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Uptake and accumulation of phosphorus by dominant plant species growing in a phosphorus mining area

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ABSTRACT

Phosphorus accumulation potentials were investigated for 12 dominant plant species growing in a phosphorus mining area in Shifang, as well as their corresponding non-mining ecotypes growing in Ya'an, China. High phosphorus concentrations were observed in the seedling and flowering stages of two species, *Pilea sinofasciata* and *Polygonum hydropiper*, up to 16.23 and 8.59 g kg^{-1}, respectively, which were 3.4 and 7 times higher than in the non-mining ecotypes. Available phosphorus levels in the respective rhizosphere soils of these plants were 112.84 and 121.78 mg kg^{-1}, 12 and 4 times higher than in the non-rhizosphere soil. Phosphorus concentrations in shoots of the mining ecotypes of all 12 species were significantly negatively correlated with available phosphorus in the rhizosphere soils ($p < 0.05$), whereas a positive correlation was observed in the non-mining ecotypes. The biomass in shoot of the mining ecotype of *P. hydropiper* was nearly 2 times that in the non-mining ecotype. The results suggested that *P. sinofasciata* and *P. hydropiper* were efficient candidates among the tested species for phosphorus accumulation in shoots, and that further studies should be conducted to investigate their potential to be adopted as phosphorus accumulators.

1. Introduction

Intensive chemical fertilizer application and animal-raising pose significant threats to surface water and therefore agricultural areas should be a priority for implementation of environmental management measures and the adoption of best management practices [1]. Water-soluble phosphorus that comes from runoff is the cause of eutrophication in the aquatic environment, which is a serious and growing environmental problem worldwide [2]. Reduction of phosphorus inputs to surface water is thus receiving much attention.

One common approach to reducing soluble phosphorus losses from cropland has been the use of chemical amendments and biosolids to immobilize phosphorus in soils [3,4,5,6]. However, chemical amendments could not prevent the accumulation of phosphorus in soils but merely reduce the amount of water-soluble phosphorus, thus regulating the runoff loss [7]. Moreover, phosphorus immobilization in soil by these amendments may not be stable on a long-term basis and, instead result in higher soluble phosphates as in case of Ca and ferric phosphate-dissolution under certain normal soil conditions [8]. Another strategy to address the excess manure phosphorus involves the treatment of animal feed with additives such as phytase and vitamin D that can increase the digestibility of phosphorus in diet [9]. Whereas, concerns have been raised that although phytase can decrease total phosphorus in litter, it could increase the water-soluble phosphorus in the litter and hence the potential for phosphorus losses to surface waters following land application [10].

Alternatively, plant-assisted removal of water-soluble phosphorus was proved to be an attractive strategy to relieve its potential hazard permanently. Utilization of grasses for phytoremediation of phosphorus from animal manure impacted soils is widely documented [11–13]. Grasses outperformed broad leafed forages in dry matter yields and nutrient uptake on application of animal manure. Grasses vary in their potential for removal of phosphorus from contaminated soils [14]. Other studies also indicate the usefulness of phytoremediation using stargrass [15] and perennial ryegrass [16] in phosphorus impacted soils. Enhanced accumulation of phosphorus by cultivars of annual ryegrass from P-enriched soil and hydroponic media has already been manifested [17,18].

Recent studies on phosphorus accumulators mostly concentrated on the phosphorus phytoremediation potential in different plant species and genotypes [19,20], while scarcely any parallel research was keen on different ecotypes of same variety. The same plant when growing in the different places could form different ecotypes. Knowledge of variations such as phosphorus concentration in different ecotypes is crucial for identifying phosphorus accumulator plants able to compete with other plant species. Current phosphorus uptake rates are low for forage grasses used to assim-

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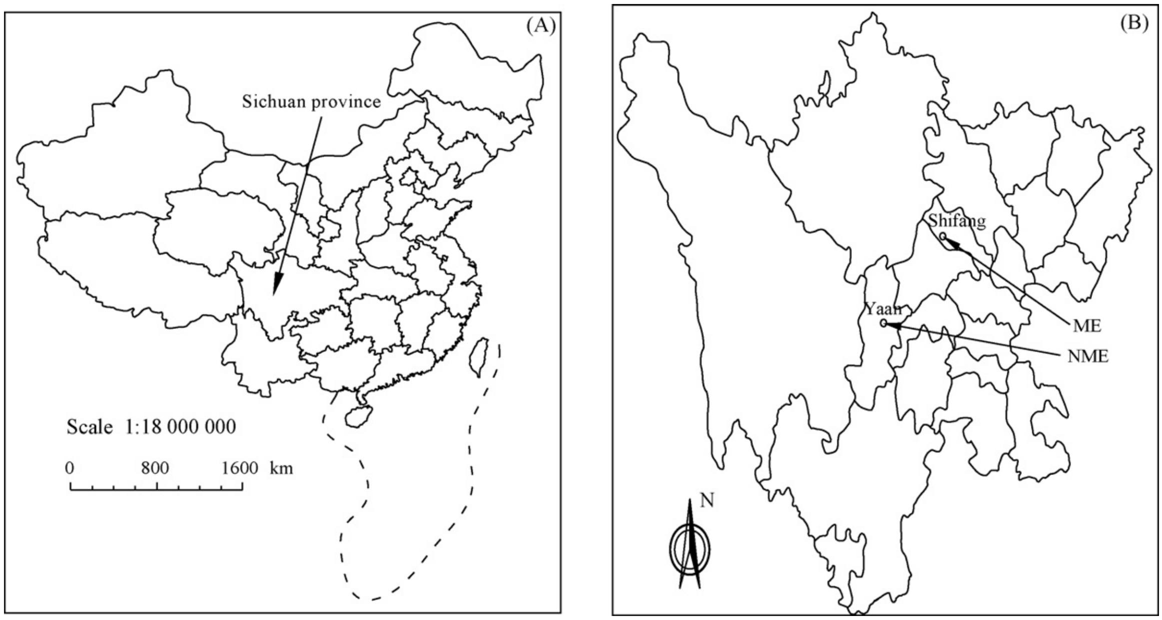

Fig. 1. (A) The map of China showing Sichuan Province. (B) Sites of the mining and non-mining ecotypes studied.

ilate phosphorus from soil [21]. Some phosphorus accumulators were screened out on the basis of phosphatic clay soil, but these plant species demonstrated low phosphorus accumulations in their shoots [11,22]. The ability of vegetation to assist in the remediation of phosphorus remains largely unknown. Annual ryegrass is one of the most efficient in extracting phosphorus and also highly productive [23]. However, annual ryegrasses are not resistant to freezing and summer drought. A hybrid grass resulting from the cross between Meadow Fescue and a ryegrass has overcome the difficulties [21], while more experiments in the form of field trials are needed to assess the utility of this grass for P phytoremediation.

Some plant species grow well in phosphorus mining areas. Hence, such species have potential to be suitable for phytoremediation of phosphorus-contaminated land. In this study, 38 plant species belonging to 7 families, collected from the phosphorus mining areas of China, were closely examined. According to accumulation concentration in plant shoots and the concentration time levels compared to plants from non-mining areas, 12 plant species were shown to have the ability to accumulate phosphorus, reflected by being the predominant plant species in their areas and growing very well during the 2 years of the study. The objectives of the present study were to determine which of the 12 species the best at accumulating phosphorus were, and to get better understanding of the accumulation capacity of the species to phosphorus in such an environment condition.

2. Materials and methods

2.1. Soil and plant sampling

The mining ecotypes were collected in a phosphorus mining area. The phosphorus mine is located in the town of Shifang, Sichuan, Southwest China (104°50′E, 30°25′N) (Fig. 1). The site has a subtropical moist monsoon climate with an average temperature of 15.9 °C. The concentration of P_2O_5 of the mine was about 27%. The relative elevation of the phosphorus mining area was nearly 1100 m. The landform gradient was 40–80°. The annual rainfall was 1259.5 mm. The non-mining ecotypes were collected from another place with similar climatic and topographic condition, in Yucheng, Ya'an (102°51′–103°12′E, 29°40′–30°14′N), Sichuan, China, also with a subtropical moist monsoon climate and an average annual temperature of 16.1 °C.

The sampling times chosen were the seedling and flowering stages. There are at least three replicates of a species. The photographs of predominant plant species were listed in Fig. 2. At least 6 individual plants of each plant species were randomly collected within the sampling area, then were mixed to give a composite whole plant sample of 1 replicate. For the collection of rhizosphere soil, the large clods were first discarded and the soil adhering to the plant roots was shaken off into a labeled plastic bags [24]. Non-rhizosphere soil was collected from the surface soil (0–20 cm depth).

The basic physiochemical characteristics of the phosphorus mining area were as follows: pH was 7–9. The content of available nitrogen and phosphorus were 7.97–33.57 and 52.43–112.84 mg kg^{-1}, respectively. The content of available potassium under 11 species were 18.30–59.11 mg kg^{-1}, that in P4 (a potassium accumulators) was 114.04 mg kg^{-1}. The content of organic matter was 2.45–5.02%.

2.2. Sample preparation and analytical methods

All plants were harvested whole and washed, first thoroughly with tap water and then 3 times with distilled water. The plants were divided into aboveground and underground parts and oven-dried at 70 °C to constant weight. The oven-dried samples were ground with a stainless steel grinder (FW-100, China) to pass through a 100 mesh sieve. The phosphorus concentrations in the plant samples and total phosphorus concentration in rhizosphere soils were measured colorimetrically at 700 nm after reaction with molybdenum blue method determined following thick H_2SO_4–H_2O_2 and H_2SO_4–$HClO_4$ digestion procedures, and the available phosphorus concentration was extracted with 0.5 M $NaHCO_3$ (soil:water = 1:20). The pH value (solid:distilled water = 1:5) of the soil samples was measured with a pH meter [25]. Three replicates of each sample were measured to ensure the precision of the determinations.

Fig. 2. Photographs of the 12 plant species used in the study, which are predominant in a phosphorus mining area of Sichuan.

2.3. Translation coefficient

Translation coefficient was calculated as follows: translation coefficient = phosphorus concentration in plant shoot/phosphorus concentration in plant root. Translation coefficient >1 indicates preferential partitioning of phosphorus to the shoot.

2.4. Bioaccumulation coefficient

Bioaccumulation coefficient was calculated as follows: bioaccumulation coefficient = phosphorus concentration in plant shoot/available phosphorus concentration in soil. Bioaccumulation coefficient can be used to evaluate the ability of plant to accumulate phosphorus.

2.5. Statistical analyses

The data on phosphorus concentrations and accumulation of the samples are presented for the three individual replicates. All data were analyzed using SPSS statistical software package (Version 11.0) by one-way ANOVA using LSD. Graphical work was carried out using Origin v.7.0.

3. Results

3.1. Phosphorus concentration in roots

The average concentration of phosphorus in the roots of the mining ecotypes was 199% higher than that of the non-mining ecotypes (Fig. 3). Almost all the plant species demonstrated a significant increase ($p < 0.05$) in the phosphorus concentration in roots over the period of growth. The amount of increase among the mining ecotypes was about 3 times that of the non-mining ecotypes. P1, P2 and P12 (Table 1) showed higher increases than the other species. A significant decrease of phosphorus concentration in roots was found in P3, P4 and P7 from the seedling to the flowering stage.

The phosphorus concentrations in the roots of the mining ecotypes varied from 1.71 to 9.28 g kg^{-1} (DW) at the seedling stage, whereas those in the non-mining ecotypes were from 1.19 to 5.42 g kg^{-1} (Fig. 3A). More than 5 g kg^{-1} of phosphorus was found

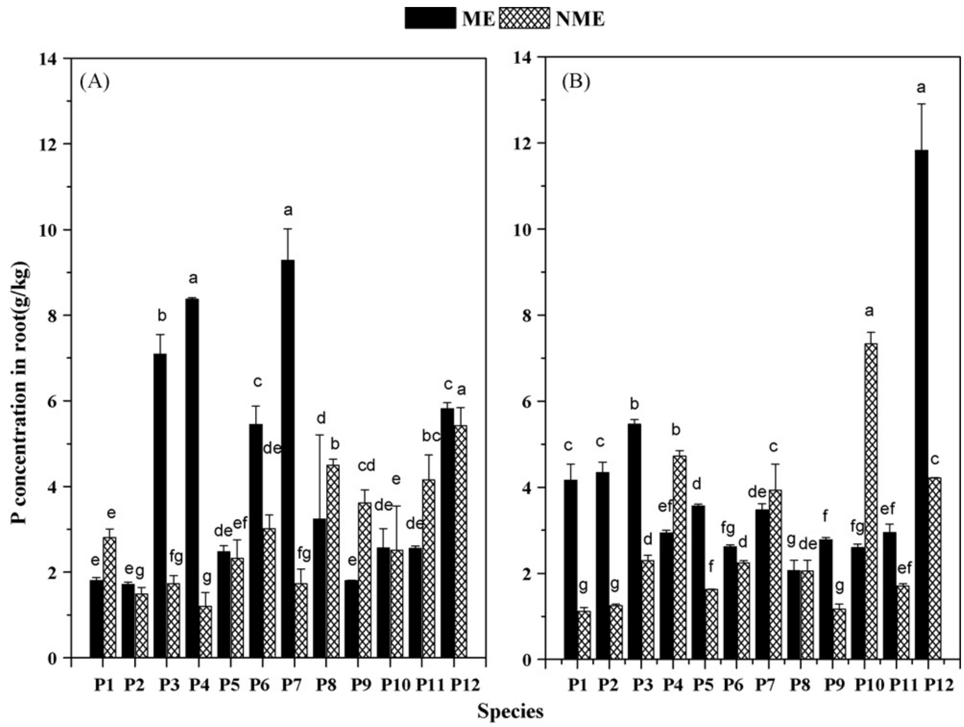

Fig. 3. Phosphorus concentrations in the roots of 12 plant species at seedling (A) and flowering stages (B). ME: mining ecotypes, NME: non-mining ecotypes. Data are means ± SD of three individual replicates. Mean values followed by different letters (a–g) are significantly different ($p < 0.05$). Flowering stage means the procreation growth stage.

in the roots of the mining ecotypes of P3, P4, P7 and P12. The highest variations between the mining and non-mining ecotypes was found for P3, P4 and P7, which were about 5 times those of the other species. The highest phosphorus concentration in the roots of a mining ecotype was found in P7. A significant difference ($p < 0.05$) was found in the phosphorus concentrations of the roots in the flowering stage, which varied from 2.06 to 11.83 g kg^{-1} (DW) in the mining ecotypes and 1.10–4.72 g kg^{-1} in the non-mining ecotypes, respectively (Fig. 3B). The average content of the mining ecotypes was 44.93% higher than that of the non-mining ecotypes. Among all the plant species, the highest root P concentration was found in P12 at the flowering stage. The phosphorus concentrations in the roots of the mining ecotypes of P1 and P12 were 3 times those of the non-mining ecotypes.

3.2. Phosphorus concentration in shoots

A significant difference ($p < 0.05$) was found in the phosphorus concentrations of the shoots for all 12 species (Fig. 4). The average concentration of phosphorus in the shoots of the mining ecotypes was 2 times that of the non-mining ecotypes. Almost all the plant species demonstrated a significant increase ($p < 0.05$) in the phosphorus concentration in the shoots from seedling to the flowering stage. The amount of increase among the mining ecotypes was about 4 times that of the non-mining ecotypes.

The phosphorus concentrations in the shoots of the mining ecotypes at the seedling stage varied from 2.46 to 8.94 g kg^{-1}, whereas those in the non-mining ecotypes were from 1.89 to 5.24 g kg^{-1} (Fig. 4A). Seven plant species showed more than 5 g kg^{-1} phosphorus concentration in the shoots of their mining ecotypes. The highest phosphorus concentration in the mining ecotype and the largest variation between the mining and the non-mining ecotypes were found in P12. A significant difference ($p < 0.05$) was also found among the phosphorus concentrations of the shoots at the flowering stage, which varied from 3.42 to 16.23 g kg^{-1} (DW) in the mining ecotypes and 1.23 to 7.33 g kg^{-1} in the non-mining ecotypes (Fig. 4B). Almost all the mining ecotypes had phosphorus concentrations of more than 5 g kg^{-1}. The largest variations between the mining and non-mining ecotypes were found in P1 and P12 (7 and 3.4 times, respectively). P12 had a phosphorus concentration of more than 10 g kg^{-1} in its shoots at the flowering stage. P1 displayed the highest phosphorus concentration variation between the mining and non-mining ecotype.

3.3. Bioaccumulation coefficient and translation coefficient

The average bioaccumulation coefficients of the 12 plant species are presented in Table 2. The variation between the coefficients of the mining ecotypes was higher than that of the non-mining ecotypes, and differed for different growth stages. The bioaccumulation coefficients of the mining ecotypes of P1, P6 and P12 were 2 times those of the corresponding non-mining ecotypes at

Table 1
Family and species composition of the predominant plants investigated in the phosphorus mining area.

Code name	Family	Species
P1	Polygonaceae	*Polygonum hydropiper*
P2	Compositae	*Anaphalis margaritacea japonica*
P3	Loganiaceae	*Buddleia davidii*
P4	Phytolaccaceae	*Phytolacca acinosa*
P5	Pteridiaceae	*Pteridium aquilinum*
P6	Onagraceae	*Epilobium fangii*
P7	Compositae	*Laggera alata*
P8	Compositae	*Senecio scandens*
P9	Compositae	*Erigeron acer*
P10	Compositae	*Hemistepta lyrata Bunge*
P11	Pteridaceae	*P. cretica var. nervosa*
P12	Urticaceae	*Pilea sinofasciata*

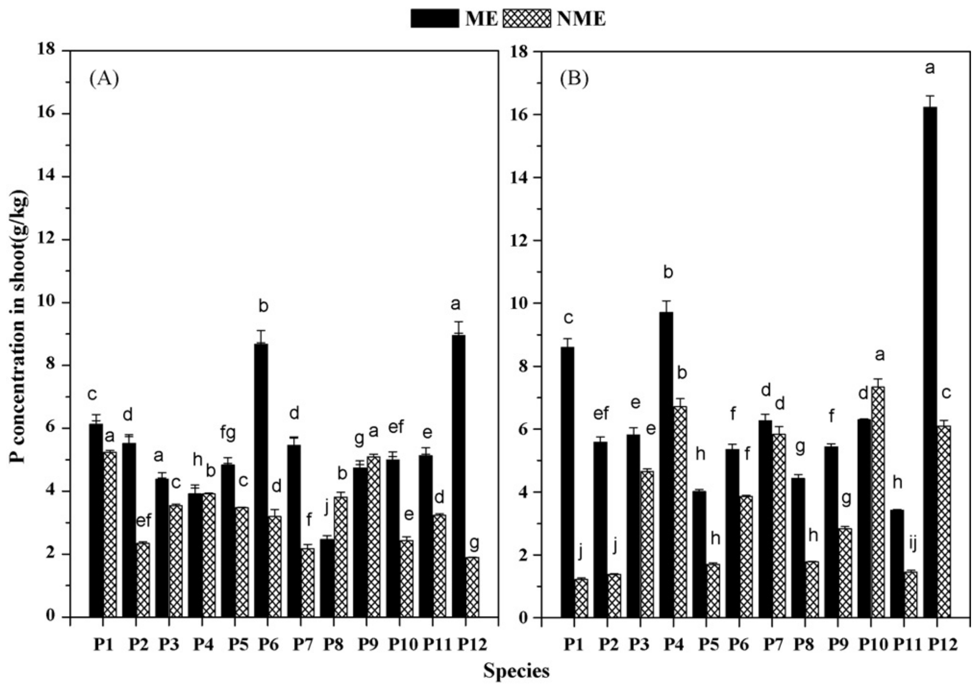

Fig. 4. Phosphorus concentration in the shoots of 12 plant species at seedling (A) and flowering stages (B). ME: mining ecotypes, NME: non-mining ecotypes. Data are means ± SD of three individual replicates. Mean values followed by different letters (a–j) are significant difference at ($p < 0.05$). Flowering stage means the procreation growing stage.

the seedling (Table 2). The highest bioaccumulation coefficients were found in P1 and P12 and the lowest in P8. The bioaccumulation coefficients of the mining ecotype of P1 were 17 times those of the corresponding non-mining ecotype at the flowering stage.

The translation coefficients of the seedlings of mining ecotypes of eight species were more than 1, and 1.3–4.4 times those of the corresponding non-mining ecotypes (Table 2), whereas those of the mining ecotype of P3, P4, P7 and P8 was less than 1. A larger variation between the plant species was found in the mining ecotypes than the non-mining ecotypes. The translation coefficients of P1, P2 and P12 were 3.39, 3.22 and 1.54, respectively, which were 1.8, 2.0 and 4.4 times those of the corresponding non-mining ecotypes at the seedling.

The translation coefficients of the mining ecotypes of P1, P4, P6, P8 and P10 were more than 2 at the flowering stage, which were higher those of the non-mining ecotype.

3.4. Phosphorus accumulation

Considerable differences were found in the phosphorus concentrations and biomass of the 12 species, leading to diversity in the phosphorus accumulation of the species (Fig. 5). Almost all the plant species demonstrated a significant increase ($p < 0.05$) in phosphorus accumulation over the period of growth. The increases of the mining ecotypes were higher than those of the non-mining ecotypes. The increment from the seedling to the flowering stage was highest for P1.

The phosphorus accumulation of the mining ecotype seedlings was 3.12–132.67 mg plant^{-1}, which was higher than that of the non-mining ecotypes (Fig. 5A). The highest phosphorus accumulation among the mining ecotypes was found in P10.

The average accumulation of the mining ecotypes at the flowering stage was 15.85–190.21 mg plant^{-1}, and the highest were found in P4 and P1 (Fig. 5B).

Table 2
Bioaccumulation coefficient and translation coefficient of the predominant plants.

Code name	Bioaccumulation coefficient				Translation coefficient			
	Seedling		Flowering stage		Seedling		Flowering stage	
	ME	NME	ME	NME	ME	NME	ME	NME
P1	568.80	196.41	798.00	45.93	3.39	1.87	2.06	1.10
P2	112.60	205.09	113.87	121.17	3.22	1.58	1.29	1.11
P3	107.98	299.96	143.50	393.72	0.62	2.05	1.06	2.03
P4	249.15	401.08	618.33	687.40	0.47	3.27	3.31	1.42
P5	195.42	321.64	162.57	157.39	1.95	1.50	1.13	1.05
P6	625.19	290.53	386.14	351.37	1.59	1.06	2.05	1.72
P7	269.86	202.88	310.05	543.13	0.59	1.26	1.81	1.48
P8	63.88	371.34	115.02	173.35	0.76	0.85	2.15	0.87
P9	104.53	252.20	120.16	140.26	2.62	1.41	1.96	2.42
P10	234.03	224.18	294.43	675.28	1.95	0.97	2.43	1.00
P11	240.37	255.31	160.43	115.25	2.01	0.78	1.16	0.86
P12	371.11	190.80	640.78	621.71	1.54	0.35	1.37	1.44

Bioaccumulation coefficient means phosphorus concentration in shoots/available phosphorus concentration in rhizosphere soils.

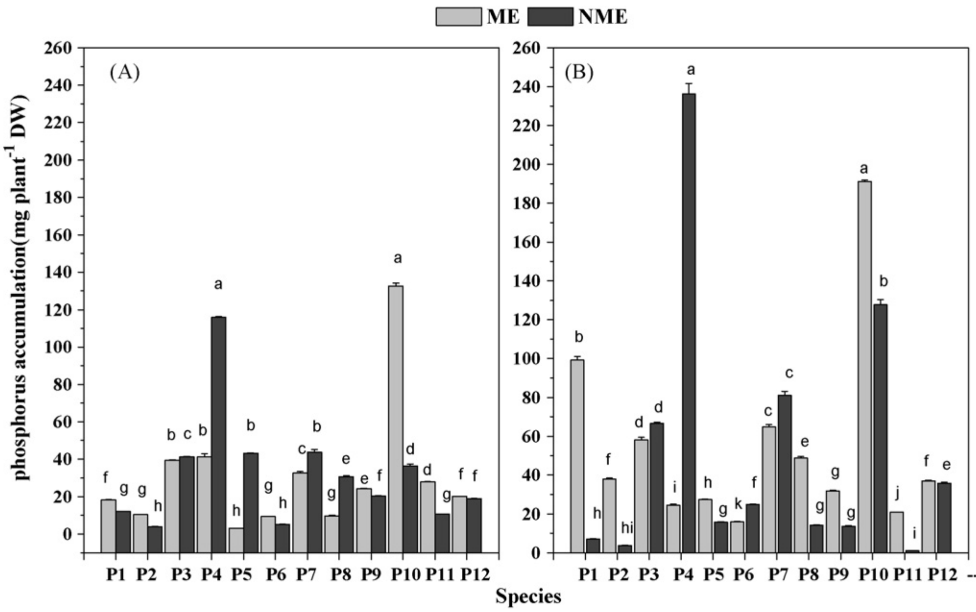

Fig. 5. The phosphorus accumulation in the shoots of 12 plant species at seedling (A) and flowering stages (B). Data are means ± SD of three individual replicates, flowering stage means procreation growing stage.

4. Discussion

4.1. Phosphorus uptake and accumulation

The plants showed a clear reaction to high phosphorus concentration in the soil, which was that the plants put more phosphorus in their shoots. The content of available phosphorus in the rhizosphere soils was higher than that of the non-rhizosphere soils (Table 3). The variation in available phosphorus content between rhizosphere and non-rhizosphere soils for the mining ecotypes was higher than for the non-mining ecotypes. The average content for the mining ecotypes was 80 mg kg^{-1}, which was 6 times more than that of the non-mining ecotypes. The highest variation in the available phosphorus content between rhizosphere and non-rhizosphere soil was founded for species P1 and P12, and was about 8 times the variation for the other plant species. The average content of total phosphorus in the mining ecotypes was 95 mg kg^{-1}, which was about 10 times that of the non-mining ecotypes. Little variation in total phosphorus existed between the rhizosphere and non-rhizosphere soils for any of the plant species, unlike the content of available phosphorus. The phosphorus concentration in shoots of the mining ecotypes was significantly negatively correlated with the content of available phosphorus in the rhizosphere ($p < 0.05$), whereas the shoot phosphorus concentration in the non-mining ecotypes was significantly correlated with the content of available phosphorus in the rhizosphere ($p < 0.01$) (Table 4). The phosphorus concentration in shoots of general plants significantly increased with ascending phosphorus concentration in soil. There was a different response to phosphorus application in the different plant parts and in the different treatments. In addition, phosphorus fertilization affected phosphorus concentration both at florescence and maturity in every plant organ, and there was also a significant effect of the change of phosphorus concentration at the two different growth stages [26].

Phosphorus supply annually increased growth and shoot phosphorus content of spring wheat [27]. These results of general plants were consistent with the non-mining ecotypes. However, the phosphorus concentration in shoots of the mining ecotypes was not directly influenced by the available phosphorus content of soil. The characteristic of accumulation of the mining ecotype is consistent with other metal hyperaccumulating plants. The potential of accumulation of the mining ecotype is controlled by the plant traits. The mining ecotypes may accumulate large amount of phosphorus under low soil phosphorus concentrations. The characteristic of accumulating phosphorus of the mining ecotype will be interpreted under pot experiment conditions.

The rhizosphere and non-rhizosphere soils were slightly calcareous (Table 3). The differences in pH between rhizosphere and non-rhizosphere soil for the mining ecotypes were greater than those for the non-mining ecotypes, and for the mining ecotypes of P1 and P12 the difference was 16 times those for the non-mining ecotypes.

All plants uptake phosphorus to varying degrees from substrates in which they are rooted. The form of phosphorus most readily accessed by plants is orthophosphates (Pi) and their forms in soil solution change according to soil pH [28]. The pK values for the dissociation of H_3PO_4 into $H_2PO_4^-$ and then into HPO_4^- are 2.1 and 7.2, respectively. Thus, below pH 6.0, most Pi will be present as the monovalent $H_2PO_4^-$ species, whereas H_3PO_4 and HPO_4^- will be available only in trace amounts [28]. In this investigation, the range of the pH values was between 7 and 9. The pH of the rhizosphere soils for the mining ecotype was lower than that of the non-rhizosphere soils. The differences in pH between rhizosphere and non-rhizosphere soil for the non-mining ecotypes were lesser than those for the mining ecotypes. The variation in pH between rhizosphere and non-rhizosphere soils for the non-mining ecotypes was less than 0.03 (Table 3). This finding implies that the different species can excrete different secretions to activate available phosphorus in the rhizosphere soil, and the mechanisms for absorbing phosphorus were not identical. It was reported that efficient use of P in the calcareous soil by P-efficient maize is related to its large root system, greater ability to acidify the rhizosphere [29].

The root traits such as root length and root volume of the mining ecotype were superior to those in the non-mining ecotype. Therefore, the capability of activating the phosphorus in rhizosphere soil for the mining ecotype was stronger than that for the non-mining ecotype. The mechanisms for phosphorus activation will be investigated under pot experiment and solution culture.

That plant uptake phosphorus is also affected by fixation of phosphorus by soil components, which is greatest in the presence

Table 3
Basic characteristics of rhizosphere and non-rhizosphere soils of the 12 plant species studied.

Code name	pH				Available phosphorus (mg kg^{-1})				Total phosphorus (g kg^{-1})			
	ME		NME		ME		NME		ME		NME	
	RS	NRS	RS	NRS	RS	NRS	RS	NRS	RS	NRS	RS	NRS
P1	8.36	8.69	7.62	7.62	112.84 ± 3.03b	95.56 ± 2.31b	26.70 ± 0.41a	14.50 ± 0.32b	96.25 ± 1.13e	96.41 ± 1.20d	9.44 ± 0.66cd	9.33 ± 0.60de
P2	8.36	8.38	5.53	5.52	83.36 ± 1.46d	80.17 ± 1.76c	11.45 ± 0.10de	10.62 ± 0.22d	90.77 ± 1.04f	89.96 ± 1.18e	8.31 ± 0.39de	8.25 ± 0.39ef
P3	7.18	7.54	7.70	7.71	82.13 ± 2.50d	78.91 ± 0.81c	11.82 ± 0.03d	9.79 ± 0.31d	97.62 ± 1.18e	97.66 ± 1.21d	10.2 ± 0.58bc	10.19 ± 0.56cd
P4	7.96	7.99	5.58	5.57	101.21 ± 3.87c	96.75 ± 1.20ab	9.76 ± 0.14h	10.09 ± 0.82d	82.2 ± 0.58g	82.25 ± 1.14f	7.44 ± 0.61e	7.29 ± 0.44f
P5	7.58	7.71	8.13	8.14	80.82 ± 1.99d	79.54 ± 1.58c	10.83 ± 0.04efg	10.17 ± 0.63d	90.98 ± 0.61f	90.82 ± 1.17e	12.42 ± 0.02a	11.76 ± 1.20ab
P6	7.25	7.34	7.74	7.71	56.30 ± 1.27e	53.13 ± 1.90de	10.99 ± 0.03ef	10.27 ± 0.11d	116.84 ± 1.19a	116.42 ± 1.76a	8.01 ± 0.58e	7.88 ± 0.07ef
P7	7.81	7.82	8.56	8.59	101.23 ± 2.37c	97.36 ± 0.64ab	10.74 ± 0.14fg	10.46 ± 0.52d	91.13 ± 1.21f	90.57 ± 1.22e	7.72 ± 0.31e	7.66 ± 0.27f
P8	7.32	7.35	7.97	7.95	98.67 ± 1.11c	97.53 ± 1.48ab	10.28 ± 0.02gh	10.11 ± 0.22d	108.87 ± 1.22bc	109.01 ± 2.30bc	10.21 ± 0.33bc	10.19 ± 0.02cd
P9	7.15	7.17	7.85	7.85	56.84 ± 1.28e	56.21 ± 1.80d	20.19 ± 0.02b	20.14 ± 0.48a	104.43 ± 1.20d	104.46 ± 1.96c	8.60 ± 0.28de	8.54 ± 0.02ef
P10	7.79	7.8	8.14	8.12	52.43 ± 3.03e	49.86 ± 2.41e	10.86 ± 0.05efg	10.28 ± 0.44d	106.31 ± 1.13cd	106.01 ± 1.75bc	11.21 ± 0.48ab	11.17 ± 0.27abc
P11	7.7	7.74	7.81	7.79	99.90 ± 1.44c	98.62 ± 0.88ab	12.66 ± 0.39c	12.14 ± 0.49c	109.91 ± 0.89b	109.88 ± 2.40b	10.76 ± 0.09b	10.56 ± 0.60bcd
P12	7.63	7.96	7.02	7.03	121.78 ± 1.48a	101.35 ± 2.26a	9.80 ± 0.49h	6.27 ± 0.40e	88.15 ± 1.03f	88.69 ± 2.30e	12.29 ± 0.35a	12.18 ± 0.56a

The data are average values of two samplings, at seedling and flowering stages. ME: mining ecotypes; NME: non-mining ecotypes; RS: rhizosphere soil; NRS: non-rhizosphere soil. Mean values followed by different letters (a–i) are significantly different ($p < 0.05$).

Table 4
Relationship of phosphorus concentrations (g kg^{-1}) between plant shoots, roots and AP of rhizosphere soils.

	Root–AP	Root–shoot	Shoot–AP
ME	−0.277	0.097	−0.370*
NME	0.055	−0.009	0.757**

AP refers available phosphorus concentration.
* Significant at $p = 0.05$.
** Significant at $p = 0.01$ according to SPSS.

of Fe- and Al-hydroxylated surfaces and, at a higher pH, calcium carbonate [30]. Most studies on the pH dependence of Pi uptake in higher plants have found that uptake rates are highest between pH 5.0 and 6.0, where plant assimilable $H_2PO_4^-$ dominates [18,28]. Changes in the pH of the rhizosphere, which is dependent on soil nutrient levels, could also be involved in absorption/assimilation aspects [31]. The pH of the rhizosphere of P1 was 8.36 and higher than the others. The pH of the rhizosphere of P4, P7, P10, P11 and P12 were higher than 7.6 (Table 3). Many researchers have indicated that the root secretions of plants had preferential effects on uptake and accumulation of nutrients by plants [32,33]. In this study, the pattern of the absorbed phosphorus was consistent with calcium carbonate. The decrease of the pH in the rhizosphere compared to the non-rhizosphere for the mining ecotypes was greater than that for the non-mining ecotypes (Table 3). The contents of total phosphorus in the rhizospheres of P1 and P12 were lower than those of the non-rhizosphere soil, whereas the contents of available phosphorus in their rhizospheres were much higher than in the non-rhizosphere soil. This indicated that P1 and P12 were able to uptake considerable amounts of phosphorus, thus indicating that secretions from the roots had reduced the pH of the rhizosphere soil and activated the phosphorus in the soil. This capability was highest in P1 and P12 (Table 3).

Both ecotype and plant species played important roles in phosphorus uptake and accumulation in this study. The variations of phosphorus concentration between different plant species and ecotypes were significant ($p < 0.01$) by analysis of variances (Table 5). In this study, the highest variations between the mining and non-mining ecotypes were found in P1 and P12, indicating that these two plant species much difference of accumulating phosphorus was found in.

It is reported that plant traits such as high biomass at low shoot phosphorus concentrations as well as the capacity to maintain high phosphorus availability in the rhizosphere by phosphorus mobilization, were able to explain the observed differences in plant growth and phosphorus uptake, although harvest date had a large effect on phosphorus uptake and its components [20]. In the present study, phosphorus accumulation was higher in the flowering than the seedling stage. This growth stage will be the focus of future studies planned to investigate the mechanism of the phosphorus accumulation in this system.

Plant phosphorus uptake has also been found to be correlated with root length and phosphorus availability in the rhizosphere,

Table 5
Analysis of variances for plant species, ecotypes and phosphorus concentration.

	Sum of squares	df	Mean squares	F	$F_{0.01}$
A	233.2462	1	233.2462	86.86**	6.84
B	268.4836	11	24.4985	9.12**	2.39
A × B	164.8382	11	14.9853	5.58**	2.39
Error	322.2318	120	2.6853		
Total	989.7998	143			

A: refers ecotypes; B: refers plant species.
** Significant at $p = 0.01$ according to SPSS.

Table 6
Biomass production in shoot and root of 12 plant species in seedling and florescence.

Species	Dry matter yield in seedling (g plant^{-1})				Dry matter yield in florescence (g plant^{-1})			
	Root		Shoot		Root		Shoot	
	ME	NME	ME	NME	ME	NME	ME	NME
P1	0.34	0.50	6.29	2.69	0.53	1.19	11.54	5.87
P2	0.91	0.99	1.88	1.70	1.51	1.20	6.77	2.68
P3	2.34	3.02	9.01	61.63	2.74	4.78	10.00	14.30
P4	17.88	11.33	10.54	29.61	0.62	9.40	2.52	35.21
P5	0.85	6.72	0.65	12.43	6.50	9.50	6.83	9.22
P6	0.24	0.83	1.08	1.65	0.53	1.98	2.96	6.46
P7	1.67	4.33	5.98	20.04	4.06	5.95	10.33	13.91
P8	0.62	1.78	3.95	8.03	5.86	0.84	11.00	8.04
P9	15.12	4.92	85.11	92.99	0.33	0.43	3.86	4.86
P10	1.85	4.01	8.52	14.99	4.94	6.82	30.39	30.41
P11	12.11	11.30	24.50	3.30	1.58	0.30	6.09	0.82
P12	0.31	2.12	3.56	5.02	0.80	0.54	5.08	5.21

particularly in the early growth stage [20]. This trait will be investigated in planned pot experiments.

4.2. Phosphorus accumulators

Some researchers suggest that for phosphorus phytoremediation to be effective, plants should have a high biomass and accumulate P at a significantly higher level (10 g kg^{-1} DW) than common plants do [12]. Many investigations of phosphorus accumulators have only concentrated on phosphorus concentration, while little has concentrated on different ecotypes of the same plant, as in the present study. However, the phosphorus accumulation potential of the different ryegrass genotypes Gulf and Marshall ryegrass has been investigated in a screening study. Gulf and Marshall ryegrass (*Lolium multiflorum*) demonstrated P accumulations >10 g kg^{-1} shoot dry weight depending on soil P concentrations (0–10 g of P kg^{-1} of soil) [18]. It was found that the herbage plants pigweed (*Amaranthus* sp.) and goosefoot (*Chenopodium* sp.) were promising phosphorus accumulators, with the ability of accumulating phosphorus in the range of 11–14 g kg^{-1} (DW) in their leaves [34].

The standard for phosphorus hyperaccumulators has not been defined scientifically. Currently, more attention has been paid to screening for plant species and genotypes which could grow well even under low phosphorus conditions, and the screening indexes include the biomass, root/shoot ratio, phosphomonoesterase, and phosphorus concentration. However, it was not precise in screening for phosphorus accumulators by using the screening index, which was applied in plants that could endure low phosphorus conditions. In our present study, four screening indexes were used: phosphorus concentration, phosphorus accumulation, the variance between the two ecotypes and translation coefficient. The concentrations of phosphorus in most plant species collected were higher than those in common plants. Using the absolute concentration previously suggested for hyperaccumulators (phosphorus >10 g kg^{-1} in shoot dry matter), P12 was found to be the sole candidate among the tested species. When translation coefficient >1 was used as a criterion, the translation coefficients of the mining ecotypes of most species exceeded this level. The translation coefficients of the mining ecotype of P1 were higher than the other tested species and were nearly 1.8 times those of their respective non-mining ecotypes. Although the translation coefficients of the mining ecotype of P12 were lower than most of the other tested species, the variation between the mining ecotype and the non-mining ecotype was higher than those in other species at the seedling. Plant species that are suited for phytoremediation should also have wide distribution, high aboveground biomass, high bioaccumulation, and high propagation rate [35]. Although the phosphorus concentration in the shoot of P1 of the florescence was only 8.54 g kg^{-1}, its phosphorus accumulation was high. The shoot biomass of P1 was only lower than P10 and higher than the other species (Table 6). Both P1 and P12 were commonly found at many of the mining sites and had high propagation rates. In addition, P12 is a perennial herbaceous plant. It is generally preferable to use a perennial, since phytoremediation will never take just a single year and the use of a perennial will prevent the need for annual planting. The capability of accumulating phosphorus was stable in P1 and P12 during the screening period.

5. Conclusion

P. sinofasciata (P12) and *P. hydropiper* (P1) demonstrated higher phosphorus accumulations in their shoots than the other tested species. A better capability of transfer phosphorus was also found in these species. Their mining ecotypes had a higher capability of accumulating phosphorus in shoots than the non-mining ecotypes. The potential of accumulating phosphorus using these accumulators is clearly an area deserving further detailed investigation, once it has been confirmed under laboratory and field trial conditions.

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