



# Targeting Low-Phytate Soybean Genotypes Without Compromising Desirable Phosphorus-Acquisition Traits

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Phytate-phosphorus (P) in food and feed is not efficiently utilized by humans and non-ruminant livestock, potentially contributing to high losses of P to the environment. Crops with high P-acquisition efficiency can access soil P effectively. It remains elusive whether crop genotypes with high P-acquisition efficiency can also have low seed phytate concentrations. A core collection of 256 soybean [*Glycine max* (L.) Merr.] genotypes from China with diverse genetic background were grown in the same environment and seeds were sampled to screen for seed phytate-P concentration. Some of these genotypes were also grown in a low-P soil in the glasshouse to measure root morphological and physiological traits related to P acquisition. Large genotypic variation was found in seed phytate-P concentration (0.69–5.49 mg P g<sup>-1</sup> dry weight), total root length, root surface area, rhizosheath carboxylates, and acid phosphatase activity in rhizosheath soil. Geographically, seed phytate-P concentration was the highest for the genotypes from Hainan Province, whereas it was the lowest for the genotypes from Inner Mongolia. Seed phytate-P concentration showed no correlation with any desirable root traits associated with enhanced P acquisition. Two genotypes (Siliyuan and Diliuhuangdou-2) with both low phytate concentrations and highly desirable P-acquisition traits were identified. This is the first study to show that some soybean genotypes have extremely low seed phytate concentrations, combined with important root traits for efficient P acquisition, offering material for breeding genotypes with low seed phytate-P concentrations.

**Keywords:** soybean germplasm, phytate, phosphorus-acquisition efficiency, root morphology, carboxylates

## INTRODUCTION

Phosphorus (P) deficiency is a major limiting factor for crop production worldwide owing to low soil P availability. It is readily sorbed by oxides and hydroxides of aluminium (Al) and iron (Fe) in acid soils, and precipitated by calcium (Ca) in alkaline soils (Hinsinger, 2001; Raghothama and Karthikeyan, 2005), leading to 70–90% of P applied as fertilizer becoming unavailable to most crop

plants (Holford, 1997). The high rates of P-fertilizer application in crop production result not only in gradually diminishing phosphate rock reserves, which is associated with decreasing P-fertilizer quality, but also in environmental issues associated with off-site effects of P fertilizers (Cordell et al., 2009; Ghaffar et al., 2017). Therefore, it is essential to enhance P-acquisition efficiency by breeding highly P-efficient crop genotypes (Cong et al., 2020).

Soybean [*Glycine max* (L.) Merr.] as a widely cultivated grain legume, is an important source of protein and vegetable oil for human consumption, and also widely used in animal feed globally (Yuan et al., 2007; Liang et al., 2010). In addition, soybean has a pivotal ecological function in cropping system, e.g., nitrogen fixation (Salvagiotti et al., 2008), soil carbon sequestration (Cong et al., 2015), enhancing soil P availability (Xia et al., 2013), and decreasing soil-borne diseases (Gao et al., 2014) for themselves and the following crops. Approximately 60–80% of total P in soybean seed accumulates as phytate (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate, PA, or IP6) (Raboy et al., 1984). A high phytate concentration in seed is an undesirable trait, because it renders zinc (Zn) and other micro-nutrients unavailable for humans and livestock, contributing to malnutrition, especially Zn and iron (Fe) deficiency (Raboy, 2001; Perera et al., 2019). Moreover, phytate cannot be efficiently utilized by humans and non-ruminant animals, contributing to high losses of P to the environment. Thus, breeding for low-phytate soybean genotypes with high P-acquisition efficiency is a highly desirable sustainable strategy. It is one of the vital and preliminary steps for improving the desired traits in breeding programs that explore the genetic variation in germplasm (Perera et al., 2019). Hence, there is a need to study the genetic variation in seed phytate-P using a large set of soybean genotypes with diverse genetic backgrounds.

Identifying P-efficient crop genotypes is a sustainable and effective way to tighten the P cycle, thereby reducing P-fertilizer input, and mitigating the risks of pollution of ground- and surface water (Cong et al., 2020). Genotypic differences in nutrient-use efficiency are closely related to differences in efficiency of nutrient acquisition by roots (Marschner, 1998). Root traits such as root size, morphology, physiology, and mycorrhizal associations play a dominant role, both in P acquisition and in the exploration of a large soil volume, particularly under low P availability in soil (Marschner, 1998). Root morphological and physiological traits may respond to P deficiency (Zhou et al., 2016). Fernandez and Rubio (2015) reported higher specific root length and smaller average root diameter in soybean where P-acquisition efficiency increased under P deficiency. Root physiological traits, mainly root exudates such as carboxylates and phosphatase enzymes, may also increase plant P-acquisition efficiency (Dinkelaker et al., 1989; Pang et al., 2018). Earlier work showed genetic variability of root architecture in a core collection of soybean germplasm, with a shallow root architecture associated with higher P efficiency (Ao et al., 2010). However, knowledge of genotypic variation in root size, morphological and physiological traits is largely unknown for soybean. Therefore, there is an urgent need to investigate the variation in root size including root dry weight and rhizosheath soil dry weight, root morphological traits including root surface area and total root length, and root physiological traits including

the total amount of carboxylates in the rhizosheath and acid phosphatase activity in a large set of soybean genotypes with diverse genetic backgrounds.

Previous studies compared the seed phytate-P concentration of soybean genotypes (Horner et al., 2005; Maharjan et al., 2019), and mainly focused on variation in seed phytate-P concentration among genotypes and its relationship with nutrients such as Fe, Zn, and calcium (Ca), and protein within a relatively small set of genotypes. Thus, there is a chance they missed identifying soybean genotypes with low seed phytate-P concentration. Wang et al. (2007) have identified a core collection of soybean germplasm, on average representing 81.5% of genetic variation. This collection provides us with a great opportunity to identify genotype with low seed phytate-P concentration and its relationship with desirable root traits related to P acquisition in soil with a low P availability. Identification of genotypes with highly desirable P-acquisition traits with low phytate-P concentrations has many benefits. First, lowering the seed phytate-P concentration would enhance the bio-availability of micro-nutrients such as Zn and Fe for humans and farm animals (Raboy, 2001), and thus improve the nutritional quality of soymeal. Increased nutritional quality of soymeal would go hand in hand with decreasing environmental pollution, especially eutrophication of aquatic environments (Sharpley et al., 2000; Brinch et al., 2002). Second, genotypes with desirable P-acquisition traits and low unavailable seed P concentrations require less P fertilization, and therefore reduce the cost for farmers and risk of P losses from the field (Cong et al., 2020).

The aims of the present study were (1) to investigate the variation in seed phytate-P concentration among 256 soybean genotypes and their geographically distributed differences; (2) to study the variation in root dry weight and rhizosheath soil dry weight, root morphological traits including total root length and surface area, as well as root physiological traits such as the amount of carboxylates and acid phosphatase activity in the rhizosheath among a subset of genotypes originally from the North China Plain (the main food producing region) under low-P condition; (3) to examine if soybean genotypes with desirable low phytate-P concentrations can simultaneously express desirable root traits associated with high P-acquisition efficiency, using a subset of genotypes. We hypothesized that (1) there is a large variation in seed phytate-P concentration and root size, morphological and physiological traits among soybean genotypes; (2) seed phytate-P concentration does not correlate with any of the measured desirable root traits related to P acquisition.

## MATERIALS AND METHODS

### Plant Material and Growing Conditions

We grew 256 genotypes (**Supplementary Table 1**) from divergent provinces of China in a single field in Hainan Province (108°37'–111°03' E, 18°10'–20°10' N), China to measure seed phytate-P concentrations. Annual mean temperature is 22°C with a minimum and maximum temperature of 17 and 27°C, respectively. Annual rainfall ranges from 1000 to 2600 mm with an average annual rainfall of 1639 mm. Annual sunshine

ranges from 1750 to 2650 h, and the total solar radiation is 4600–5800 MJ m<sup>-2</sup> year<sup>-1</sup>. The soil is classified latosol. Information on these genotypes including Province of origin is described in **Supplementary Table 1**.

Of the 256 genotypes, 43 from five provinces in the Huang-Huai-Hai plain and Middle-Lower Yangtze plain were used to study six root traits that are associated with P acquisition (**Supplementary Table 1**). Those 43 genotypes are representative, because they include both ones that show the lowest and the highest phytate concentration, and the coefficient of variation (CV) is similar to that of the data set comprising 256 genotypes.

Pot experiment was conducted to determine root traits in a glasshouse at China Agricultural University, Beijing, with a day temperature of 25–30°C and a night temperature of 18–22°C. River sand and a soil with a low P availability were collected from Changping in the Beijing area. River sand was washed, and then both river sand and soil were air-dried and sieved (2-mm mesh size) to remove coarse fragments and macroarthropods prior to potting. Soil and sand were sterilized by gamma irradiation (>25 K Gray gamma irradiation before potting). Each pot (85 cm × 85 mm × 180 mm) was filled with 1.2 kg mixture of sterilized washed river sand and soil with a low P availability (in a ratio of 3:7, w/w). The pot experiment followed a completed randomized design with soybean genotype as the main factor. Each of 43 genotypes was replicated in four pots. Seeds were surface sterilized with 10% (v/v) hydrogen peroxide for 3 min, then washed with deionized water and germinated in the dark on moist filter paper at 25/20°C (day/night). In each pot, we planted four seeds at 20 mm depth and inoculated with *Rhizobium leguminosarum* bv. NM353 (provided by the Culture Collection of China Agricultural University). Seedlings were thinned to one plant per pot at 7 days after sowing. Each pot was watered with deionized water to 60% field capacity by weighting every other day.

## Determination of Seed Phytate Concentration

Grains of each soybean genotype were collected at maturity, dried and ground to a fine powder. The powder was then passed through a 0.5 mm sieve. The phytate concentration was determined using the trichloroacetic acid method (Perera et al., 2019). Briefly, 4 g of seed powder was added to 50 ml of 30 g l<sup>-1</sup> trichloroacetic acid and kept for 2 h for digestion, and then shaken for 30 min, and filtered using dry filter paper. Ten ml of supernatant was treated with 4 ml of ferric chloride solution (FeCl<sub>3</sub>) in a boiling water bath for 45 min. After cooling, samples were centrifuged at 1,710 × g for 10 min. The supernatant was removed, and 20–25 ml of 0.18 M trichloroacetic acid solution was added to wash the samples; after that, the samples were boiled in a water bath for 10 min twice. The precipitate was treated with 20 ml of water and 3 ml of 1.5 M NaOH in a boiling water bath for 30 min. After centrifugation, 5–10 ml of supernatant was treated with 3 ml of a mixture of di-acids (2 nitric acid + 1 perchloric acid) for digestion on an electric furnace at low temperature until there were white fumes. After cooling, samples were washed with 30 ml water several times; then, 3 ml of nitric acid solution

and 10 ml of chromogenic agent were added. After 20 min, the absorbance was measured spectrophotometrically at 420 nm.

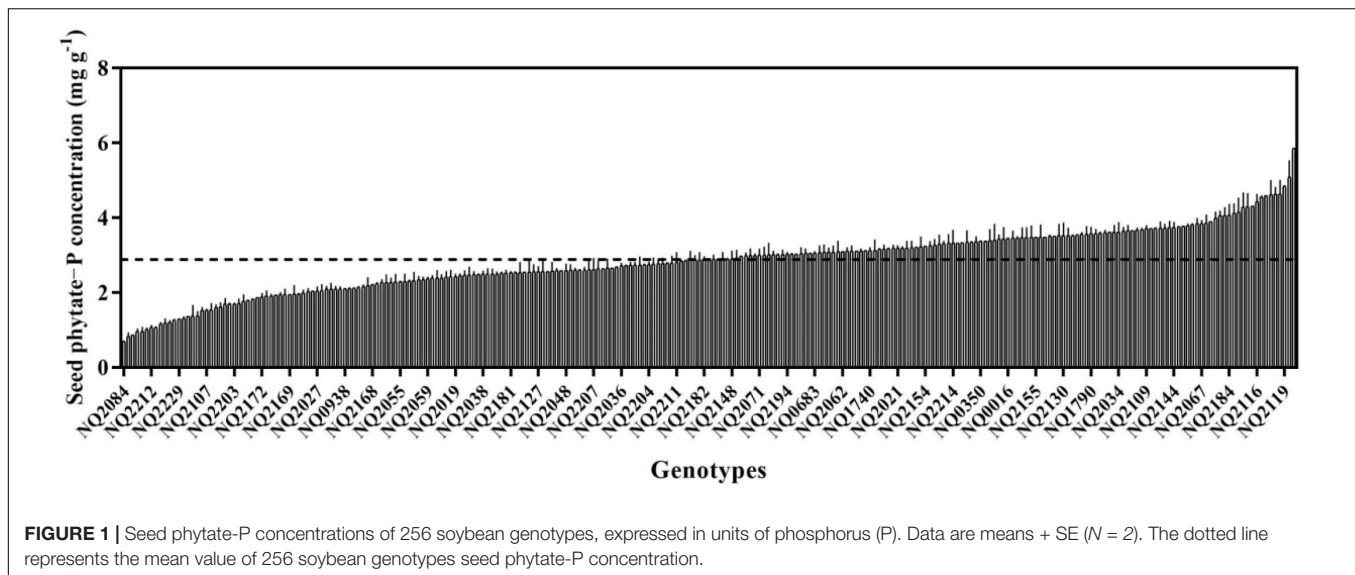
## Plant Harvest and Measurements

Plants grown in the glasshouse were harvested 45 days after sowing. Shoots were separated from the roots. In order to collect rhizosheath exudates, the method of Pearse et al. (2007) was modified. Briefly, at harvest, each pot was squeezed gently to allow dislodgement of the soil column and loosening of soil around the roots. The roots were shaken lightly to remove excess bulk soil; the soil and sand remaining attached to the roots was defined as rhizosheath soil (Pang et al., 2017). The root system was then transferred into a beaker containing 50 ml of 0.2 mM CaCl<sub>2</sub> to avoid cell damage and gently shaken for 60 s to remove as much of the rhizosheath soil as possible. For the determination of acid phosphomonoesterase (APase) activity in the rhizosheath soil, a 0.5 ml subsample of the rhizosheath extract was transferred into a 2 ml centrifuge tube (Alvey et al., 2001). This solution was incubated at 30°C for 30 min after adding 0.4 ml of 200 mM sodium acetate and 0.1 ml of 150 mM pNPP. After incubation, 0.5 ml of 0.5 M NaOH was added and the solution was filtered. Then, the absorbance of this solution was measured spectrophotometrically at 405 nm, representing rhizosphere APase (Nuruzzaman et al., 2006; Lyu et al., 2016). For the analysis of carboxylates, 10 ml of supernatant was taken and three drops of concentrated phosphoric acid and a microbial inhibitor, Micropur (Sicheres Trinkwasser, Graz, Austria) were added, and filtered through a 0.22 μm syringe filter into high performance liquid chromatography (HPLC) vials according to Shen et al. (2003). HPLC samples were frozen at –20°C until analysis. The rhizosheath soil in the rhizosphere exudate solution after extraction of APase and carboxylates was kept in the greenhouse to allow evaporation of excess solution. The rhizosheath soil was oven-dried at 105°C for 72 h, and the dry weight was recorded.

After collection of rhizosheath exudates, roots were washed free of remaining soil, spread out on a transparent plastic tray, and root images were obtained by Epson Perfection V700 dual lens scanning system at a resolution of 600 dpi (dots per inch). Root images were analyzed for total root length and total root surface area using WinRHIZO software (Pro 2009b, Regent Instruments Inc., Quebec City, Canada). After analyzing root images, roots were oven-dried at 70°C for 72 h until constant weight to measure biomass.

## Classification of Soybean Genotypes

Soybean genotypes were classified into four categories as proposed by Bilal et al. (2018). These categories include (i) a low-phytate group with desirable root traits, (ii) a low-phytate group with undesirable root traits, (iii) a high-phytate group with desirable root traits, and (iv) a high phytate-P group with undesirable root traits. Low phytate-P refers to genotypes having seed phytate-P concentration lower than the mean seed phytate-P concentration, and desirable root traits means genotypes having more desirable root traits than the mean root traits and *vice versa*.



## Statistical Analyses

Analysis of variance was used to analyse the differences in seed phytate-P concentration between genotypes used in different agroecological regions using SPSS19. Tukey's *post hoc* test was further used to examine the differences in case the effect was significant. Principal component analysis (PCA) of all plant traits comprising seed phytate-P concentration and root traits based on Pearson's correlation matrix was conducted by Genstat (version 18.2, Genstat Procedure Library Release PL26.2, VSN International, Hemel Hempstead, United Kingdom, 2016) to investigate their correlations.

## RESULTS

### Seed Phytate-P Concentration

Seed phytate-P concentrations from plants grown in the same environment varied greatly, with approximately a seven-fold difference among 256 varieties, ranging from 0.69 mg P g<sup>-1</sup> DW to 5.49 mg P g<sup>-1</sup> DW, with a mean value of 2.88 mg P g<sup>-1</sup> DW (Figure 1). Over half of the genotypes had a higher phytate concentration than the mean value. Two genotypes (Pixianlayinghuang and Heikewudou) had the highest seed phytate-P concentration (>5 mg g<sup>-1</sup> DW), whereas five varieties (Dabaimaodou, Longchuanhuangniumao, Liushiribaidou, Siliyuan, and Lvcaodou) had very low values (<1 mg g<sup>-1</sup> DW) (Figure 1).

### Geographic Background of Genotypes With Specific Seed Phytate-P Concentrations

There was a large variation in seed phytate-P concentration among genotypes from 26 provinces ( $P < 0.05$ ). Averaged for all provinces, the mean seed phytate concentration in the 26 provinces ranged from 2.31 mg P g<sup>-1</sup> DW in Gansu to 5.08 mg P g<sup>-1</sup> DW in Hainan Province, with a mean value of

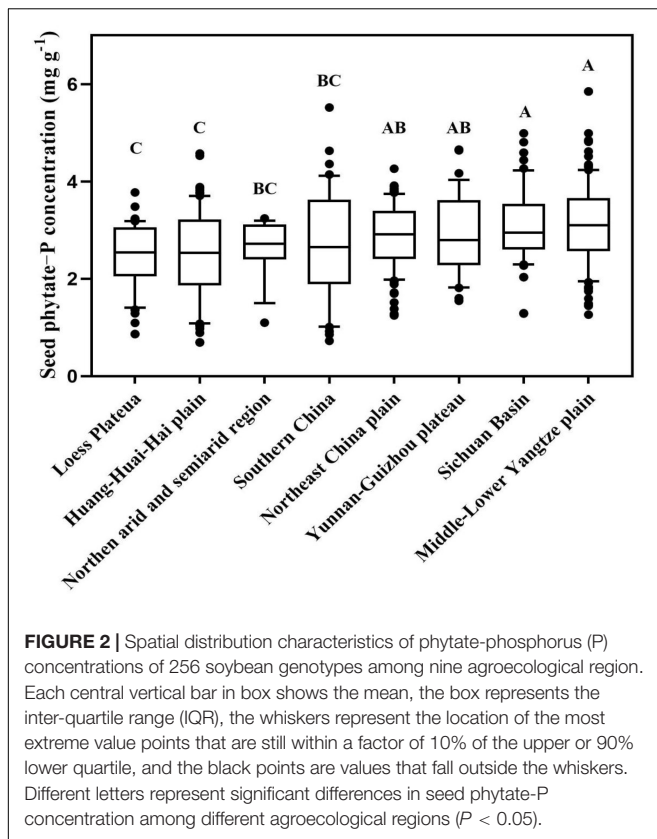
2.94 mg P g<sup>-1</sup> DW across China (Supplementary Figure 1 and Supplementary Table 2). The seed phytate-P concentration for genotypes from Hainan Province was 24~54% greater than that in other provinces ( $P < 0.05$ ). The coefficient of variation of seed phytate-P concentration in each Province ranged from 0 to 52%. Among nine agroecological region, seed phytate-P concentration ranged from 2.50 mg P g<sup>-1</sup> DW on the Loess Plateau to 3.11 mg P g<sup>-1</sup> DW on the Middle-Lower Yangtze Plain ( $P < 0.05$ , Figure 2).

### Root Traits

There was a large variation among 43 genotypes in root dry weight (DW) and rhizosheath soil dry weight ( $P < 0.001$  for both) (Figures 3A,B). Root dry weight varied 2.5-fold, ranging from 0.18 g DW plant<sup>-1</sup> in Pingdingheito 0.45 g DW plant<sup>-1</sup> in Dadunxiaohaidou (Figure 3A). Rhizosheath soil dry weight varied 2.7-fold, from 2.0 g DW plant<sup>-1</sup> in Binhaidahuangkezijia to 5.4 g DW plant<sup>-1</sup> in Siliyuan (Figure 3B).

An eight-fold difference in total root length was found among 43 soybean varieties, ranging from 0.77 to 6.88 m plant<sup>-1</sup> ( $P < 0.001$ , Figure 3C). Similarly, root surface area also varied greatly among 43 soybean genotypes, ranging from  $1.8 \times 10^{-2}$  m<sup>2</sup> plant<sup>-1</sup> in Xichuanjiwohuang to  $4.4 \times 10^{-2}$  m<sup>2</sup> plant<sup>-1</sup> in Heidou ( $P < 0.001$ , Figure 3D).

There was a large variation among the 43 genotypes in root physiological traits including the total amount of carboxylates and acid phosphatase activity ( $P < 0.001$  for both) (Figures 3E,F). We found a 35-fold difference in the activity of acid phosphatase in the rhizosheath among 43 genotypes, ranging from 0.19 nkat g<sup>-1</sup> soil DW in Baiqidawandou to 6.63 nkat g<sup>-1</sup> soil DW in Xinyangyangandou ( $P < 0.001$ , Figure 3E). Similarly, the amount of carboxylates in the rhizosheath soil relative to root dry weight also differed seven-fold among genotypes, ranging from 14.9 μmol g<sup>-1</sup> root DW in Pixianlayanghuang to 110.8 μmol g<sup>-1</sup> root DW in Datunxiaohaidou ( $P < 0.001$ , Figure 3F). The composition of carboxylates showed a large variation among 43 genotypes ( $P < 0.001$ , Figure 3G). All 43 genotypes showed



a predominant combination of malonate and malate in the rhizosphere soil, whereas other carboxylates including fumarate and *trans*-aconitate only accounted for 0.25–67.5% of total carboxylates (Supplementary Figure 2). Malonate accounted for the largest proportion of the total carboxylates in the rhizosphere soil (19–85%,  $P < 0.001$ ) (Supplementary Figure 2). There was a large variation in the proportion of malate (10–43%,  $P < 0.001$ ) and succinate (0–33%,  $P < 0.001$ , Supplementary Figure 2).

### Correlations Among Traits

Based on nine plant traits of 43 genotypes, PCA explained 74% of the variation in the first, second, and third components (Figures 4A,B and Supplementary Table 3). The first component (PC1) represented 44% of the variance and primarily comprised root DW, total root length and root surface area. The second component represented 16% of variability and accounted mainly for shoot DW, root: shoot ratio, acid phosphatase activity and rhizosphere soil DW. The third component (PC3) represented 14% of variability, and primarily accounted for seed phytate-P concentration, acid phosphatase activity and shoot DW (Figures 4A–C and Supplementary Table 3).

Seed phytate-P concentrations showed no significant correlation with either root size, root morphological traits, physiological traits, or shoot DW (Table 1). Root DW had a significant positive correlation with all plant traits, except acid phosphatase activity, whereas acid phosphatase activity was only significantly correlated with rhizosphere soil DW. Total root

length, root surface area and the total amount of carboxylates showed a significant positive correlation with all other traits, except seed phytate-P concentration and acid phosphatase activity (Table 1).

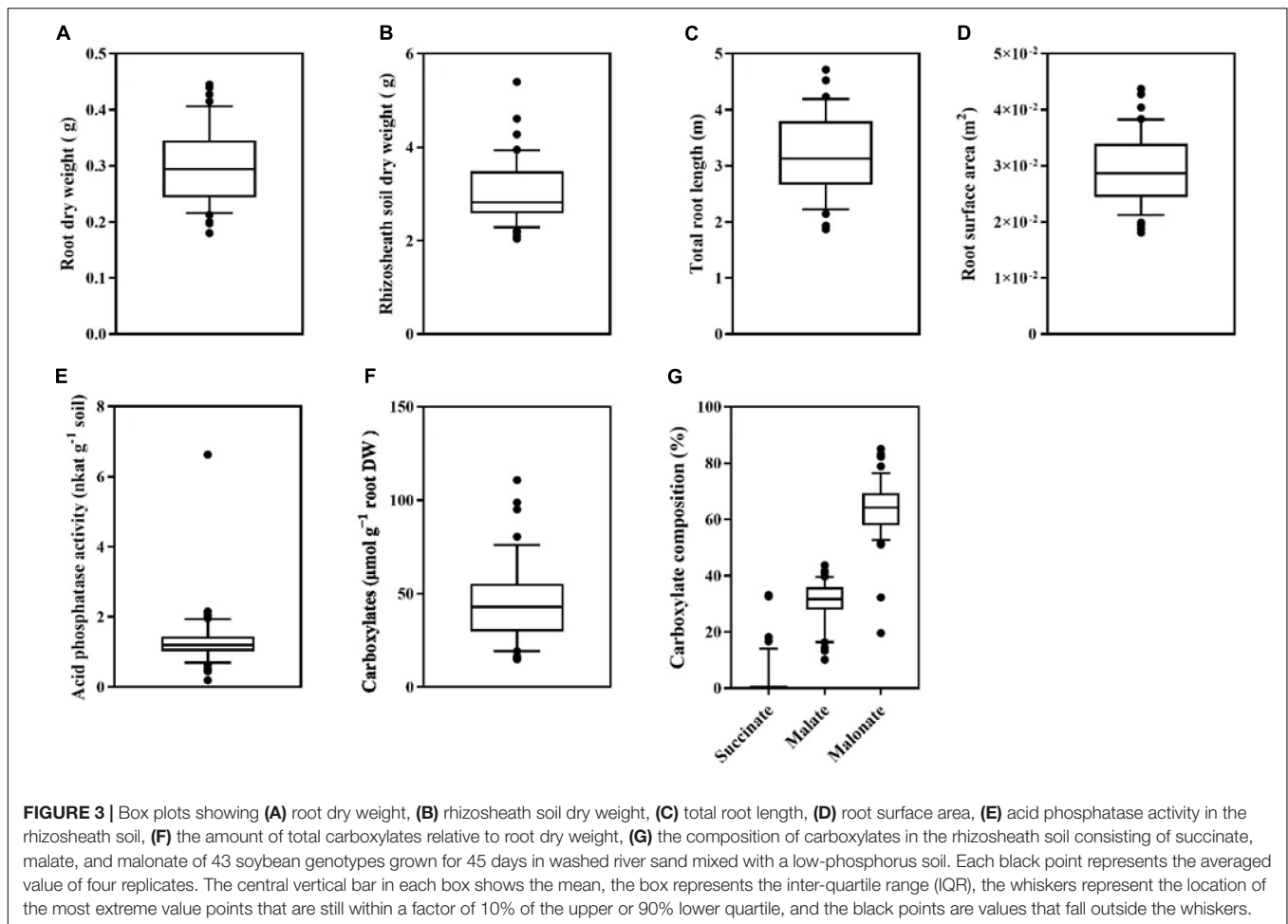
### Classification of Soybean Genotypes

There were 19 (out of 43) soybean genotypes that had a lower seed phytate-P concentration and at least one desirable P-acquisition trait (Table 2). The soybean genotypes showed a large variation with respect to each trait (Supplementary Figure 3 and Table 2). The maximum and minimum comprehensive score was gained by genotype Diliuhuangdou-2, Siliyuan and Jidou no 7 (5 out of 6), and genotype Lvcaodou, Zheng84240-B1, Miyangxiaozihuang, Binhaidahuangkezijia, Baiqidawandou and Zheng8516 (1 out of 6), respectively. Three soybean genotypes showed a higher score (5 out of 6) of these, two genotypes had seed phytate-P concentration below  $1.53 \text{ mg g}^{-1} \text{ DW}$  including Siliyuan and Diliuhuangdou-2. The high-score genotypes all exhibited efficient root morphological traits (Table 2).

### DISCUSSION

A vital first step for improving trait in breeding programs is to explore the genetic variation in germplasm (Perera et al., 2019). The soybean core collections with different sample size were established based on the genetic diversity existed among its originated country of China (Wang et al., 2006; Qiu et al., 2013), in which the mini core collection had the rich diversity with the least sample size (Song et al., 2010). Thus, they had priority for discovering new traits or genes. The present study shows a large variation in seed phytate-P concentration among 256 soybean genotypes (Figure 1) because almost all mini core collection was included. Since the present genotypes were grown at the same time in the same field, we minimized environmental variables, growing location, irrigation condition, fertilizer applications soil type, and planting time (Boehm et al., 2017). Therefore, variation in seed phytate-P concentration in the present study can be primarily attributed to the broad genotypic variability of 256 soybean genotypes. This agrees with results of Raboy et al. (1984), who found extensive variation in soybean seed phytate-P concentration among 38 genotypes that were grown at the same time in the same field. Seed phytate P concentration ranged from  $13.9$  to  $23.0 \text{ mg g}^{-1} \text{ DW}$  with a mean of  $17.6 \text{ mg g}^{-1} \text{ DW}$ . Likewise, Horner et al. (2005) found a large variation in phytate-P concentration among 86 soybean genotypes that were grown at the same time at three locations, ranging from  $7.7$  to  $22.2 \text{ mg g}^{-1} \text{ DW}$  with a mean of  $14.5 \text{ mg g}^{-1} \text{ DW}$ . These results provide compelling evidence that genotypic variation in phytate-P concentration exists within soybean germplasm, despite a previous study showing that soybean has higher concentrations of seed phytate-P compared with our study (Wilcox et al., 2000).

Importantly, we found that more than half of the studied genotypes had a lower seed phytate P concentration than that of Gm-lpa-ZC-2 (range from  $5.6$  to  $9.7 \text{ mg g}^{-1} \text{ DW}$ ) and Gm-lpa-TW75-1 (range from  $3.0$  to  $6.4 \text{ mg g}^{-1} \text{ DW}$ ), although both Gm-lpa-ZC-2 and Gm-lpa-TW75-1 were previously identified as

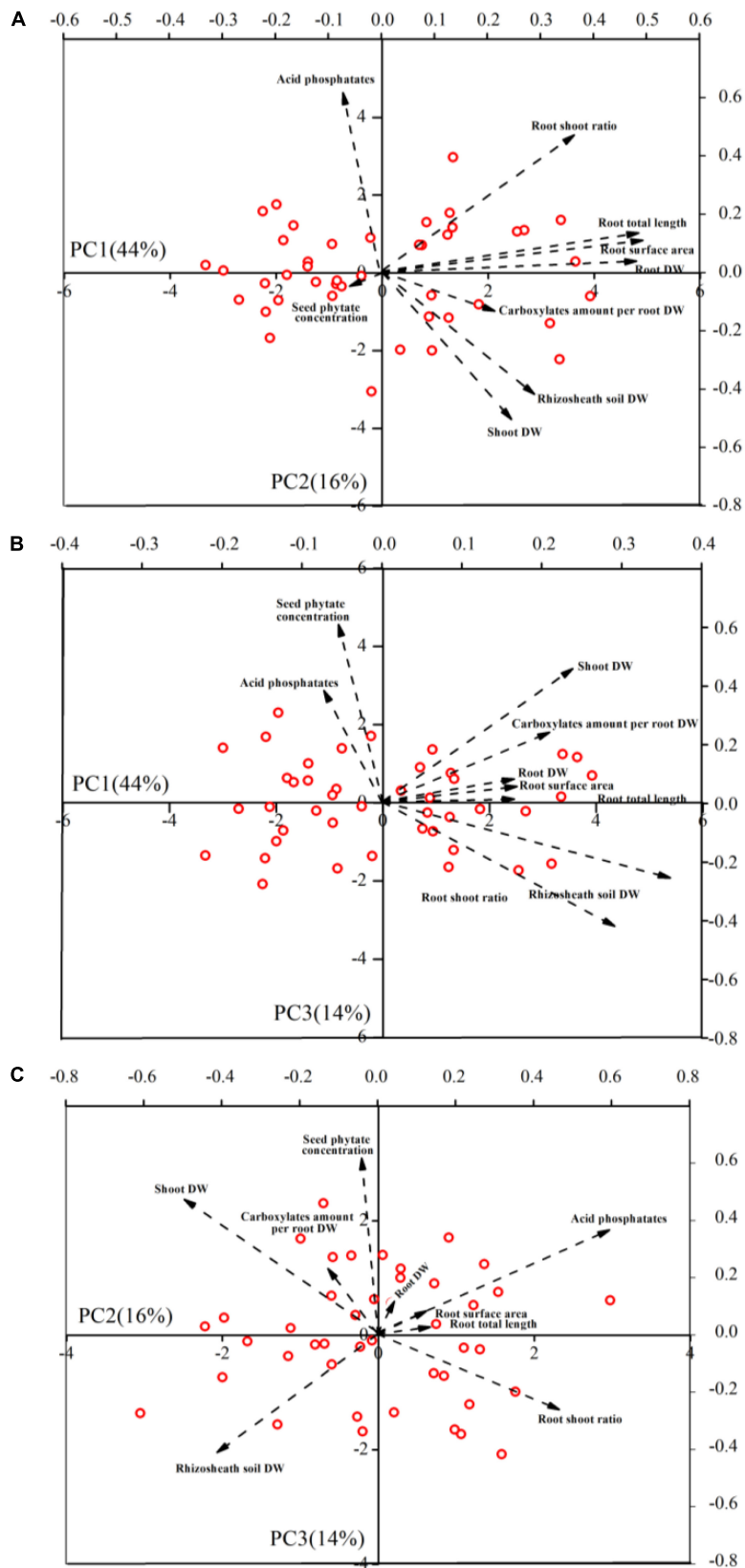


low-phytate soybean mutants (Yuan et al., 2007, 2009). This offers the exciting possibility of identifying genotypes with even lower phytate-P concentrations than known before. This information is highly valuable for breeding and identifying soybean genotypes with low phytate-P concentrations, because seed phytate-P not only reduces the availability of micro-nutrients, especially Zn, Fe, and Cu, for humans and livestock, contributing to malnutrition, but also cannot be efficiently utilized by humans and non-ruminant animals, contributing to high losses of P to the environment (Raboy, 2001).

As we hypothesized, we found that under low-P conditions, root size (root dry weight), rhizosphere soil dry weight, morphological (total root length and root surface area) and physiological (carboxylate exudation and acid phosphate activity in rhizosphere soil) traits showed significant variations among 43 soybean genotypes (Figure 3), confirming a large genotypic variability for these traits. Root traits are critically important by determining soil exploration and therefore nutrient acquisition (Lynch, 2007). They affect a plant's acquisition of P including through the release of root exudates such as carboxylates and enzymes that alter the rhizosphere soil properties and increase the amount of available nutrients, distribution of roots, and morphological characteristic of the root system such as

root surface area and specific root length. All of these traits influence the soil volume that is explored by the root system for acquisition of nutrients (Lambers et al., 2006; Richardson et al., 2011; White et al., 2013; Krishnapriya and Pandey, 2016; Zhou et al., 2016; Lynch, 2019; Wang et al., 2019; White, 2019). Therefore, this information provides a scientific basis for breeding and identifying soybean genotypes with greater P-acquisition efficiency.

The absence of correlations between desirable root traits and seed phytate-P concentration gives valuable insight for selection in breeding programs. In this study, principal component analysis explained 74% of variation in the first, second, and third components (Figures 4A–C and Supplementary Table 3). The first (PC1) and second (PC2) components primarily comprised all of the root traits measured, whereas the third component (PC3) comprised seed phytate-P concentration. Our study shows that seed phytate-P concentration had no significant correlation with any root trait measured, while there was a significant correlation among all of these root traits measured (Table 1 and Supplementary Figure 3). This suggests that there is potential to breed low seed phytate-P soybean genotypes without compromising desirable P-acquisition traits. Classifying the genotypes based on the seed phytate-P concentration and



**FIGURE 4 |** Principal component analyses of 9 plant traits for 43 soybean genotypes. Biplot vectors are trait factor loadings, whereas the position of each genotype is shown. Panels refers to PC1 vs. PC2 (A), PC1 vs. PC3 (B), and PC2 vs. PC3 (C), respectively.

root traits is a useful tool in selecting potential genotypes to be used in biofortification of well-adapted genotypes with enhanced P acquisition. In the present study, we identified 19 soybean genotypes belonging to a low-phytate group with desirable root traits based on the plant performance score (Table 2 and Supplementary Figure 3), while some genotypes only had a low score (1 out of 6). The low-phytate soybean genotypes with desirable root traits identified in the present study are recommended to be used as genetic resource for breeding programs, whereas the low-phytate soybean genotypes with undesirable traits could be used as experimental materials in future genomic studies on low seed phytate. Although there were three genotypes that had a higher score (5 out of 6), we selected two soybean genotypes including Siliyuan and Diliuhuangdou-2 with both highly desirable P-acquisition traits

and low phytate-P concentrations (Table 2), because these had far lower seed phytate-P concentration than the other two genotypes. In addition, seed phytate-P concentrations of these genotypes were lower than that of Gm-lpa-ZC-2 and Gm-lpa-TW75-1, which were previously identified as low phytate-P soybean mutants (Yuan et al., 2007, 2009), suggesting the possibility of identifying and selecting further low-phytate soybean genotypes with highly desirable P-acquisition traits. Importantly, these two genotypes have crop yields of more than 3 t ha<sup>-1</sup> (unpublished), holding promise for application in agriculture. Further work on agronomic and physiological traits as well as seed quality parameters of the low-phytate P genotypes is needed. For example, it is important to examine whether seed P content of the low-phytate P genotype is sufficient for seed establishment.

**TABLE 1** | Pearson's correlation matrix for 9 plant traits in 43 soybean genotypes.

	Phytate-P	Root DW	Shoot DW	RhiS DW	Root/shoot ratio	TRL	RSA	APase
Root DW	-0.04							
Shoot DW	0.08	<b>0.54***</b>						
RhiS DW	-0.25	<b>0.40**</b>	<b>0.36**</b>					
Root/shoot ratio	-0.11	<b>0.71***</b>	-0.21	0.16				
TRL	-0.05	<b>0.83***</b>	<b>0.34*</b>	<b>0.47**</b>	<b>0.68***</b>			
RSA	-0.04	<b>0.90***</b>	<b>0.41**</b>	<b>0.43**</b>	<b>0.70***</b>	<b>0.97***</b>		
APase	0.10	-0.12	-0.11	<b>-0.38**</b>	-0.06	-0.06	-0.06	
Carb_root	-0.10	<b>0.31*</b>	0.28	0.21	0.11	0.30	<b>0.33*</b>	-0.01

Root DW, root dry weight; Shoot DW, shoot dry weight; RhiS DW, rhizosphere soil DW; TRL, total root length; RSA, root surface area; APase, acid phosphatase activity in the rhizosphere soil; Carb\_root, the amount of total carboxylates relative to root dry weight. Significant correlations are shown in bold, \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

**TABLE 2** | Comprehensive plant performance scores of low phytate-P soybean genotypes.

Genotype name	Phytate-P Conc mg g <sup>-1</sup>	Root size		Root morphological trait		Root physiological trait	
		Root DW	RhiS DW	TRL	RSA	APase	Carb_root
Lvcaodou	0.69					✓	
<b>Siliyuan</b>	0.95	✓	✓	✓	✓		✓
Pingdinghei	1.01		✓	✓	✓	✓	
Huaheihu	1.07	✓		✓	✓		
Gaozuoxuan no. 1	1.16	✓	✓	✓	✓		
<b>Diliuhuangdou-2</b>	1.53	✓	✓	✓	✓	✓	
Pixiannianzhuangliuyuxian	2.09		✓			✓	✓
Zhechengxiaohuangdou	2.11					✓	✓
Jidou no. 7	2.27	✓	✓	✓	✓		✓
Chadou	2.28	✓		✓	✓		
Bo'aiahongpizaojiaozi	2.48					✓	✓
Tongshanqingdadou	2.49	✓	✓		✓		✓
Miyangxiaozihuang	2.50					✓	
Zheng8516	2.55		✓				
Pixiansilicao	2.56	✓		✓	✓	✓	
Yangtianxiaohuangdou	2.58		✓	✓	✓		✓
Binhaidahuangkezijia	2.77					✓	
Miyangniumaohuang	2.79	✓	✓				✓
Baiqidawandou	2.87		✓				

Root DW, root dry weight; RhiS DW, rhizosphere soil dry weight; TRL, total root length; RSA, root surface area; APase, acid phosphate activity in the rhizosphere soil; Carb\_root, the total amount of total carboxylates relative to root DW. Ticks mean that genotypes have lower seed phytate-P concentration than the mean seed phytate-P concentration of 43 soybean genotypes, and higher amount or larger root traits than the average value. Genotype with bold is the recommended genotypes.



In summary, selecting low phytate-P soybean genotypes with desirable P-acquisition traits will tighten the P cycle in crop production systems, animal production systems as well as human consumption, and lead to reduction of P-fertilizer cost, increase of nutritional quality of soymeal, and decrease of P-related environmental pollution.

## CONCLUSION

We show substantial genotypic variation in seed phytate-P concentration and a range of root traits related to P acquisition. Some of the present genotypes showed even lower seed phytate-P concentrations than mutants that were considered the best available in terms of low seed phytate-P. Most importantly, seed phytate-P concentration was not correlated with any desirable root traits measured in this study.

## DATA AVAILABILITY STATEMENT

The generated datasets available by request to the corresponding author.

## AUTHOR CONTRIBUTIONS

MK, HL, JP, and W-FC designed the study. MK and WJ performed the experiments and collected the data. MK analyzed the data. MK, HL, JJ, L-JQ, JP, WD, W-FC, and FZ interpreted the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2020.574547/full#supplementary-material>

**Supplementary Figure 1** | Spatial distribution characteristics of phytate phosphorus (P) concentrations of 256 soybean genotypes. Abbreviations used along the x-axis refer to the provinces, spelled out toward the right of the figure panel.

**Supplementary Figure 2** | The proportion of carboxylates in the rhizosphere soil consisting of malate, citrate, fumarate, succinate, and *trans*-aconitate of 43 soybean genotypes.

**Supplementary Figure 3** | Plant performance scores of 43 soybean genotypes grown in washed river sand with a low supply of phosphorus (P).

**Supplementary Table 1** | Detailed information on the 256 soybean genotypes used in the present study to measure seed phytate phosphorus concentration, and to study six root traits, associated with phosphorus acquisition.

**Supplementary Table 2** | Descriptive statistics of phytate-phosphorus (P) concentration of 256 soybean genotypes commonly cultivated in China.

**Supplementary Table 3** | Variable scores of nine plants traits in 43 soybean genotypes and proportion of variation of each component.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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