly increase the total root length, root surface area, root tip number, root branch number, root crossing number, and root volume, which can subsequently enhance nutrient uptake, plant growth and yield in crops (Lu et al., 2019; Cao et al., 2021). Moreover, plant roots recruit beneficial bacteria through the release of root exudates, in turn enhancing rhizosphere activity, promoting P mineralization, and increasing plant tolerance to P stress (Zhang et al., 2014; 2016; 2018). Phosphate solubilizing bacteria (PSB) secrete organic acids and enzymes that mineralize inorganic and organic P in the soil to increase the availability of rhizosphere Pi (Rezakhani et al., 2019; Zheng et al., 2019). PSB have effectively released insoluble Pi to promote biomass, yield and P accumulation in wheat (Zheng et al., 2019). In oilseed rape, a combination of PSB and biochar has also effectively improved the bacterial community activity, and significantly increased biomass and P accumulation (Wang et al., 2013).

In this study, four cultivars were used to characterize the root morphology, biomass, seed yield and P accumulation in oilseed rape when grown with different Pi fertilizer application rates in two years and at two experimental sites; and one of these cultivars was used to study the effect of a root agent (RA) and PSB on the above traits with a 20% reduction of farmers' fertilizer practice (FFP, $P_{26.2}$, 26.2 kg P ha^{-1}) in year 1 and a 40% reduction of FFP in year 2 at the same experimental site.

2. Materials and methods

2.1. Materials

Semi-winter oilseed rape (*Brassica napus* L.) cultivars Zhongshuang 11 (ZS11), Zhongyouza 19 (ZYZ19), Huayouza 9 (HYZ9) and Shengguang 168 (SG168) were employed in this study. They are widely planted in Yangtze river basin. ZS11 is an open-pollinated cultivar. ZYZ19, HYZ9 and SG168 are hybrid cultivars. ZS11 and ZYZ19 were provided by the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, and HYZ9 and SG168 were provided by the National Key Lab of Crop Genetic Improvement, Huazhong Agricultural University.

2.2. Experiment 1 (Expt. 1)

Expt. 1 was conducted to evaluate the effect of the Pi fertilizer application rates on shoot and root growth, seed yield and yield related traits, and Pi uptake of four oilseed rape cultivars. The field experiments were conducted at Wuxue Experimental Station (WX) (30.11°N, 115.61°E) and Qianjiang Experimental Station (QJ) (30.37°N, 112.91°E) in Hubei province, China, from Sep. 2017 to May 2018 and from Sep. 2018 to May 2019, respectively. The monthly average precipitation and monthly average temperatures (T_{avg}) in the growth seasons of oilseed rape at WX and QJ are shown in **Fig. S1**. In WX and QJ, the average monthly rainfall varied from 26.7 to 280.3 mm and from 16.8 to 205.3 mm, respectively; and the average monthly temperature ranged from 3.5 to 24.1℃ and from 4.0 to 24.5℃. The soil types at WX and QJ were sandy paddy soil and loamy paddy soil, respectively. At WX, the topsoil layer (0-20 cm) contained 8.93 g kg^{-1} organic matter, 61.50 mg kg^{-1} alkaline-hydrolysable nitrogen, 11.40 mg kg^{-1} Olsen-P, 78.67 mg kg^{-1} NH_4Ac-K, 1.12 g kg^{-1} total-P with soil pH of 6.38. The chemical characteristics at QJ were as follows: 4.66 g kg^{-1} organic matter, 37.40 mg kg^{-1} alkaline-hydrolysable nitrogen, 22.5 mg kg^{-1} Olsen-P, 95.98 mg kg^{-1} NH_4Ac-K, 0.63 g kg^{-1} total-P with soil pH of 6.01. The methods for analysis of soil nutrients all follow Bao (2016).

Field experiments at each experimental site were established by two factor split plot design with four replicates. The whole-plot factor was cultivar and the split-plot factor was Pi fertilizer application rate. The subplot size was 7.5 m length × 1.6 m width, with 30 cm row spacing and 30 cm plant spacing. Each subplot had 24 rows and 5 plants each row. The first row and the last row were edge plants. All the four cultivars were sown in a nursery bed in the field in mid-September and the seedlings were transplanted by hand 30 d after sowing.

There were six Pi treatments, namely (1) no Pi supply (P_0, 0 kg P ha^{-1}), (2) a quarter of the Pi application rate of the Farmers' fertilizer practice (FFP) ($P_{6.6}$, 6.6 kg P ha^{-1}), (3) a half of the Pi application rate of the FFP ($P_{13.1}$, 13.1 kg P ha^{-1}), (4) three-quarters the Pi application rate of FFP ($P_{19.7}$, 19.7 kg P ha^{-1}), (5) full FFP Pi application rate ($P_{26.2}$, 26.2 kg P ha^{-1}, based on a FFP rate of 26.2 kg P ha^{-1}) and (6) one and a quarter times the Pi application rate of FFP ($P_{32.8}$, 32.8 kg P ha^{-1}). All the subplots received basal fertilizer, including 60% of the total N applied (supplied as urea, N≥46%), and all the Pi as detailed above (supplied as monoammonium phosphate, P≥21%, N≥11%), K (supplied as potassium chloride, K≥49.8%) and boron (supplied as $Na_2B_4O_7 \cdot 10H_2O$). The application rates were as follows: N 108 kg N ha^{-1}, K 62.3 kg K ha^{-1} in QJ and 86.9 kg K ha^{-1} in WX and B 15 kg ha^{-1} borax. These fertilizers were thoroughly mixed and applied in bands near the crop rows as basal fertilizer before planting. The remaining 40% N was top dressed as urea during overwintering stage. Sowing date and harvest date were 26 September 2017 and 7 May 2018 in WX, and 25 September 2018 and 5 May 2019 in QJ. Other field management protocols were carried out in accordance with local practices (e.g. irrigation, application of herbicides and pesticides).

2.2. Experiment 2 (Expt. 2)

Expt. 2 was conducted to evaluate the effect of RA and PSB on shoot and root growth, seed yield and yield related traits, and Pi uptake of oilseed rape cultivar ZS11 with 20% (Expt. 2-1) or 40% (Expt. 2-2) less Pi application than FFP. The field experiments were conducted at WX (30.11°N, 115.61°E; 30.31°N, 115.75°E) in Hubei province, China, from September 2017 to May 2018 (Expt. 2-1) and from September 2018 to May 2019 (Expt. 2-2), respectively. This study was established as a complete randomized block design with three replicates. The plot size was 12.5 m length × 1.6 m width with 30 cm row spacing and 30 cm plant spacing. Each plot had 41 rows. The first row and the last row were edge plants. In Expt. 2-1, there were six treatments (1) no Pi supply (P_0, 0 kg P ha^{-1}), (2) P_0+PSB ($4.5*10^{\wedge 9}$ cfu (colony forming units) ha^{-1}), (3) Four-fifth the P application rate of FFP ($P_{21.0}$, 21.0 kg P ha^{-1}), (4) $P_{21.0}$+RA (1.5 L ha^{-1}), (5) $P_{21.0}$+PSB ($4.5*10^{\wedge 9}$ cfu ha^{-1}), (6) FFP ($P_{26.2}$, 26.2 kg P ha^{-1}). In Expt. 2-2, there were five treatments based on the results of Expt. 2-1 (1) no Pi supply (P_0, 0 kg P ha^{-1}), (2) Three-fifth the P application rate of FFP ($P_{15.7}$, 15.7 kg P ha^{-1}), (3) $P_{15.7}$+RA (1.5 L ha^{-1}), (4) $P_{15.7}$+PSB ($4.5*10^{\wedge 9}$ cfu ha^{-1}), (5) FFP ($P_{26.2}$, 26.2 kg P ha^{-1}). PSB was mainly *Bacillus amyloliquefaciens* that was mixed with maize straw, and the content of PSB was 3*10^9 cfu kg^{-1} straw. PSB was provided by Prof. Duanwei Zhu, Huazhong Agricultural University (Jiang, 2020). RA (根多乐, Root power) included mainly natural metabolites from functional microorganisms and a little boron and zinc, which was provided by Stoller (Qingdao) Agriculture Technology Co., Ltd (https://stollerchina.com/products.aspx). Other fertilization and field management practices were the same as Expt. 1.

In Expt. 2-1, the topsoil layer (0-20 cm) contained 9.5 g kg^{-1} organic matter, 57.2 mg kg^{-1} alkaline-hydrolysable nitrogen, 11.2 mg kg^{-1} Olsen-P, 82.9 mg kg^{-1} NH_4Ac-K and 0.76 g kg^{-1} total-P with soil pH of 6.20; in Expt. 2-2, the topsoil layer (0-20 cm) contained 6.60 g kg^{-1} organic matter, 75.10 mg kg^{-1} alkaline-hydrolysable nitrogen, 14.10 mg kg^{-1} Olsen-P, 159 mg kg^{-1} NH_4Ac-K and 0.86 g kg^{-1} total-P with soil pH of 5.80. In addition, in WX from September 2018 to May 2019 (Expt. 2-2), the average monthly rainfall varied from 19.5 to 213.5 mm; and the average monthly temperature ranged from 5.15 to 25.4℃.

2.3. Plant dry weight and P determination

Three plants per replicate were sampled at the leaf development stage, stem elongation stage, flowering stage and ripening stage according to the BBCH development scale for oilseed rape, respectively (Feller et al., 1994; Böttcher et al., 2016) **(Table S1)**. At the ripening stage, the sampled shoot was divided into stem, pericarp and seed. All the samples were dried at 105°C for 30 min and then at 65°C until a constant weight was attained. The dry weights were recorded and subsamples were milled and taken to measure tissue P concentration.

2.4. Root morphology

Roots were taken from excavated soil cubes at the leaf development stage, which were from a soil monolith of 30 cm × 30 cm × 30 cm. The roots in each cube were carefully removed from the bulk soil and kept in a nylon net bag and labeled, then the roots were washed with clean water to remove the rhizosphere soil. For morphological analyses, roots were placed in a plexiglass tray with a film of distilled water and scanned with a flat-panel scanner (Epson V700, Nagano-ken, Japan). The root images were analyzed using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). The root samples were washed with ddH_2O, then dried at 105℃ for 30 min and finally at

65℃ until a constant weight was attained to obtain root dry weight.

2.5. Seed yield and yield components

At ripening stage, the seed yield of oilseed rape in an area of 4.5 m^2 of each replicate was harvested to determine the seed yield. Among them, three plants from each plot were harvested to measure plant height (PH), branch number (BN), pod number (PN), 1000-seed weight (TSW) and seed yield per plant (DW-Se).

2.6. P determination and P efficiency

Tissue P concentrations were measured using the vanado-molybdate method (Westerman et al., 1990). The P concentration in plant tissues was expressed as mg g^{-1} DW. Tissue P accumulation equal to biomass of tissue multiplying by P concentration in same tissue.

P recovery efficiency (PRE) = (total P accumulation (kg P ha^{-1}) for shoot at Px – total P accumulation (kg P ha^{-1}) for shoot at P_0) / P_x (kg P ha^{-1}) at the ripening stage, here P_x are Pi supplies (kg P ha^{-1}); P0 is the plot without Pi supply (Chen et al., 2022)

P partial factor productivity (PPFP) = seed yield at P_x / P_x (kg P ha^{-1}), here P_x are Pi supplies (Chen et al., 2022)

P efficiency (PE) = P content per plant / total root length (0-0.3 m soil layer) (Duan et al., 2020)

Specific root length (SRL) = total root length/root dry weight (Fitter et al., 1976)

$P_{balance}$ = P_{input} (kg P ha^{-1}) – P_{output} (kg P ha^{-1}); where P_{input} included total P input to the soil from Pi fertilizers at the ripening stage, and P_{output} included the total P output from the soil at the ripening stage (Ma et al., 2018)

2.7. Statistical analysis

In Expt. 1, main and interaction effects of experimental factors, including experimental site, cultivar and Pi fertilizer application rate, were determined from analysis of variance (ANOVA) using the general linear model (GLM) procedure in SPSS 22.0 software (SPSS, USA). The univariate within SPSS was used to test the assumptions of ANOVA. Experimental site, cultivar and Pi fertilizer application rate were fixed factors. Main and interaction effects were given statistical significance at $p \leqslant 0.05$ by F-test. When the effects were significant, multiple comparisons using Tukey's HSD test were conducted and differences in different experimental sites, different cultivars and different Pi fertilizer application rates were considered to be significant at $p \leqslant 0.05$ (Wacker-Fester et al., 2019). Origin software (Origin 2022, USA) was used to fit models for seed yield with Pi fertilizer application rate, and DW-Se with shoot dry weight at each growth stage. The best-fit models returned the lowest *p*-value and the largest coefficient of determination (R^2) (Greenland, 2019). Graphs were generated with GraphPad 8.0 software (GraphPad, USA). The data are presented as mean ± standard error (SE) in the figures. Principal component analysis (PCA) was used to visually demonstrate separation of contrasting cultivars, based on key traits including TRL, RSA, TRL, RAD, SRL, PE, TP-L and SDW-L at the leaf development stage in six Pi supplies at WX and QJ, respectively. Correlation analysis was used to characterize the correlations among root morphologies (e.g. TRL, RSA and TRV, etc.), and seed yield and yield-related traits of all cultivars in all Pi supplies at WX and QJ, respectively. Pearson correlation analysis was performed on the tutools platform (https://www.cloudtutu.com) function “corr_heatmap”.

In Expt. 2, a one-way ANOVA was used to test the differences of dry weight, seed yield, tissue P content, P efficiency and root morphological traits among the six (or five) Pi treatments with least significant difference (Tukey’s HSD) at $p = 0.05$ level.

3. Results

3.1 Root morphology of four oilseed rape cultivars at the leaf development stage under different Pi application rates

Total root length (TRL), root surface area (RSA), total root volume (TRV), root average diameter (RAD) and P efficiency (PE) of all the four cultivars increased with increasing Pi application rate up to 19.7 kg P ha^{-1}, but TRL, RSA, RAD and PE did not change significantly when the P application rate was greater than 19.7 kg P ha^{-1} (**Fig. 1**). Specific root length (SRL) of all cultivars decreased significantly with increasing Pi application rate (**Fig. 1**). Compared with ZS11 and ZYZ19, TRL, RSA, TRV, RAD and PE of HYZ9 and SG168 increased by 14.5-37.6%, 25.3-59.4%, 25.9-54.0%, 10.0-14.4% and 14.0-41.3%, but SRL decreased by 13.4-16.9% under the different Pi application rates at WX, respectively (**Fig. 1a ~ f**). At QJ, compared with ZS11 and ZYZ19, TRL, RSA, TRV, RAD and PE of HYZ9 and SG168 increased by 23.5-28.1%, 19.5-33.7%, 13.4-30.1%, 11.7-17.9% and 26.5-39.1%, but SRL decreased by 22.9-43.4% under the different Pi application rates, respectively **(Fig. 1g ~ i)**. In addition, all the root morphological traits were significantly affected by experiment sites, Pi application rates, cultivars, and their interactions **(Table S2)**.

3.2 Differences in shoot dry weight and shoot P content among four oilseed rape cultivars grown with six Pi application rates

At both WX and QJ, the shoot dry weight of all cultivars increased with increasing Pi application rate at all the growth stages, and all cultivars had the largest shoot dry weight at the ripening stage at all Pi application rates **(Fig. 2)**. Shoot P content of all cultivars increased up until the flowering stage and then either did not change at WX, or decreased at QJ from the flowering stage to the ripening stage at all Pi application rates **(Fig. S2)**. ZS11 had the lowest shoot dry weight and shoot P content among all the cultivars from the leaf development stage to the ripening stage at all Pi application rates at both experiment sites **(Fig. 2 and S2)**. SG168 had the largest shoot dry weight among all cultivars from the leaf development stage to the ripening stage at all Pi application rates WX **(Fig. 2)**. Shoot dry weight and shoot P content were also significantly affected by experiment sites, Pi application rates and cultivars, and their interactions (**Table S2**).

Seed yield per plant had significant (p <0.05) positive correlations with shoot dry weight at all Pi application rates at all growth stages at both sites (**Fig. S3**). Biomass to yield conversion efficiency (seed yield per plant/ shoot dry weight per plant at each growth stage) was the highest at the leaf development stage at all Pi application rates, and decreased gradually with increasing Pi applications rates at each growth stage **(Fig. S3 and Table S3)**. At both experiment sites, DW-Se of HYZ9 and SG168 were greater than ZS11 and ZYZ19 at the same development stage at all Pi application rates **(Table S3)**.

3.3 Difference in the seed yield and seed yield related traits among four oilseed rape cultivars grown with six Pi application rates

Seed yield (SY) was significantly affected by experimental sites, Pi application rates, cultivars, and their interaction (**Table S2**). Pi application rate increased seed yield of all cultivars at both experiment sites **(Fig. 3)**. Moreover, seed yield was more at QJ than WX at all Pi application rates **(Fig. 3)**. Regression analysis of seed yield and Pi application rate showed that the Pi supplies with the highest yield of ZS11, ZYZ19 and SG168 were 15.2 kg P ha^{-1}, 18.5 kg P ha^{-1} and 12.4 kg P ha^{-}

^{1} at WX, respectively; and 13.1 kg P ha^{-1}, 14.9 kg P ha^{-1} and 12.2 kg P ha^{-1}at QJ, respectively **(Fig. 3 and Table S4)**. HYZ9 had the highest seed yield at 16.6 kg P ha^{-1} at QJ **(Fig. 3b and Table S4)**. Seed yield of HYZ9 increased with the increase of P application rate at WX, but did not reach the highest seed yield at $P_{32.8}$. HYZ9 and SG168 had higher seed yield than ZS11 and ZYZ19 at all Pi application rates **(Fig. 3)**. The Pi application rates to achieve the largest seed yield of SG168 were the lowest, and the highest for HYZ9 **(Table S4)**.

Significant interactions among experiment sites, Pi application rates and cultivars were observed for plant height, branch number, pod number, 1000-seed weight, seed weight per plant (DW-Se) and harvest index **(Table S2)**. The change of DW-Se and yield related traits, and root morphology traits of all cultivars with the increasing of Pi application rates were consistent at both experiment sites **(Fig. 1 and Table S5)**. The effect of Pi application rates on plant height and 1000-seed weight for all cultivars was small (<5%), but that on branch number and DW-Se was large **(Table S6)**. ZS11 had the largest coefficient of variations for plant height, branch number and DW-Se than other cultivars at both experiment sites **(Table S6)**. These indicated that ZS11 was more sensitive to Pi application rate. The coefficients of variations for these traits were lower at QJ than at WX for all cultivars **(Table S6)**.

3.4 Differences in P uptake and distribution among four oilseed rape cultivars grown with six Pi application rates during ripening stage

Total P content in seed (TP-Se), total P content in pericarp (TP-Pe) and total P content in stem (TP-St) of all cultivars increased with increasing Pi application rates at both experiment sites **(Fig. S4a and b)**. The TP-Se distribution ratio in shoot of all cultivars was higher than TP-Pe and TP-St at all Pi application rates, and the coefficients of variation associated with TP-Se for all cultivars were lower than those for TP-Pe and TP-St among all Pi application rates at both experiment sites **(Fig. S4c and d and Table S6)**. TP-Se distribution percentage decreased with increasing of Pi supply in all cultivars at both sites **(Fig. S4c and d)**. Moreover, the TP-Se distribution percentages in SG168 and HYZ9 were higher than those in ZS11 and ZYZ19 **(Fig. S4c and d).** Increasing Pi application rates had a greater impact on the TP-Se of ZS11 and SG168 than ZYZ19 and HYZ9 at both experiment sites **(Fig. S4a, b, c, d and Table S6)**. P contents (PC) of seed, pericarp and stem were significantly affected by experiment sites, Pi applications rates, cultivars, and their interactions

(Table S2).

3.5 Difference in P efficiency and $P_{balance}$ among four oilseed rape cultivars grown with six Pi application rates

P recovery efficiency (PRE), P partial factor productivity (PPFP) and $P_{balance}$ were also significantly affected by experiment sites, Pi applications rates, cultivars, and their interactions **(Table S2)**. The PRE and PPFP of all cultivars decreased gradually when the Pi application rate increased from 0 kg P ha^{-1} to 32.8 kg P ha^{-1} at both experiment sites **(Fig. 4a, b, d and e)**. HYZ9 and SG168 had higher PRE than ZS11 and ZYZ19 at all Pi application rates at both experiment sites **(Fig. 4a, and d)**.

$P_{balance}$ of all cultivars increased with increasing Pi application rate, and the $P_{balance}$ was lower in HYZ9 and SG168 than ZS11 and ZYZ19 at all Pi application rates at both experiment sites **(Fig. 4c and f)**. The threshold of Pi application rate for ZS11, which was used to represent the equivalent amount of Pi input and output, was the lowest, and highest for SG168 at both experiment sites **(Table S7)**. Furthermore, the thresholds of Pi application rates were lower than that of the Pi application rates of all cultivars that reached the highest seed yield in both experiments except for SG168 at QJ **(Table S4 and S7)**. The Pi application rate that gave the highest yield of all cultivars except for SG168 was more than the plant P content, and the remaining P could improve the basic fertility of the soil. The P absorption capacity in shoot of ZS11 at P_0 was the lowest (5.56 and 6.53 kg P ha^{-1} at WX and QJ, respectively), and that of HYZ9 was the highest (7.81 kg P ha^{-1} at WX) and that of SG168 was the highest (10.68 kg P ha^{-1} at QJ) among these cultivars **(Table S7)**. Therefore, HYZ9 and SG168 had a greater ability to take up P from the soil than other cultivars.

The relationships between target seed yield and soil Olsen-P concentration were determined based on the analysis of the seed yield and Pi application rates on two soil types with different Olsen-P concentrations **(Table 1, S4 and S7)**. The target seed yield of minimum, mean and maximum have been defined to be seed yield at no Pi supply, seed yield at the threshold Pi supply and the highest seed yield among these Pi supplies, respectively. The target seed yield of minimum, mean and maximum of four cultivars were greater grown in the soil with higher Olsen-P than in the soil with the low Olsen-P. In addition, the target seed yield of minimum, mean and maximum of SG168 and HYZ9 were all greater than that of ZS11 and ZYZ19 at both QJ and WX. The P application rates of the target seed yield of maximum was lower, but shoot P content was larger in SG168 and HYZ9 than ZS11 and ZYZ19 **(Table 1)**. $P_{balance}$ of the target seed yield of minimum was lower in SG168 and HYZ9 than ZS11 and ZYZ19 at both sites. The $P_{balance}$ of the target seed yield of maximum was the lowest in SG168 at both sites, and the P_{output} is greater than the P_{input} in SG168 at QJ **(Table 1)**. P required ratio (ratio between P application rate and shoot P content) of the target seed yield of maximum was lower at QJ than WX; and that of SG168 was the lowest and that of HYZ9 was the largest among the four cultivars **(Table 1)**.

3.6 Correlation between seed yield and yield related traits of oilseed rape and root morphology parameters at the development stage

Pearson's correlation showed that seed yield, DW-Se, DW-Pe, DW-St and shoot dry weight were significantly correlated (positive) with TRL, RSA, TRV, RAD and P efficiency, but correlated (negative) with specific root length at both experiment sites (p<0.001) (Figs. 5c and d). Principal components analysis (PCA) was also conducted to analyze the relationship between Pi uptake and root morphology traits in the four oilseed rape cultivars at WX and QJ **(Fig. 5a and b)**. The variance explained by the first two principal components was 79.9% for PC1 and 7.1% for PC2 at WX, and 70.2% for PC1and 21.1% for PC2 at QJ **(Fig. 5a and b)**. SG168 and HYZ9 were located in the left, and ZS11 and ZYZ19 were located in the right on the PCA plot **(Fig. 5a and b).** PCA explained 87% and 91.3% of the phenotypic variation of genotype at WX and QJ, respectively. These analyses demonstrate that the root system traits at the leaf development stage could be used as a positive indicator of seed yield and agronomic traits. Promoting the growth of roots at the leaf development stage could be an important way to reduce the input of Pi fertilizer, and increase PRE and PPFP, while decreasing the $P_{balance}$, and producing higher seed yield.

3.7 Effect of RA and PSB on ZS11 at a reduction of 20% (or 40%) P application rate of FFP

In Expt. 2-1, compared with P_0, Pi application increased TRL, RSA, TRV and RAD of ZS11 **(Table 2)**. Addition of PSB at P_0 also significantly enhanced root growth, reduced the SRL by 16.6% and increased the PE by 63.6%. However, addition of PSB with $P_{21.0}$ had no effect on root traits compared with $P_{21.0}$ **(Table 2)**. Compared with $P_{21.0}$, addition of RA with $P_{21.0}$ significantly improved the root growth and PE (by 49.4%), and reduced the SRL ratio by 24.5% **(Table 2)**. There was no significant difference in the investigated root traits between the treatment of $P_{21.0}$+RA and $P_{26.2}$

(Table 2). In Expt. 2-2, addition of PSB or RA with $P_{15.7}$ both significantly increased root traits including TRL, RSA, TRV and RAD **(Table 2)**.

The effects of Pi application on shoot dry weight, shoot P content and yield-related traits and on root morphology traits were consistent across all growth stages in both Expt. 2-1 and Expt. 2-2 **(Table 2, S8 and Fig. S5)**. Addition of PSB at P_0 significantly increased biomass, yield related traits and total P content in seed and pericarp of ZS11 at the ripening stage, and finally increased seed yield by 13.7% and decreased $P_{balance}$ by 32.2% **(Fig. 6a ~ c, g and Table S8)**. However, addition of PSB at $P_{21.0}$ did not increase these traits compared with $P_{21.0}$ in Expt. 2-1 **(Fig. 6a ~ c, g and Table S8)**. Interestingly, addition of PSB at $P_{15.7}$ increased pod number, dry weight, total P content, and finally the seed yield compared with $P_{15.7}$ in Expt. 2-2 (**Fig. 6d ~ f, i and Table S8**). Application of RA at $P_{21.0}$ in Expt. 2-1 and at $P_{15.7}$ in Expt. 2-2 both increased biomass and P content in all tissues of ZS11, promoted seed yield by 5.3% and 6.1%, respectively, and greatly reduced $P_{balance}$ by 20.7% and 200%, respectively **(Fig. 6a ~ f, g and i)**. There was no significant difference in biomass, tissue P content and seed yield of ZS11 between $P_{21.0}$+RA and $P_{26.2}$ in Expt. 2-1 and between $P_{15.7}$+RA and $P_{26.2}$ in Expt.2-2, however, addition of RA at $P_{21.0}$ in Expt. 2-1 and at $P_{15.7}$ in Expt. 2-2 both sharply reduced $P_{balance}$ compared to $P_{26.2}$ **(Fig. 6a ~ f, g and i)**. Seed yield and yield related traits were significantly correlated with the root morphology traits (e.g. TRL, RSA, TRV) **(Figs. 6h and j)**.

4. Discussion

4.1 Reducing the P application rate with P-efficient oilseed rape cultivars

The challenge of supplying sufficient P to meet agricultural demands worldwide without degrading freshwater resources is a key issue for agriculture in the 21st century (Macdonald et al., 2011). In this study, the seed yield of four oilseed rape cultivars all showed the characteristics of an initial linear increase followed by a plateau with increasing Pi application rate **(Fig. 3)**, which are in accordance with reports by Lewis et al. (1987) and Zhao et al. (2021). Pi fertilizers are applied to soils to meet the nutritional requirement of crops and replace nutrients lost in grain and straw at harvest (Gilbert et al., 2009). In this study, the Pi application rates to achieve the highest yield of all cultivars were lower than the Pi application rate of FFP (26.2 kg P ha^{-1}) at both WX and QJ **(Table S4)**. Thus, the current Pi application rate of FFP in oilseed rape is not supported by our data, as has also been observed by Li et al. (2011), Ren et al. (2015) and Xie et al. (2016). Olsen-P at QJ (22.5

mg kg^{-1}) was higher than that at WX (11.4 mg kg^{-1}), which is why SG168 could achieve the highest yield at less P application at QJ than at WX **(Table S4 and S7)**.

There were significant differences in the seed yield of the four oilseed rape cultivars at different Pi application rates. Both SG168 and HYZ9 had larger root morphology traits (e.g. TRL, RSA and TRV, etc.) and achieved higher seed yield than ZS11 and ZYZ19 at all Pi application rates at both experiment sites **(Fig. 1 and 3)**. In this study, the Pi application rates to achieve the highest yield of SG168 were lower than that of ZS11 at both experiment sites **(Fig. 3)**. In a previous study, the seed yield of the P-efficient cultivar Bristol was also found to be higher than the P-inefficient cultivar Lirajet at the same Pi supply, and the Pi supply producing the highest seed yield of Bristol was lower than Lirajet (Lickfett et al., 1999). These observations suggested that employing P-efficient cultivars of oilseed rape could be an important strategy for reducing Pi application rates for zero-pollution agriculture.

4.2 Reducing the P application rate with RA and PSB

Barraclough et al (1989) reported that the average root length density (RLD) of oilseed rape in the top 20 cm of soil is 8 cm cm^{-3}, and only 0.35 cm cm^{-3} at 60–180 cm of soil. In another study, the root length of oilseed rape in the top 40 cm of soil accounted for 70% of the total root length in the total 100 cm of soil (White et al 2015). The 0-30 cm soil layer root of oilseed rape was sampled in the studies of Gu et al (2016, 2019) and Arifuzzaman et al (2019). Although the 0-30 cm soil layer root does not include all the root system of oilseed rape in the soil, it should reflect the difference in the root morphology of oilseed rape between different treatments (Liu et al 2011; Chen et al 2021). Thus, in the present study, the root system of oilseed rape was excavated to a depth of 30 cm in the field to measure root morphological traits in this layer.

Due to the low mobility of Pi in the soil, root system architecture is closely related to Pi acquisition (Shen et al., 2011; Lynch et al., 2011; Lambers et al., 2006; White et al., 2013; Wang et al., 2017). Previous results have shown that seed yield, shoot dry weight and total P content of oilseed rape are strongly correlated with root morphological traits in the surface soil (Koscielny et al., 2012; Thomas et al., 2016; Duan et al., 2020). In this study, DW-Se and yield related traits of all oilseed rape cultivars were significantly correlated (positive) with their root morphology traits including TRL, RSA, TRV and RAD at the leaf development stage **(Fig. 5c and d)**. Therefore, in this study, RA was adopted to promote a larger root system for increasing the acquisition of Pi in the soil **(Table 2)**.

In the current study, there was no significant difference in biomass, tissue P content and seed yield of oilseed rape between $P_{21.0}$+RA and $P_{26.2}$ in Expt. 2-1 and between $P_{15.7}$+RA and $P_{26.2}$ in Expt.2-2 **(Fig. 6a ~ f)**. Application of RA at reduced Pi supply significantly improved the root morphological traits **(Table 2)** and the seed yield **(Fig. 6c and f)**. Root growth regulators have also been reported to greatly increase TRL, TRV, RSA, root tip number, root branch number and root crossing number of vegetables, cash crops and food crops, and ultimately enhance plant growth (Lu et al., 2019; Cao et al., 2021). For example, β-cyclocitral promotes the growth of main roots and lateral roots of *Arabidopsis* and rice to enhance its tolerance to environmental stresses, and auxin regulators promote the development of root hairs of *Arabidopsis* (Dickinson et al., 2019, Rahman et al., 2002). These indicated that RA could be an effective strategy to maintain the seed yield of oilseed rape at reduced Pi supply **(Fig. 6c and f)**.

In addition, addition of PSB effectively stimulated root growth, increased shoot dry weight and P content at each growth stage, and finally enhanced seed yield with no additional Pi applied in Expt. 2-1 and at $P_{15.7}$ in Expt. 2-2 **(Table 2, Fig. 6c, f and S5)**. PSB have been reported to promote the mineralization of soil P, increases the availability of soil available P, and improve plant P absorption and biomass accumulation in oilseed rape, wheat and maize (Zheng et al., 2019; Rezakhani et al., 2019; Pande et al., 2017). However, addition of PSB with $P_{21.0}$ had no significant effect on shoot dry weight, tissue P content and seed yield compared to $P_{21.0}$ in Expt. 2-1 **(Fig. 6a ~ c)**. The reasons could be attributed to two aspects, one is that sufficient soil available Pi inhibited PSB growth, or sufficient soil Pi was available to drive crop growth. PSB can release Pi in the soil and promote Pi uptake by plants in soils with low Pi availabilities (Zhang et al., 2016; 2018), however, the relative abundance of PSB was decreased, and PSB does not participate in the process of P solubilization in soils with sufficient Pi availabilities (Adnan et al., 2020; Tan et al., 2013; Mander et al., 2012). These observations indicate that it is valuable to evaluate the optimal conditions for PSB in oilseed rape production.

5. Conclusions

Pi application increased the seed yield of oilseed rape at both experiment sites. Under the current status of soil fertility, a reduction of 25.0-52.3% Pi at WX and 36.7-53.3% Pi at QJ compared with FFP ($P_{26.2}$) could be achieved for the oilseed rape cultivars tested here. P-efficient cultivars, RA and PSB could offer nature-based solutions to reduce the Pi fertilizer application rates in the future. Novel innovation packages should be generated combining these nature-based solutions to enhance P utilization efficiency in oilseed rape cropping systems, reduce nutrient loss from the system and allow for recapture and recycling of nutrients contributing to waste and pollution from existing systems.

Competing financial interests

The authors declare no competing financial interests.

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References

Adnan, M., Fahad, S., Zamin, M., Shah, S., Battaglia, M.L., Mian, I.A., Danish, S., Zafar-Ul-Hye , M., Battaglia, M.L., Naz, R.M.M, Saeed, B., Saud, S., Ahmad, I., Yue, Z., Brtnicky, M., Holatko, J., Datta, R. (2020). Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. Plants, 9(7), 900. https://doi.org/10.3390/plants9070900.

Arifuzzaman, M., Oladzadabbasabadi, A., McClean, P., Rahman, M. (2019). Shovelomics for phenotyping root architectural traits of rapeseed/canola (*Brassica napus* L.) and genome-wide association mapping. Molecular Genetics and Genomics, 294(4), 985–1000. https://doi.org/10.1007/s00438-019-01563-x

Barraclough, P.B. (1989). Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. Plant and Soil, 119(1), 59–70. https://doi.org/10.1007/BF02370269

Bayle, V., Arrighi, J.F., Creff, A., Nespoulous, C., Vialaret, J., Rossignol, M., Gonzalez, E., Paz-Ares, J., Nussaume, L. (2011). *Arabidopsis thaliana* high-affinity phosphate transporters exhibit multiple levels of posttranslational regulation. Plant Cell, 23(4), 1523–1535. https://doi.org/10.1105/tpc.110.081067

Bao, S.D. (2016). Soil and agricultural chemistry analysis (third edition). Beijing: China Agricultural Press, pp. 25-114.

Böttcher, U., Rampin, E., Hartmann, K., Zanetti, F., Flenet, F., Morison, M., Kage, H. (2016). A phenological model of winter oilseed rape according to the BBCH scale. Crop and Pasture Science, 67(3), 345–358. https://doi.org/10.1071/CP15321

Cathcart, J.B. (2015). World phosphate reserves and resources. The Role of Phosphorus in Agriculture, 1-18. https://doi.org/10.2134/1980.roleofphosphorus.c1

Chen, J., Wu, Z., Zhao, T., Yang, H., Long, Q., He, Y. (2021). Rotation crop root performance and its effect on soil hydraulic properties in a clayey Utisol. Soil and Tillage Research, 213(6), 105136. https://doi.org/10.1016/j.still.2021.105136

Chen, X., Liu, P., Zhao, B., Zhang, J., Ren, B., Li, Z., Wang, Z. (2022). Root physiological adaptations that enhance the grain yield and nutrient use efficiency of maize (*Zea mays* L) and their dependency on phosphorus placement depth. Field Crops Research, 1(276), 108378. https://doi.org/10.1016/j.fcr.2021.108378

Cao, J., Liu, B., Xu, X., Zhang, X., Zhu, C., Li, Y., Ding, X. (2021). Plant endophytic fungus extract znc improved potato immunity, yield, and quality. Frontiers in Plant Science, 12(9), 1–13. https://doi.org/10.3389/fpls.2021.707256

Dickinson, A.J., Lehner, K., Mi, J., Jia, K.P., Mijar, M., Dinneny, J., Al-Babili, S., Benfey, P.N. (2019). β-Cyclocitral is a conserved root growth regulator. Proceedings of the National Academy of Sciences of the United States of America, 116(21), 10563-10567. https://doi.org/10.1073/pnas.1821445116

Duan, X., Jin, K.M., Ding, G.D., Wang, C., Cai, H.M., Wang, S.L., White, P.J., Xu, F.S., Shi, L. (2020). The impact of different morphological and biochemical root traits on phosphorus acquisition and seed yield of *Brassica napus*. Field Crops Research, 1(258), 107960. https://doi.org/10.1016/j.fcr.2020.107960

Feller, C., Bleiholder, H., Frau, E., Hess, M., Wicke, H., Meier, U., Lancashire, P. D. (1994). Growth stages of mono-and dicotyledonous plants. BBCH monograph. Federal Biological Research Centre for Agriculture and Forestry. https://www.reterurale.it/downloads/BBCH_engl_2001.pdf

Feng, G., Gai, J., Feng, X., Li, H., Zhang, L., Yi, K., Lv, J., Zhu, Y., Tang, L., Li, Y. (2019). Strategies for improving fertilizer phosphorus use efficiency in Chinese cropping systems. Frontiers of Agricultural Science and Engineering, 6(4), 341-347. https://doi.org/10.15302/J-FASE-2019280

Fitter, A.H. (1976). Effects of nutrient supply and competition from other species on root growth of *Lolium perenne* in soil. Plant and Soil, 45(1), 177-189. https://doi.org/10.1007/BF00011140

Friedt, W., Tu, J., Fu, T. (2018). Academic and economic importance of *Brassica napus* rapeseed. The *Brassica napus* Genome. 1-20. https://doi.org/10.1007/978-3-319-43694-4_1

Gilbert, N. (2009). The disappearing nutrient. Nature, 461(7265), 716-718. https://doi.org/10.1038/461716a

Greenland, S. (2019). Valid p-values behave exactly as they should: some misleading criticisms of p-values and their resolution with s-values. The American Statistician, 73(1), 106–114. https://doi.org/10.1080/00031305.2018.1529625

Gu, X.B., Li, Y.N., Du, Y.D. (2016). Continuous ridges with film mulching improve soil water content, root growth, seed yield and water use efficiency of winter oilseed rape. Industrial Crops and Products, 85, 139–148. https://doi.org/10.1016/j.indcrop.2016.02.056

Gu, X.B., Cai, H.J., Du, Y.D., Li, Y.N. (2019). Effects of film mulching and nitrogen fertilization on rhizosphere soil environment, root growth and nutrient uptake of winter oilseed rape in northwest China. Soil and Tillage Research, 187(23), 194–203. https://doi.org/10.1016/j.still.2018.12.009

Hu, Q., Hua, W., Yin, Y., Zhang, X.K., Liu, L.J., Shi, J.Q., Zhao, Y.G., Qin, L., Chen, C., Wang, H.Z. (2017). Rapeseed research and production in china. Crop Journal, 5, 127-135. https://doi.org/10.1016/j.cj.2016.06.005

Jiang, R. (2020). Study on optimization of fermentation process, preservation technology and application for production of microbial agent of phosphate-solubilizing bacteria PC06 by using biogas slurry from pig farm. https://doi.org/10.27158/d.cnki.ghznu.2019.000547

Keesstra, S., Nunes, J., Novara, A., Finger, D., Avelar, D., Kalantari, Z., Cerdà, A. (2018). The superior effect of nature based solutions in land management for enhancing ecosystem services. Science of the Total Environment, 610, 997-1009. https://doi.org/10.1016/j.scitotenv.2017.08.077

Koscielny, C.B., Gulden, R.H. (2012). Seedling root length in *Brassica napus* L. is indicative of seed yield. Canadian Journal of Plant Science, 92, 1229-1237. https://doi.org/10.4141/cjps2012-070

Lynch, J. (1995). Root architecture and plant productivity. Plant Physiology, 109(1), 7–13. https://doi.org/10.1104/pp.109.1.7

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J. (2006). Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Annals of Botany, 98, 693-713. https://doi.org/10.1093/aob/mcl114

Lambers, H., Clements, J.C., Nelson, M.N. (2013). How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus, Fabaceae*). American Journal of Botany, 100(2), 263–288. https://doi.org/10.3732/ajb.1200474

Lewis, D.C., Potter, T.D., Weckert, S.E., Grant, I.L. (1987). Effect of nitrogen and phosphorus fertilizers on the seed yield and oil concentration of oilseed rape (*Brassica napus* L.) and the prediction of responses by soil tests and past paddock use. Australian Journal of Experimental Agriculture, 27(5), 713-720. https://doi.org/10.1071/EA9870713

Li, Y.S. (2011). Effect of phosphorus application rate on yield and fertilizer-phosphorus utilization efficiency in rapeseed. Chinese Journal of Oil Crop Sciences, 33(1), 52-56.

Li, H., Huang, G., Meng, Q., Ma, L., Yuan, L., Wang, F., Zhang, W., Cui, Z., Shen, J., Chen, X., Jiang, R., Zhang, F. (2011). Integrated soil and plant phosphorus management for crop and environment in china. Plant and Soil, 349(1), 157-167. https://doi.org/10.1007/s11104-011-0909-5

Lickfett, T., Matthäus, B., Velasco, L., Möllersc, C. (1999). Seed yield, oil and phytate concentration in the seeds of two oilseed rape cultivars as affected by different phosphorus supply. European Journal of Agronomy, 11(3), 293-299. https://doi.org/10.1016/S1161-0301(99)00038-6

Liu, H.J., Wang, J.C., Zhang, B.B, Yang, X.Y, Hammond, J.P., Ding, G.D., Wang, S.L., Cai, H.M., Wang, C., Xu, F.S., Shi, L. (2021). Genome-wide association study dissects the genetic control of plant height and branch number in response to low-phosphorus stress in *Brassica napus*. Annals of Botany, 128(7), 919-930. https://doi.org/10.1093/aob/mcab115

Liu, L., Gan, Y., Bueckert, R., Van Rees, K. (2011). Rooting systems of oilseed and pulse crops. II: Vertical distribution patterns across the soil profile. Field Crops Research, 122(3), 248–255. https://doi.org/10.1016/j.fcr.2011.04.003

López-Arredondo, D.L., Leyva-González, M.A., González-Morales, S.I., López-Bucio, J., Herrera-Estrella, L. (2014). Phosphate nutrition: improving low-phosphate tolerance in crops. Annual Review of Plant Biology, 65, 95-123. https://doi.org/10.1146/annurev-arplant-050213-035949

López-Bucio, J., De la Vega, O.M., Guevara-García, A., Herrera-Estrella, L. (2000). Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nature Biotechnology, 18(4), 450-453. https://doi.org/10.1038/74531

Lu, C., Liu, H., Jiang, D., Wang, L., Jiang, Y., Tang, S., Ding, X. (2019). *Paecilomyces variotii* extracts (ZNC) enhance plant immunity and promote plant growth. Plant and Soil, 441(1), 383–397. https://doi.org/10.1007/s11104-019-04130-w

Lynch, J.P. (2011). Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. Plant Physiology. 156, 1041-1049. https://doi.org/10.1104/pp.111.175414

Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W.R., Shen, J. (2016). Major crop species show differential balance between root morphological and physiological responses to variable phosphorus supply. Frontiers in Plant Science, 7(1), 1-15. https://doi.org/10.3389/fpls.2016.01939

Ma, J., Liu, Y., He, W., He, P., Haygarth, P.M., Surridge, B.W.J., Lei, Q., Zhou, W. (2018). The long-term soil phosphorus balance across Chinese arable land. Soil Use and Management, 34(3), 306-315. https://doi.org/10.1111/sum.12438

MacDonald, G.K., Bennett, E.M., Potter, P.A., Ramankutty, N. (2011). Agronomic phosphorus imbalances across the world's croplands. Proceedings of the National Academy of Sciences of the United States of America, 108(7), 3086-3091. https://doi.org/10.1073/pnas.1010808108

Mander, C., Wakelin, S., Young, S., Condron, L., O'Callaghan, M. (2012). Incidence and diversity of phosphate-solubilizing bacteria are linked to phosphorus status in grassland soils. Soil Biology and Biochemistry, 44(1), 93–101. https://doi.org/10.1016/j.soilbio.2011.09.009

Niu, Y.F., Chai, R.S., Jin, G.L., Wang, H., Tang, C.X., Zhang, Y.S. (2013). Responses of root architecture development to low phosphorus availability: A review. Annals of Botany, 112(2), 391–408. https://doi.org/10.1093/aob/mcs285

Nourgholipour, F., Mirseyed Hosseini, H., Tehrani, M.M., Motesharezadeh, B., Moshiri, F. (2018). Comparison of phosphorus efficiency among spring oilseed rape cultivars in response to phosphorus deficiency. New Zealand Journal of Crop and Horticultural Science, 46(1), 54-71. https://doi.org/10.1080/01140671.2017.1343192

Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S. (2017). Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. Journal of Genetic Engineering and Biotechnology, 15(2), 379-391. https://doi.org/10.1016/j.jgeb.2017.06.005

Rahman, A., Hosokawa, S., Oono, Y., Amakawa, T., Goto, N., Tsurumi, S. (2002). Auxin and ethylene response interactions during *Arabidopsis* root hair development dissected by auxin influx modulators. Plant Physiology, 130(4), 1908-1917. https://doi.org/10.1104/pp.010546

Ramchiary, N., Kole, C. (2017). Compendium of plant genomes the Brassica genome. The Brassica Genome. https://link.springer.com/content/pdf/10.1007%2F978-3-319-66117-9.pdf

Ren, T., Li, H., Lu, J., Bu, R., Li, X., Cong, R., Lu, M. (2015). Crop rotation-dependent yield responses to fertilization in winter oilseed rape (*Brassica napus* L.). Crop Journal, 3(5), 396-404. https://doi.org/10.1016/j.cj.2015.04.007

Rezakhani, L., Motesharezadeh, B., Tehrani, M.M., Etesami, H., Hosseini, H.M. (2019). Phosphate-solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (*Triticum aestivum* L.) plant fertilized with soluble or insoluble P source. Ecotoxicology and Environmental Safety, 173, 504-513. https://doi.org/10.1016/j.ecoenv.2019.02.060

Shen, J.B., Yuan, L.X., Zhang, J.L., Li, H.G., Bai, Z.H., Chen, X.P., Zhang, W.F., Zhang, F.S. (2011). Phosphorus dynamics: From soil to plant. Plant Physiology. 111, 997-1005. https://doi.org/10.1104/pp.111.175232

Thomas, C.L., Graham, N.S., Hayden, R., Meacham, M.C., Neugebauer, K., Nightingale, M., Dupuy, L.X., Hammond, J.P., White, P.J., Broadley, M.R. (2016). High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (*Brassica napus* L.). Annals of Botany, 118, 655-665. https://doi.org/10.1093/aob/mcw046

Tan, H., Barret, M., Mooij, M. J., Rice, O., Morrissey, J. P., Dobson, A., Griffiths, B., O'Gara, F. (2013). Long-term phosphorus fertilization increased the diversity of the total bacterial community and the phoD phosphorus mineralizer group in pasture soils. Biology and Fertility of Soils, 49(6), 661–672. https://doi.org/10.1007/s00374-012-0755-5

Van Vuuren, D.P., Bouwman, A.F., Beusen, A.H.W. (2010). Phosphorus demand for the 1970-2100 period: a scenario analysis of resource depletion. Global Environmental Change, 20(3), 428-439. https://doi.org/10.1016/j.gloenvcha.2010.04.004

Wacker-Fester, K., Uptmoor, R., Pfahler, V., Dehmer, K.J., Bachmann-Pfabe, S., Kavka, M. (2019). Genotype-Specific Differences in Phosphorus Efficiency of Potato (*Solanum tuberosum* L.). Frontiers in Plant Science, 10. https://doi.org/10.3389/fpls.2019.01029

Wang, F., Jiang, R., Kertesz, M.A., Zhang, F., Feng, G. (2013). Arbuscular mycorrhizal fungal hyphae mediating acidification can promote phytate mineralization in the hyphosphere of maize (*Zea mays* L.). Soil Biology and Biochemistry, 65, 69-74. https://doi.org/10.1016/j.soilbio.2013.05.010

Wang, X.H., Chen, Y.L., Thomas, C.L., Ding, G.D., Xu, P., Shi, D.X., Grandke, F., Jin, K.M., Cai, H.M., Xu, F.S., Yi, B., Broadley, M.R., Shi, L. (2017). Genetic variants associated with the root system architecture of oilseed rape (*Brassica napus* L.) under contrasting phosphate supply. DNA Research, 24(4), 407-417. https://doi.org/10.1093/dnares/dsx013

Wang, W., Ding, G.D., White, P.J., Wang, X.H., Jin, K.M., Xu, F.S., Shi, L. (2019). Mapping and cloning of quantitative trait loci for phosphorus efficiency in crops: opportunities and challenges. Plant and Soil, 439(1-2), 91-112. https://doi.org/10.1007/s11104-018-3706-6

Westerman, R.L. (1990). Soil testing and plant analysis. 3rd Edition. WI: Soil Science Society of America. https://doi.org/10.2136/sssabookser3.3ed

White, P.J., George, T.S., Dupuy, L.X., Karley, A.J., Valentine, T.A., Wiesel, L., Wishart, J. (2013). Root traits for infertile soils. Frontier in Plant Science. 4, 193. https://doi.org/10.3389/fpls.2013.00193

White, C.A., Sylvester-Bradley, R., Berry, P.M. (2015). Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. Journal of Experimental Botany, 66(8), 2293–2303. https://doi.org/10.1093/jxb/erv077

Xie, Y., Niu, X., Niu, J. (2016). Effect of phosphorus fertilizer on growth, phosphorus uptake, seed yield, yield components, and phosphorus use efficiency of oilseed flax. Agronomy Journal, 108(3), 1257-1266. https://doi.org/10.2134/agronj2015.0537

Yan, X.L., Wu, P., Ling, H.Q., Xu, G.H., Xu, F.S., Zhang, Q.F. (2006). Plant nutriomics in China: An overview. Annals of Botany, 98, 473-482. https://doi.org/10.1093/aob/mcl116

Yuan, P., Ding, G.Da, Cai, H.M., Jin, K.M., Broadley, M.R., Xu, F.S., Shi, L. (2016). A novel brassica-rhizotron system to unravel the dynamic changes in root system architecture of oilseed rape under phosphorus deficiency. Annals of Botany, 118(2), 173-184. https://doi.org/10.1093/aob/mcw083

Zhang, H.W., Huang, Y., Ye, X.S., Xu, F.S. (2010). Analysis of the contribution of acid phosphatase to P efficiency in *Brassica napus* under low phosphorus conditions. Science China Life Sciences, 53(6), 709-717. https://doi.org/10.1007/s11427-010-4008-2

Zhang, F., Wang, J., Zhang, W., Cui, Z., Ma, W., Chen, X., Jiang, R. (2008). Nutrient use efficiencies of major cereal crops in china and measures for improvement. Acta Pedologica Sinica, 45. 915-924.

Zhang, F., Cui, Z., Chen, X., Ju, X., Shen, J., Chen, Q., Liu, X., Zhang, W., Mi, G., Fan, M., Jiang, R. (2012). Integrated nutrient management for food security and environmental quality in China. In Advances in Agronomy, 116, 1-40. https://doi.org/10.1016/B978-0-12-394277-7.00001-4

Zhang, L., Fan, J., Ding, X., He, X., Zhang, F., Feng, G. (2014). Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium promote phytate mineralization in soil. Soil Biology and Biochemistry, 74, 177-183. https://doi.org/10.1016/j.soilbio.2014.03.004

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., Feng, G. (2016). Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytologist, 210(3), 1022-1032. https://doi.org/10.1111/nph.13838

Zhang, L., Feng, G., Declerck, S. (2018). Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate-solubilizing bacterium. Multidisciplinary Journal of Microbial Ecology, 12(10), 2339-2351. https://doi.org/10.1038/s41396-018-0171-4

Zhao, Z., Wang, Y.Q., Shi, J.Q., Wang, S.L., White, P.J., Shi, L., Xu, F.S. (2021). Effect of balanced application of boron and phosphorus fertilizers on soil bacterial community, seed yield and phosphorus use efficiency of *Brassica napus*. Science of the Total Environment, 751, 141644. https://doi.org/10.1016/j.scitotenv.2020.141644

Zheng, B.X., Ding, K., Yang, X.R., Wadaan, M.A.M., Hozzein, W.N., Peñuelas, J., Zhu, Y.G. (2019). Straw biochar increases the abundance of inorganic phosphate solubilizing bacterial community for better rape (*Brassica napus*) growth and phosphate uptake. Science of the Total Environment, 647, 1113-1120. https://doi.org/10.1016/j.scitotenv.2018.07.454

Zhong, X.Y., Zhao, X.R., Bao, B.J., Li, H.H., Li, G.T., Lin, L. (2004). The evaluation of phosphorus leaching risk of 23 Chinese soils I. Leaching criterion. Acta Ecologica Sinica, 24(10), 2270-2280.

Figure Legends

Fig. 1. TRL (a, g), RSA (b, h), TRV (c, i), RAD (d, j), SRL (e, k) and PE (f, i) of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at leaf development stage under six phosphate (Pi) application rates at Wuxue (a, b, c, d, e, f) and Qianjiang (g, h, i, j, k, l), respectively.

Fig. 2. Shoot dry weight of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 grown under six phosphate (Pi) application rates at Wuxue (a, b, c, d) and Qianjiang (e, f, g, h), respectively.

Fig. 3. Effect of phosphate (Pi) application rates on seed yield of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at Wuxue (a) and Qianjiang (b), respectively.

Fig. 4. Phosphorus (P) recovery efficiency (PRE) (a, d), P partial factor productivity (PPFP) (b, e) and $P_{balance}$ (c, f) of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at ripening stage under six Pi application rates at Wuxue (a, b, c) and Qianjiang (d, e, f), respectively.

Fig. 5. Principal component analysis (PCA) plot showing the relationship among root morphology traits and P uptake in all oilseed rape cultivars at Wuxue (a) and Qianjiang (b), respectively; and the correlation among seed yield and yield related traits of oilseed rape cultivars and the root morphology traits at leaf development stage at Wuxue (c) and Qianjiang (d), respectively.

Fig. 6. Difference in the investigated traits of oilseed rape cultivar ZS11 among six treatments in 2017-2018 (Expt. 2-1) (a, b, c, g, h) and 2017-2018 (Expt. 2-2) (d, e, f, i, j), respectively.

Table Legends

Table 1. Target seed yields and the Pi fertilizer application rates of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 under P deplete (Olsen P $\leq$15) and replete (Olsen P>15) conditions.

Table 2. Responses of root morphology of oilseed rape cultivar ZS11 to different Pi application and nature-based treatments at the leaf development stage.

Supplementary information.

Fig. S1. Precipitation, monthly average temperatures (T_{avg}) from late September to early May at Wuxue (a, 2017-2018; c, 2018-2019) and Qianjiang (b, 2018-2019), Hubei province, China.

Fig. S2. Shoot P content of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 grown under six P supplies at Wuxue (a, b, c, d) and Qianjiang (e, f, g, h), respectively.

Fig. S3. Pearson's correlation between seed yield per plant (DW-Se) and shoot dry weight (SDW) of oilseed rape cultivars ZS11 and ZYZ19, HYZ9 and SG168 grown at six phosphate (Pi) application rates at for growth stages S1, leaf development stage; S2, stem elongation stage; S3, flowering stage; S4, ripening stage at Wuxue (a, b, c, d, e, f) and Qianjiang (g, h, i, j, k, l), respectively.

Fig. S4. Effects of P application rates on P content (a, b) and distribution (c, d) in stem, pericarp and seed of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at the ripening stage at Wuxue (a, c) and Qianjiang (b, d), respectively.

Fig. S5. Shoot dry weight (a) and shoot P content (b) of oilseed rape cultivar ZS11 under six treatments in 2017-2018 (Expt. 2-1) (a, c) and 2018-2019 (Expt. 2-2) (b, d), respectively.

Table S1. Sampling stages according to the BBCH development scale for oilseed rape cultivars.

Table S2. Variance analysis of Pi treatments (P), cultivars (C), experiment sites (S) for oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 grown under six Pi application rates.

Table S3. Regression analysis of shoot dry weight at four growth stages and seed yield at the ripening stage at six P supplies at Wuxue (WX) and Qianjiang (QJ) in oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168.

Table S4. Regression analysis of P application rates and seed yield in oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at the ripening stage at Wuxue (WX) and Qianjiang (QJ).

Table S5. Effect of P supply on seed yield related traits of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at the ripening stage at Wuxue (WX) and Qianjiang (QJ).

Table S6. The coefficient of variation of P supply on seed yield-related traits of oilseed rape cultivars

ZS11, ZYZ19, HYZ9 and SG168 at the ripening stage at Wuxue (WX) and Qianjiang (QJ).

Table S7. Regression analysis of phosphate application rate and $P_{balance}$ in oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at the ripening stage at Wuxue (WX) and Qianjiang (QJ).

Table S8. Effect of P supply on yield-related traits of oilseed rape cultivar ZS11 at the ripening stage.

Table 1. Target seed yields and the Pi fertilizer application rates of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 under P deplete (Olsen P ≤15) and replete (Olsen P>15) conditions.

Soil Olsen-P (mg kg^{-1})	Cultivar	Yield grade	Target yield (kg ha^{-1})	Pi fertilizer application rate (kg P ha^{-1})	Shoot P content (kg ha^{-1})	$P_{balance}$ (kg P ha^{-1})	P required ratio
≤15	ZS11	minimum	1483.5	0	5.56	-5.56	-
		mean	1656.27	7.11	7.11	0	1
		maximum	1853.1	15.21	8.88	6.33	1.71
	ZYZ19	minimum	1746.1	0	6.62	-6.62	-
		mean	1851.94	7.84	7.84	0	1
		maximum	1995.4	18.53	9.5	9.03	1.95
	HYZ9	minimum	2260	0	7.81	-7.81	-
		mean	2394.83	9.11	9.11	0	1
		maximum	2746.3	32.78	12.49	20.29	2.62
	SG168	minimum	2241.5	0	6.94	-7.38	-
		mean	2607.1	9.14	9.14	0	1
		maximum	2738.9	12.5	9.85	2.65	1.27
>15	ZS11	minimum	1945.4	0	6.53	-6.53	-
		mean	2190.47	8.48	8.48	0	1
		maximum	2324.4	13.11	9.54	3.57	1.37
	ZYZ19	minimum	2186.4	0	8.08	-8.08	-
		mean	2462.84	10.97	10.97	0	1
		maximum	2562	14.86	12	2.86	1.24
	HYZ9	minimum	3111.3	0	8.85	-8.85	-
		mean	3306.51	12.05	12.05	0	1
		maximum	3380.1	16.61	13.26	3.35	1.25
	SG168	minimum	2919.2	0	10.68	-10.68	-
		mean	3369.5	13.56	13.56	0	1
		maximum	3369.5	12.24	13.28	-1.04	0.92

ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168; minimum, mean and maximum are defined to be seed yield at no Pi supply, seed yield at the threshold Pi supply and the highest seed yield among these Pi supplies, respectively; P required ratio, ratio of P input in soil to shoot P content of oilseed rape cultivars.

Table 2. Responses of root morphology of oilseed rape cultivar ZS11 to different Pi application and nature-based treatments at the leaf development stage.

Year	P supplies (kg P ha^{-1})	TRL (cm)	RSA (cm^2)	TRV (cm^3)	RAD (mm)	SRL	PE (mg m^{-1} TRL)
2017-2018	P_0	517.20d	103.29d	1.63d	0.63c	18.40a	1.00e
(*Expt. 2-1*)	P_0+PSB	697.92c	144.38c	2.26c	0.65b	14.90b	1.70d
	$P_{21.0}$	904.77b	185.64b	3.26b	0.76a	9.00c	3.00c
	$P_{21.0}$+RA	1052.39a	238.32a	4.01a	0.82a	7.00d	4.00a
	$P_{21.0}$+PSB	900.00b	185.76b	3.33b	0.76a	8.30c	3.00bc
	$P_{26.2}$	983.33a	236.75a	4.06a	0.82a	6.10d	4.30b
2018-2019	P_0	440.45c	85.48c	1.06c	0.64b	173.62a	0.1c
(*Expt. 2-2*)	$P_{15.7}$	505.68b	91.38b	1.55b	0.73b	154.74b	0.15b
	$P_{15.7}$+RA	559.63a	102.25a	2.53a	1.5a	152.02b	0.18a
	$P_{15.7}$+PSB	561.5ab	101.3a	2.36a	1.38a	151.5b	0.17a
	$P_{26.2}$	591.08a	108.8a	2.56a	1.48a	157.87b	0.18a

ZS11, Zhongshuang 11; TRL, total root length; RSA, root surface area; TRV, total root volume; RAD, root average diameter; SRL, special root length; PE, total phosphorus uptake per total root length at leaf development stage; P_0, 0 kg P ha^{-1} (control); P_0+PSB, addition of phosphate solubilizing bacteria (4.5*10$^{\wedge 9}$ cfu kg^{-1} ha^{-1}) at P_0; $P_{21.0}$, 21.0 kg P ha^{-1}; $P_{21.0}$+RA, addition of rooting agent (RA, 1.5 L ha^{-1}) at $P_{21.0}$; $P_{21.0}$+PSB, addition of phosphate solubilizing bacteria (4.5*10$^{\wedge 9}$ cfu kg^{-1} ha^{-1}) at $P_{21.0}$; $P_{26.2}$, 26.2 kg P ha^{-1} (FFP, Farmers' fertilizer practice); $P_{15.7}$, 15.7 kg P ha^{-1}; $P_{15.7}$+RA, addition of rooting agent (RA, 1.5 L ha^{-1}) at $P_{15.7}$; $P_{15.7}$+PSB, addition of phosphate solubilizing bacteria (4.5*10$^{\wedge 9}$ cfu kg^{-1} ha^{-1}) at $P_{15.7}$. The different lower-case letters above the column indicate significant difference among the P supplies (Tukey's test, P <0.05).

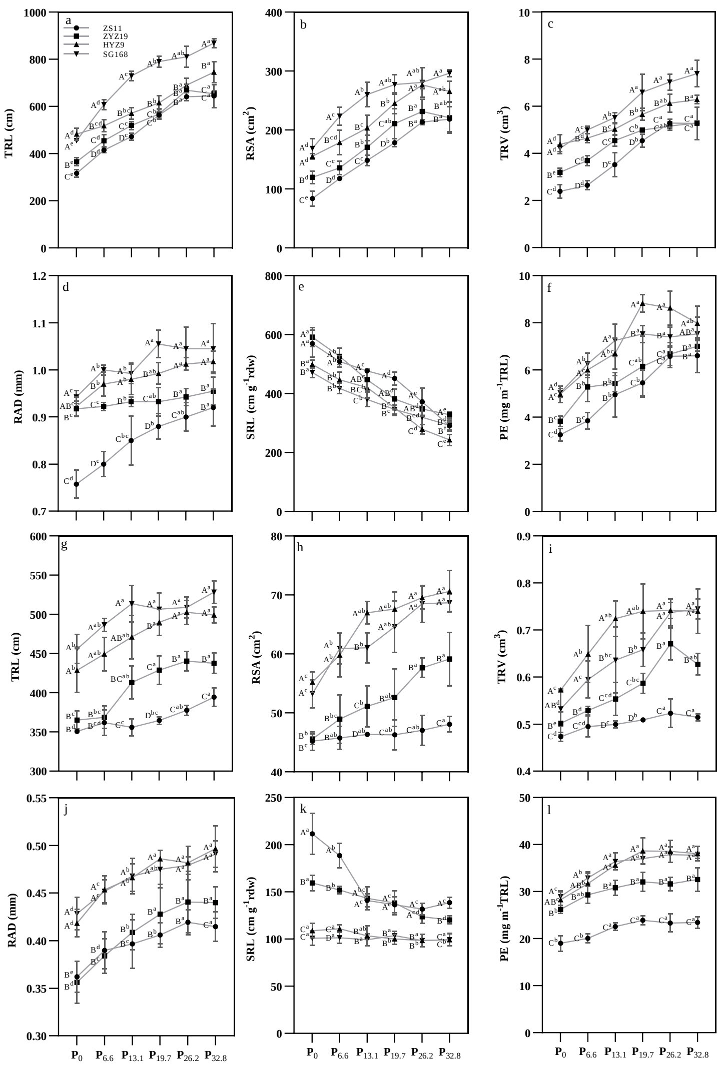


Fig. 1. TRL (a, g), RSA (b, h), TRV (c, i), RAD (d, j), SRL (e, k) and PE (f, i) of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at leaf development stage under six phosphate (Pi) application rates at Wuxue (a, b, c, d, e, f) and Qianjiang (g, h, i, j, k, l), respectively. TRL, total root length; RSA, root surface area; TRV, total root volume; RAD, root average diameter; SRL, specific root length; PE, phosphorus efficiency; ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168; The six Pi application rates were P_0, 0 kg P ha^{-1} (control); $P_{6.6}$, 6.6 kg P ha^{-1}; $P_{13.1}$, 13.1 kg P ha^{-1}; $P_{19.7}$, 19.7 kg P ha^{-1}; $P_{26.2}$, 26.2 kg P ha^{-1} (Farmers' fertilizer practice, FFP); $P_{32.8}$, 32.8 kg P ha^{-1}. Each value is the mean (±SE) of four replicates. The different lower-case letters above the column indicate significant differences between Pi application rates within the same cultivar, and different capital letters indicate significant differences between the four cultivars within the same Pi application rate (Tukey's test, P <0.05).

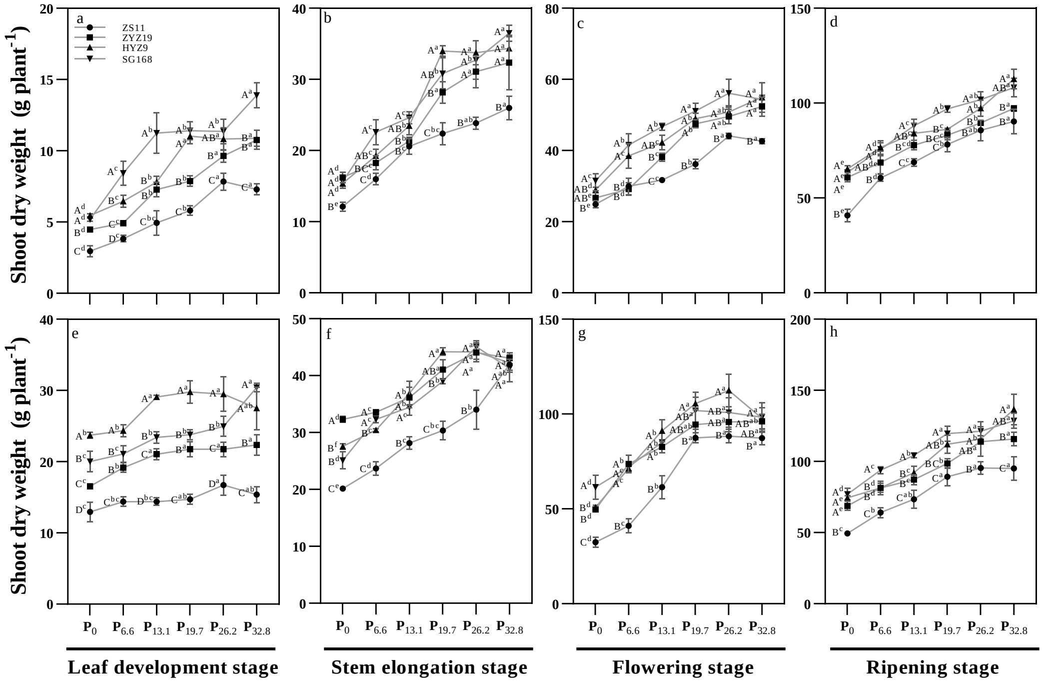
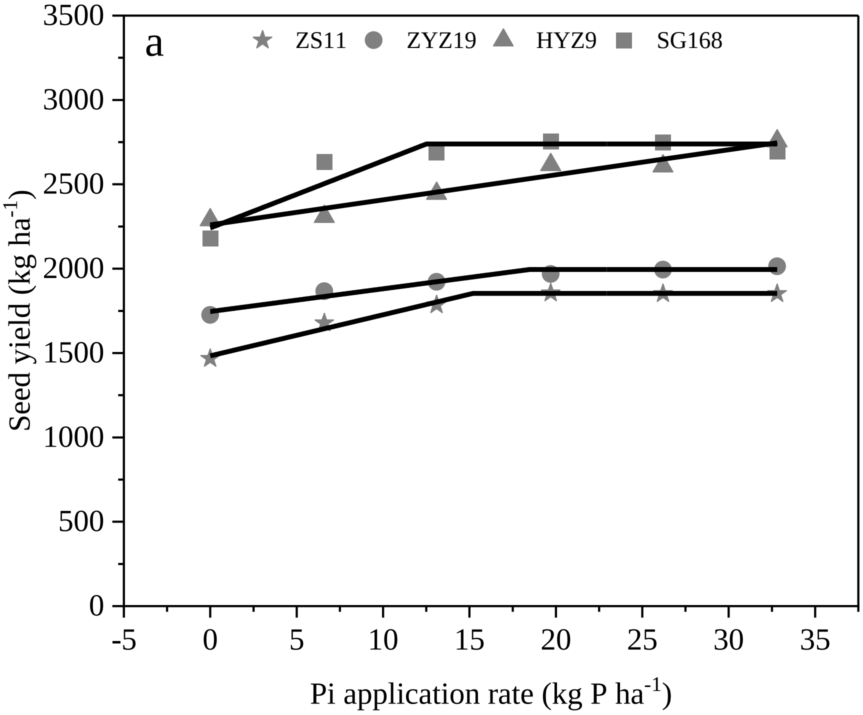


Fig. 2. Shoot dry weight of *oilseed rape* cultivars ZS11, ZYZ19, HYZ9 and SG168 grown under six phosphate (Pi) application rates at Wuxue (a, b, c, d) and Qianjiang (e, f, g, h), respectively. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168; P_0, 0 kg P ha^{-1} (control); $P_{6.6}$, 6.6 kg P ha^{-1}; $P_{13.1}$, 13.1 kg P ha^{-1}; $P_{19.7}$, 19.7 kg P ha^{-1}; $P_{26.2}$, 26.2 kg P ha^{-1} (Farmers' fertilizer practice, FFP); $P_{32.8}$, 32.8 kg P ha^{-1}. Each value is the mean (±SE) of four replicates. The different lower-case letters above the column indicate significant differences between Pi application rates within the same cultivar at the same growth stage, and different capital letters indicate significant differences between the four cultivars within the same Pi application rate at the same growth stage (Tukey's test, P <0.05)



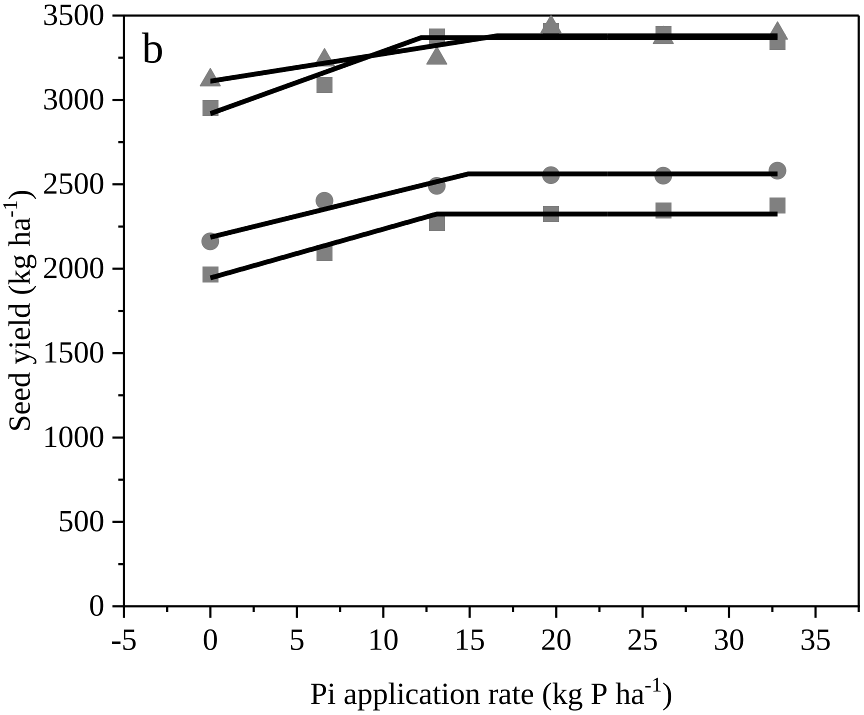


Fig. 3. Effect of phosphate (Pi) application rates on seed yield of oilseed rape cultivars ZS11, ZYZ19, HYZ9 and SG168 at Wuxue (a) and Qianjiang (b), respectively. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168. Each value is the mean of four replicates.

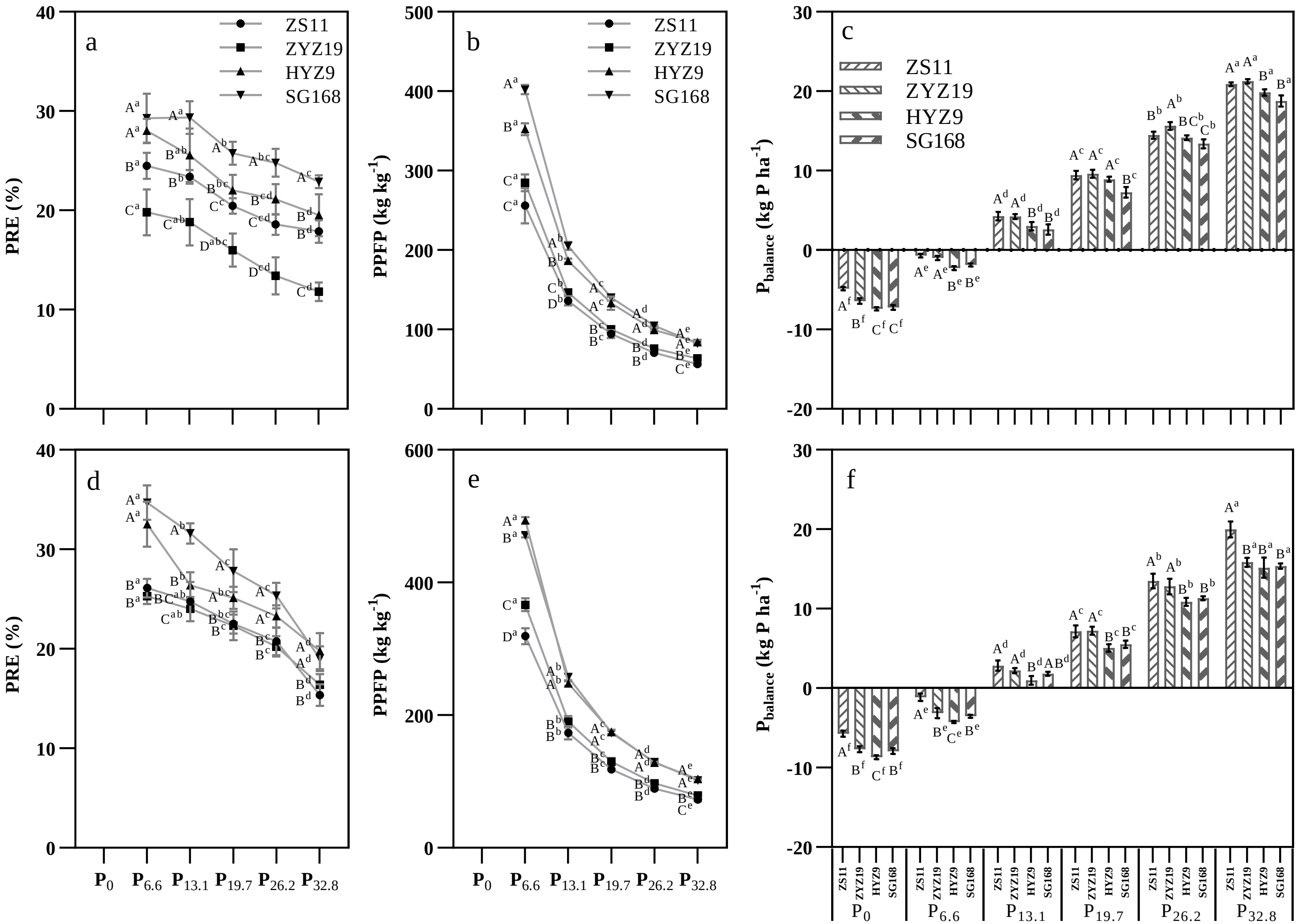
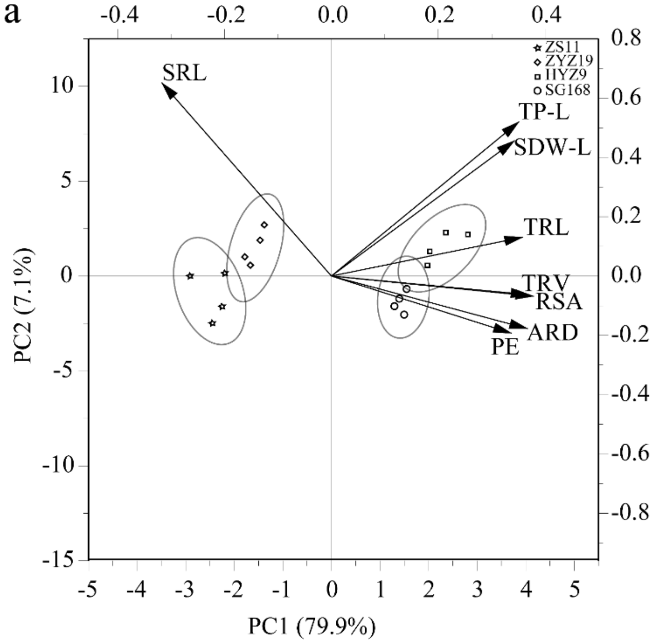
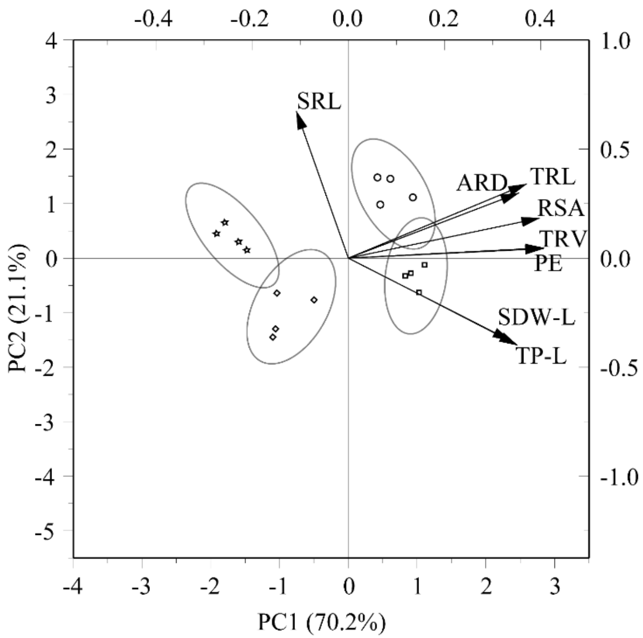

a
b
c
d
e
f
ZS11
ZYZ19
HYZ9
SG168
PRE (%)
PPFP (kg kg-1)
Pbalance (kg P ha-1)
P0
P6.6
P13.1
P19.7
P26.2
P32.8
0
10
20
30
40
100
200
300
400
500
600
-20
-10

Fig. 4. Phosphorus (P) recovery efficiency (PRE) (a, d), P partial factor productivity (PPFP) (b, e) and $P_{balance}$ (c, f) of *oilseed rape* cultivars ZS11, ZYZ19, HYZ9 and SG168 at ripening stage under six Pi application rates at Wuxue (a, b, c) and Qianjiang (d, e, f), respectively. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168; P_0, 0 kg P ha^{-1} (control); $P_{6.6}$, 6.6 kg P ha^{-1}; $P_{13.1}$, 13.1 kg P ha^{-1}; $P_{19.7}$, 19.7 kg P ha^{-1}; $P_{26.2}$, 26.2 kg P ha^{-1} (Farmers' fertilizer practice, FFP); $P_{32.8}$, 32.8 kg P ha^{-1}. Each value is the mean (±SE) of four replicates. The different lower-case letters above the column indicate significant differences between Pi application rates within the same cultivar, and different capital letters indicate significant differences between the four cultivars within the same Pi application rate (Tukey's test, P <0.05).

a



b



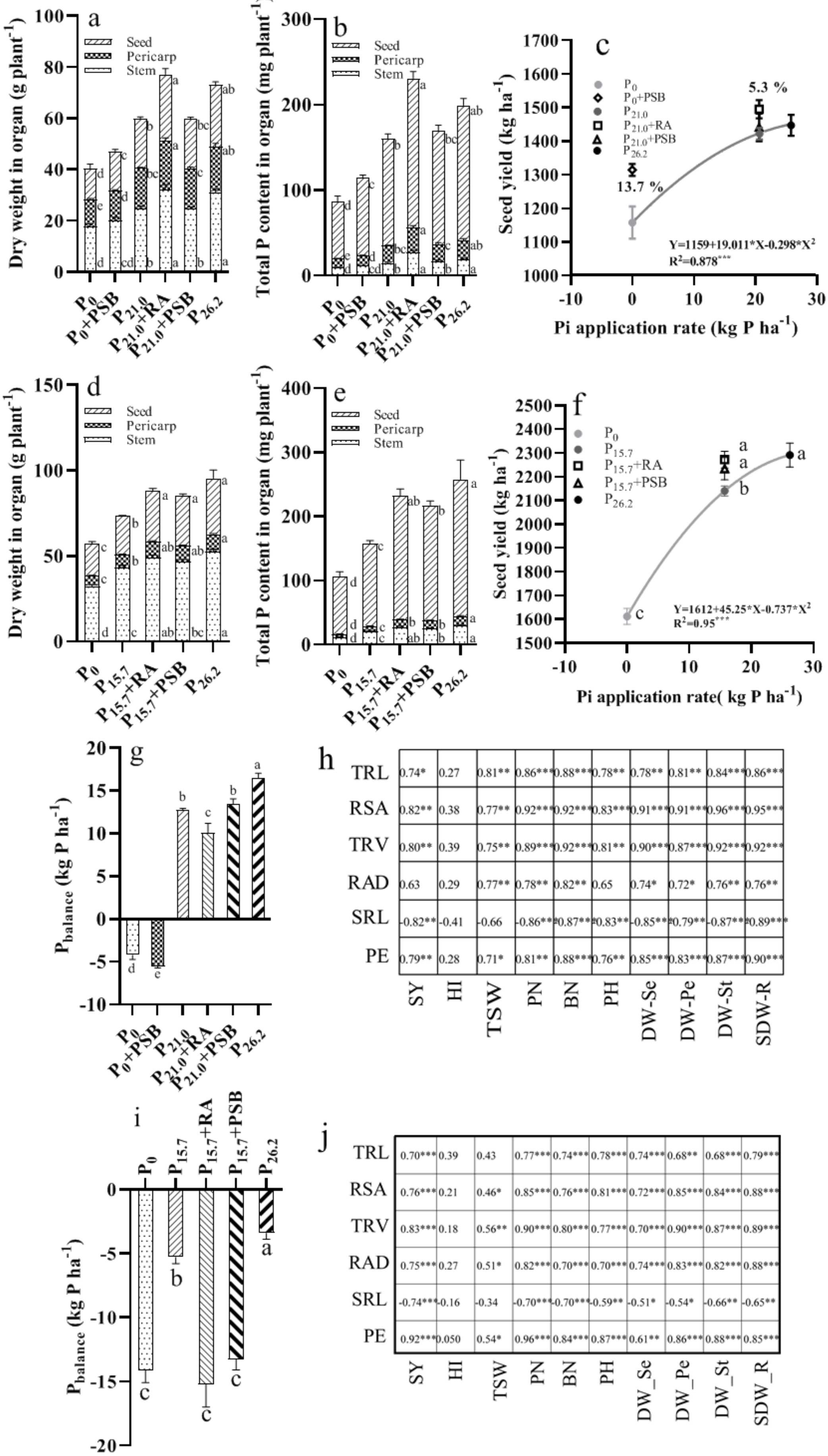
c

	SY	HI	TSW	PN	BN	PH	DW-Se	DW-Pe	DW-St	SDW-R
TRL	0.70***	-0.16	0.28	0.85***	0.77***	0.75***	0.69***	0.73***	0.77***	0.82***
RSA	0.69***	-0.16	0.17	0.85***	0.74***	0.75***	0.68***	0.70***	0.72***	0.79***
TRV	0.71***	-0.17	0.13	0.86***	0.79***	0.74***	0.66***	0.65***	0.76***	0.79***
RAD	0.71***	-0.11	0.08	0.72***	0.73***	0.65***	0.50***	0.45***	0.58***	0.58***
SRL	-0.56***	0.30	-0.06	-0.75***	-0.69***	-0.61***	-0.65***	-0.68***	-0.71***	-0.77***
PE	0.62***	-0.17	-0.06	0.76***	0.68***	0.68***	0.67***	0.50***	0.66***	0.70***

d

	SY	HI	TSW	PN	BN	PH	DW-Se	DW-Pe	DW-St	SDW-R
TRL	0.84***	0.09	0.03	0.67***	0.64***	0.75***	0.69***	0.48***	0.53***	0.65***
RSA	0.80***	-0.05	-0.10	0.67***	0.59***	0.76***	0.58***	0.54***	0.54***	0.65***
TRV	0.70***	-0.12	-0.08	0.68***	0.58***	0.72***	0.59***	0.54***	0.55***	0.65***
RAD	0.69***	-0.06	-0.05	0.66***	0.62***	0.65***	0.57***	0.46***	0.61***	0.65***
SRL	-0.07	0.50***	0.30	-0.27	-0.29	-0.29	-0.05	-0.26	-0.31	-0.26
PE	0.78***	0.02	0.03	0.58***	0.60***	0.78***	0.62***	0.57***	0.52***	0.66***

Fig. 5. Principal component analysis (PCA) plot showing the relationship among root morphology traits and P uptake in all oilseed rape cultivars at Wuxue (a) and Qianjiang (b), respectively; and the correlation among seed yield and yield related traits of oilseed rape cultivars and the root morphology traits at leaf development stage at Wuxue (c) and Qianjiang (d), respectively. ZS11, Zhongshuang 11; ZYZ19, Zhongyouza 19; HYZ9, Huayouza 9; SG168, Shengguang 168; SDW-L, shoot dry weight at leaf development stage; TP-L, shoot phosphorus accumulation per plant at leaf development stage; TRL, total root length; RSA, root surface area; TRV, total root volume; RAD, root average diameter; SRL, special root length; PE, total phosphorus uptake per total root length at leaf development stage; SDW-R, shoot dry weight at ripening stage; PH, plant height; BN, branch number; PN, pod number; TSW, 1000-seed weight; DW-St, dry weight of stem; DW-Pe, dry weight of pericarp ; DW-Se, seed yield per plant; SY, seed yield; HI, harvest index. *P <0.05, **P <0.01 and ***P <0.001.


a
Seed
Pericarp
Stem
Dry weight in organ (g plant-1)
P0
P0+PSB
P21.0
P21.0+RA
P21.0+PSB
P26.2
b
Total P content in organ (mg plant-1)
c
Seed yield (kg ha-1)
Pi application rate (kg P ha-1)
5.3 %
13.7 %
Y=1159+19.011*X-0.298*X2
R2=0.878***
d
e
f
P15.7
P15.7+RA
P15.7+PSB
Y=1612+45.25*X-0.737*X2
R2=0.95***
Pi application rate(kg P ha-1)
g
Pbalance (kg P ha-1)
h
TRL 0.74* 0.27 0.81** 0.86*** 0.88*** 0.78** 0.78** 0.81** 0.84*** 0.86***
RSA 0.82** 0.38 0.77** 0.92*** 0.92*** 0.83*** 0.91*** 0.91*** 0.96*** 0.95***
TRV 0.80** 0.39 0.75** 0.89*** 0.92*** 0.81** 0.90*** 0.87*** 0.92*** 0.92***
RAD 0.63 0.29 0.77** 0.78** 0.82** 0.65 0.74* 0.72* 0.76** 0.76**
SRL -0.82** -0.41 -0.66 -0.86*** -0.87*** -0.83** -0.85*** -0.79** -0.87*** -0.89***
PE 0.79** 0.28 0.71* 0.81** 0.88*** 0.76** 0.85*** 0.83*** 0.87*** 0.90***
SY HI TSW PN BN PH DW-Se DW-Pe DW-St SDW-R
i
j
TRL 0.70*** 0.39 0.43 0.77*** 0.74*** 0.78*** 0.74*** 0.68** 0.68*** 0.79***
RSA 0.76*** 0.21 0.46* 0.85*** 0.76*** 0.81*** 0.72*** 0.85*** 0.84*** 0.88***
TRV 0.83*** 0.18 0.56** 0.90*** 0.80*** 0.77*** 0.70*** 0.90*** 0.87*** 0.89***
RAD 0.75*** 0.27 0.51* 0.82*** 0.70*** 0.70*** 0.74*** 0.83*** 0.82*** 0.88***
SRL -0.74*** -0.16 -0.34 -0.70*** -0.70*** -0.59** -0.51* -0.54* -0.66** -0.65**
PE 0.92*** 0.050 0.54* 0.96*** 0.84*** 0.87*** 0.61** 0.86*** 0.88*** 0.85***
SY HI TSW PN BN PH DW_Se DW_Pe DW_St SDW_R

1

Fig. 6. Difference in the investigated traits of oilseed rape cultivar ZS11 among six treatments in 2017-2018 (Expt. 2-1) (a, b, c, g, h) and 2017-2018 (Expt. 2-2) (d, e, f, i, j), respectively. Dry weight (DW) (a) and total P content in stem, pericarp and seed (b), Seed yield (c) and $P_{balance}$ (d) at ripening stage for six treatments; P_0, 0 kg P ha^{-1} (control); P_0+PSB, addition of phosphate solubilizing bacteria (4.5*10^9 cfu kg^{-1} ha^{-1}) at P_0; $P_{21.0}$, 21.0 kg P ha^{-1}; $P_{21.0}$+RA, addition of rooting agent (RA, 1.5 L ha^{-1}) at $P_{21.0}$; $P_{21.0}$+PSB, addition of phosphate solubilizing bacteria (4.5*10^9 cfu kg^{-1} ha^{-1}) at $P_{21.0}$; $P_{15.7}$, 15.7 kg P ha^{-1}; $P_{15.7}$+RA, addition of rooting agent (RA, 1.5 L ha^{-1}) at $P_{15.7}$; $P_{15.7}$+PSB, addition of phosphate solubilizing bacteria (4.5*10^9 cfu kg^{-1} ha^{-1}) at $P_{15.7}$; $P_{26.2}$, 26.2 kg P ha^{-1} (FFP, Farmers' fertilizer practice). (e) The relationship among the SY and yield related traits of *Brassica napus* cultivar ZS11 at ripening stage and the root morphology traits (TRL, RSA, TRV, RAD, SRL and PE) at leaf development stage. ZS11, Zhongshuang 11;TRL, total root length; RSA, root surface area; TRV, total root volume; RAD, root average diameter; SRL, special root length; PE, total phosphorus uptake per total root length at leaf development stage; SDW-R, shoot dry weight at ripening stage; PH, plant height; BN, branch number; PN, pod number; TSW, 1000-seed weight; DW-St, dry weight of stem; DW-Pe, dry weight of pericarp; DW-Se, seed yield per plant; SY, seed yield; HI, harvest index; Each value is the mean (±SE) of three replicates. The different lower-case letters above the column indicate significant differences among the P supplies (Tukey's test, $P < 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.