African Journal of Biotechnology Vol. 11(13), pp. 3061-3069,14 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3043
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Genotypic variation of rape in phosphorus uptake from sparingly soluble phosphate and its active mechanism

Zhou Xin-Bin[1,2,3], Huang Jian-Guo[2], Zhou Yong-xiang[2] and Shi Wei-Ming[1]*

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences,
Nanjing 210008, China.
[2]College of Resources and Environment, Southwest University, Chongqing 400716, China.
[3]Institute of Agricultural Resources and Regional Planning, Chinese Academy
of Agricultural Science, Beijing 100081, China.

Accepted 17 January, 2012

Phosphorus deficiency is one of the most growth-limiting factors in soils in various parts of the world. Two rape cultivars, which differed in Phosphorus (P) uptake from Fe-P ($FePO_4 \cdot 4H_2O$) and Al-P ($AlPO_4$), were investigated to elucidate the contributions of root morphology and organic acids exudation to P uptake by rape from iron phosphate and aluminum phosphate. By solution culture and sand culture experiments, the activation capacity of insoluble Fe-P and Al-P of organic acids secreted by different rape genotypes roots was studied. The results show that two rape cultivars has significant genotypic variations in both plant dry weight and P uptake per plant when supplied with Fe-P and Al-P as the P source. When supplied with sparingly soluble phosphate (Fe-P and Al-P), the root length, surface area and number of roots tips of genotype HG (phosphorus efficient rape) were significantly higher than genotype LG (phosphorus inefficient rape). The rape grew better in Al-P treatment than Fe-P treatment. Root exudates of rape has certain ability to activate insoluble P and it had better activation capacity of insoluble Fe-P under the condition of P stress than normal supply of P. Hence, the large differences show traits for more phosphorus efficient plants between in the tested rape genotypes. The genotype HG showed increased P acquisition from the Al-P and Fe-P than the genotype LG. This opens the possibility to breed for more P uptake-efficient varieties as a way to bring more sparingly soluble soil P into cycling in crop production.

Key words: Sparingly soluble phosphate, phosphorus efficiency, genotypic differences, rape.

INTRODUCTION

Rape is one of the major oil crops in China and worldwide. It is extremely sensitive to P deficiency, and soil phosphorus supply is one of the most important factors affecting rape production. However, phosphorus is usually the most limiting nutrient for growth of rape crops in tropical and sub-tropical regions. Phosphorus fertilizer is applied to mitigate soil phosphorus deficiency. However, the P utilization is very low (generally less than 20%), indicating that it is not a sustainable approach to mitigate soil P deficiency. As P fertilizer in soil is fixed by soil iron and aluminum oxide, P is a strongly bound and largely unavailable forms for crop uptake that resulted in the lower efficiency of P fertilizers (Ae et al., 1990a). In many of the so-called P-deficient soils in the world, total soil P level are 40 to 100 fold higher than the crop requirements, but sparingly soluble P pools are not immediately available to support plant growth. Moderate applications of P fertilizer often have only marginal effect on yields due to P fixation by Fe- and Al-oxides in the soils (Sample et al., 1980). Different genotypes vary significantly in the capacity of using sparingly soluble phosphate, some of the phosphorus efficient plants such as melons (Fita et al., 2011), barley (Gahoonia et al., 2000), tea (Lin et al., 2011), cowpea (Krasilnikoff et al., 2003) and wheat (Batten, 1992) can secrete large amounts of organic acids to enhance the utilization of

---

*Corresponding author. E-mail: zxbissas@swu.edu.cn.

sparingly soluble phosphate. In the respect of different wheat varieties, P efficient cultivar has significantly higher ability to dissolve calcium phosphate than phosphate inefficient cultivar (Liu et al., 1996). Therefore, development of crop varieties that can efficiently utilize these fixed forms of soil P (mostly iron-and aluminum bound-P) would result in lower input and an eco-friendly sustainable production systems both for developed and developing countries.

When studying phosphorus efficiency of plants, no matter whether it is cultured with conventional or modern biotechnology application, firstly it is important to reveal the mechanism of plants adapting to soil phosphorus stress, and further to find the specific physiological and biochemical indicators relevant to plants tolerance to low phosphorus. Effectiveness of soil P largely depends on the plant's ability to use insoluble P. Feature of root exudates (organic acids in particular) is one important factor to affect plant P uptake (Marschner et al., 1987). For example, Gahoonia et al. (2000) results showed that winter barley cultivars Marinka absorbed nearly twice as much P from the strongly adsorbed soil P fraction. The higher P uptake by Marinka than Sonate can be attributed to its ability to acquire P from strongly adsorbed soil P, by releasing more organic acids, especially citric acid, from its roots. Root exudates play an important role in the mobilization of soil mineral nutrients from sparingly soluble phosphate. Organic acid exudation by rape (*Brassica napus*) was shown to be an effective strategy to increase P uptake from rock phosphate (Hoffland et al., 1992). Rape utilized P from $Ca_3(PO_4)_2$ much better than from $AlPO_4$ (Zhang, 1997). Malonic acid exuded from the roots of pigeon pea could facilitate P release from $FePO_4$ and $AlPO_4$ (Otani et al., 1996). Recent studies suggested that organic acid anions can desorbs P from mineral surfaces and solubilize P from Al-, Fe- and Ca-phosphates by chelating metals (Ryan et al., 2001). Thus, the root release of organic acid anions is considered a principal mechanism in alleviating P deficiency. However, the study of Subbarao et al. (1997) showed that different Fe-P activation capacity of different genotype pigeon pea, root exudates cannot well explain the genotype difference of pigeon pea's ability to absorb Fe-P.

Based on the above literatures described, we guessed that exudation of organic acids by rape roots are probably involved in P mobilization and plant uptake. Whether different P activation capacity and organic acid content of different genotype rape root exudates can reflect the genotype difference of rape's ability to absorb sparingly soluble phosphate still needs further study.

In this present study, we analyzed different use of sparingly soluble phosphate (Al-P and Fe-P) of different phosphorus efficiency rape clarifying the relationship between rape's capacity of absorbing sparingly soluble phosphate and root morphology and root secretion of organic acids. Also, defining utilization patterns and physiological characteristics of different rapes' genotype and the ability to absorb sparingly soluble phosphate could provide a theoretical basis for the selection and cultivation of P efficiency rape.

MATERIALS AND METHODS

Plant materials and seedling growth

Two cultivars of rapes (*Brassica napus* L. B56 and B10) that differ in P-uptake efficiency in the field were selected for this study. Field test results showed that phosphorus absorption and use efficiency of rape B56 was significantly higher than rape B10 (Wang et al., 2011). For the convenience of writing, phosphorus efficient rape B56 was recorded as HG, and phosphorus inefficient rape B10 recorded as LG. A sand pot experiment was carried out at the Southwest University, Chongqing, China. Rape seeds were surface-sterilized for 5 min in 20 g/kg $HgCl_2$ solution and sown in sand and grown for 14 days with deionized water only. Seedlings were washed from the sand culture and carefully transferred to a solution containing [in mM]: 0.75 K_2SO_4, 0.65 $MgSO_4$, 1.0 KH_2PO_4, 2.0 $Ca(NO_3)_2$, 1.0 KCl; and [in μM]: 50 FeEDTA, 10 H_3BO_3, 1.0 $MnSO_4$, 0.5 $ZnSO_4 \cdot 5H_2O$, $0.05(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. The solution was adjusted to pH 6.0 with 1.0 mM HCl or NaOH and renewed every 2 days. After a 8-day growth period in the aerated nutrient solution, seedlings of similar size were selected and transplanted into the same aerated nutrient solution in a 2 L plastic pot (6 seedlings per pot) subjected to P treatments of different treatments (0, 1, 10, 20, 200, 500 μM) for additional 3 days before root exudates were collected. The seedlings were placed in a growth chamber with relative humidity of 65%, the mean day/night temperatures in the growth chamber were 25/20° cycles and the mean day/night temperature in the growth chamber was 14/10 h cycles. The photon fluency rate was 300 μmol photon/($m^2 \cdot s$). Each experiment was conducted with three replicates.

Sand culture experiment

For each pot, 2 kg quartz was measured, mixed thoroughly with treatment level of P and filled into the pots. Two genotypes were used in this experiment, respectively. Four surface sterilized seeds of each genotype were sown into the quartz filled pots. Four treatments were imposed with three replicates per pot: 87.8 mg KH_2PO_4; 78.6 mg $AlPO_4$; 143.5 $FePO_4 \bullet 4H_2O$ and CK (without P). To remove the water soluble P from the Fe-P and Al-P sample, which were washed repeatedly with water 10 times, dried in an oven 70°C before use. The total amount of P (10 mg P/kg) was the same for the three treatments. For convenience of description, the above P treatments will be designated as K-P, Al-P, Fe-P and CK, respectively. Pots were watered with demineralized water as and when required. Growth conditions were the same as those in solution culture experiment. After 30 day growth, the plants were harvested and P content in the shoots was determined by the molybdenum blue method (Murphy and Riley, 1962).

Estimation of root morphology

After the above experiment harvest, roots were washed with distilled water and transferred to 25% ethanol immediately. Then the roots were scanned with a STD 1600 scanner (Epson, Japan). The morphological parameters of roots, including length, surface area, volume, and the number of lateral roots, were examined by applying root system image analysis software (Win-Rhizo, Regent Instruments, Canada). After scanning, the roots were washed with distilled water, and prepared for the determination of dry weight and

P content as described above for shoot samples.

Solution culture experiment

Two rape's seedling preculturing were the same as in the case of sand culture experiment. After 10 days, healthy and uniform seedling of each rape cultivars were transferred to large-volume (50 L) containers supplied with either complete (10 mg P/L) or P-free nutrient solution. The nutrient solution was adjusted to pH 5.0 with 0.5 mM NaOH or HCl daily and renewed every two days.

Phosphate depletion experiment

Phosphate depletion experiment was carried out according to Claassen and Barber (1974) with some modifications. On day 20 after transferring, the groups of ten plants were placed into a 3 L container with P-free nutrient solution. The concentrations of other nutrients were one fourth of complete nutrient solution. After 36 h of P starvation, the P-free nutrient solution was replaced with a nutrient solution containing 2.5 mg P/L as NaH_2PO_4. The phosphate depletion experiment was carried out at 7:00 AM. Aliquots of nutrient solution were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 h after the beginning of depletion procedure, immediately filtered through a 0.45 μm syringe filter, and used for the determination of P concentration by molybdate blue method (Murphy and Riley, 1962). During the depletion procedure, the volume of nutrient solution was kept constant by adding distilled water every 30 min. This experiment was replicated three times at 25°C day temperature for 14 h and 22°C night temperature for 10 h, a humidity of 70%, and a light intensity of 300 μmol photon/(m^2·s). After the depletion procedure, the roots were rinsed three times with distilled water, dried at 105°C for 30 min, and then oven-dried at 70°C to a constant weight. Dry weight of root samples were recorded (Li et al., 2007).

Collection of root-exuded organic acids

Seedlings were pre-cultured in Hoagland's solution for six days, and then divided into two groups. One group of seedlings received P at 10 mg/L to produce P-sufficient plants; the other group which did not receive any P were P-deficient plants. After growing under these conditions for 16 days, to collect root exudates from whole root systems, four uniform seedlings of each genotype were taken from the pot, their seedlings were washed thoroughly in distilled water and transferred to a 1-L plastic container of 0.5 mmol/L $Ca(NO_3)_2$ solution at pH 5.3 for 10 min. Root exudates were collected for 24 h under aeration in a growth chamber at 27°C, 16-/8-h day/night cycles, 300 μmol photon/(m^2·s) light intensity and 70% relative humidity. The root exudates were divided into two groups. One group was evaporated to dryness under pressure at 40°C, dissolved in 5 ml of distilled water and then stored in a refrigerator at -20°C until organic acid analysis. Root exudates were standardized with root fresh weight, which was measured immediately after the treatment. The other group was used to do experiment mobilization of sparingly soluble inorganic phosphate.

Determination of organic acids

All the root exudates samples collected from rape genotypes grown in solution culture as described above were passed through a cation exchange column (16 ×14 mm) filled with 5 g of Amberlite IR-120B resin (H^+ form), followed by an anion-exchange column filled with 2 g of Dowex 1 × 8 resin in a cold room. The anionic fraction was eluted with 5 M HCOOH and evaporated to dryness using a rotary evaporator at 40°C. The residue was dissolved in 1 ml dilute H_2SO_4 and passed through a membrane filter (0.45 μm). The organic acid concentrations in root exudates were determined by HPLC equipped with an ion-exclusion column.

Estimation of solubilizing activity in root exudates

In order to examine the Fe-P, Al-P solubilizing activity by root exudates, Fe-P and Al-P solubilizing activity of the root exudates was estimated according to the method of Ae et al. (1990). A 25 ml of concentrated root exudates and 5 ml sodium acetate buffer (1 M; pH 5.6) was mixed with 0.1g $AlPO_4$, $FePO_4·4H_2O$ (which was washed repeatedly with distilled water to remove the water soluble P) in a plastic centrifuge tube. To this tube, 0.1 ml of chloroform was added to prevent microbial degradation of the root exudate compounds and the samples were loaded onto a shaker for 24 h at 25°C. Distilled water instead of the root exudates sample was used as the control. The P-solubilizing activity of root exudates of root exudates is expressed as the difference in P release from Al-P and Fe-P between root exudates and distilled water. A 25 ml of citrate (initial pH ≤ 4.0) with different concentrations (the concentrations were determined according to the concentration of citrate in P-deficient root exudates) was mixed with 0.1 g $AlPO_4$, $FePO_4·4H_2O$ in a plastic centrifuge tube as above methods. After shaking the samples for 24 h at 25°C, the samples were centrifuged at 16000 × g for 10 min and the P content in the supernatant was measured using the method by Murphy and Riley (1962). The P content in the root exudates was also estimated using the molybdenum blue method, and taken into account while calculating the Fe-P and Al-P solubilizing activity of the root exudates was considered to express the solubilizing activity of the root exudates.

Data analysis

The experiments were arranged in completely randomized design, and the data were analyzed in SPSS 18.0. Analysis of variance was done for each variable with comparison of means by Duncan's multiple range tests or "t" test.

RESULTS

Plant growth and P uptake

Rape genotypes differed significantly in their utilization of K-P, Al-P, and Fe-P as P sources. The dry matter production and P accumulation between the two genotypes treated with three P fertilizer sources is shown in Table 1. The K-P was the most available P sources, followed by Al-P, Fe-P and CK (without P addition). The two genotypes displayed a similar pattern in response to different P treatments, but there was a significant genotypic difference in response to given P treatments. Higher phosphorus efficiency rape showed higher plant dry weights in comparison with those of low phosphorus efficiency rape. For Al-P and Fe-P, plant dry weights of HG were 52.1 and 27.1% of that of the K-P, respectively.

The corresponding values of LG genotypes were 70.4 and 44.5%, respectively. The total P uptake by the two rape genotypes under the three P fertilizer treatments is given in Table 1. The P accumulation of two genotypes showed a similar pattern to that of plant dry weight. In

Table 1. Dry weight and P accumulation of Brassica genotypes with different P treatments in sand culture at 50 days.

Treatment	Dry weight (g $plant^{-1}$)		P accumulation (mg $plant^{-1}$)	
	HG	LG	HG	LG
K-P	2.42^a	1.83^a	10.41^a	6.95^a
Al-P	1.83^b	1.34^b	5.12^b	3.62^b
Fe-P	1.27^c	1.12^b	2.21^c	1.49^c
CK	0.56^d	0.47^c	0.62^d	0.47^d
Treatments (T)	**		**	
Genotypes (G)	*		**	
T×G	**		**	

Data are means (n = 4). Different letters within a column are significantly different at $P < 0.05$ by Duncan Multiple Range Test.
Significant effects: ** $P < 0.01$,* $P < 0.05$; n.s. non-significant effect.

Table 2. Genotypic variations in different root morphological parameters of two rape genotypes under different P treatments.

Item	P- resource	HG	LG
Root length (cm)	CK	234.4^b	286.6^c
	K-P	422.3^a	429.3^a
	Fe-P	324.5^c	302.2^b
	Al-P	364.3^{bc}	311.7^b
Root surface area (cm^2/plant)	CK	31.2^d	29.2^c
	K-P	121.1^a	131.6^a
	Fe-P	44.5^c	32.1^b
	Al-P	61.1^b	57.6^b
Root volume (cm^3/plant)	CK	1.67^a	1.12^c
	K-P	2.65^a	2.72^a
	Fe-P	2.71^a	2.82^a
	Al-P	1.89^a	1.78^b
AvgDiam (mm)	CK	0.65^b	0.64^b
	K-P	0.87^a	0.91^a
	Fe-P	0.75^a	0.71^a
	Al-P	0.81^a	0.73^a
Tips	CK	223.4^c	223.3^c
	K-P	443.3^a	375.2^a
	Fe-P	298.3^b	275.2^{bc}
	Al-P	311.3^b	276.3^{bc}

Different letters within a row are significantly different at $P < 0.05$ by t test (n = 3).

terms of P uptake of the two rape varieties, P uptake with K-P treatment is more than Al-P treatment, and P uptake with Fe-P treatment was less than Al-P treatment. In the K-P and Al-P treatment, P accumulation of HG was significantly higher than those of LG in different P resources. There were significant differences ($P < 0.05$) in dry matter production among the rape genotypes studied. Rape had much better ability to absorb P from Al-P than from Fe-P.

Differences of rape roots with different P treatments

As seen from Table 2, when adding Al-P and Fe-P in sand culture, the indicators reduce differently compared with normal supply of phosphorus, in this condition; two

Table 3. Mobilization of Al-P and Fe-P by root exudates of two genotypes of rape. Vertical bars represent ±S.D. (n = 4).

Genotype	Normal P supply treatment		P deficiency treatment	
	Al-P	Fe-P	Al-P	Fe-P
HG	1887.1^a	1278.5^a	2765.4^a	2131.4^a
LG	778.4^b	521.4^b	1026.7^b	865.3^b
Treatments (T)	**		**	
Genotypes (G)	**		**	
T×G	**		**	

Data are means (n = 4). Different letters within a column are significantly different at $P < 0.05$ by Duncan Multiple Range Test. Significant effects: ** $P < 0.01$,* $P < 0.05$; n.s. non-significant effect.

Table 4. Exudation of organic acids by roots of rape genotypes in the present (CK+P) or absence (CK) of phosphorus.

Treatment	Type of organic acid	Organic acid exudation (µg / pot / 4 h)	
		HG	LG
CK+P	Malic acid	3.28^a	3.26^a
	Succinic acid	0.25^a	0.23^a
	Citric acid	3.21^a	3.18^a
	Acetate acid	2.12^a	2.04^a
	Total organic acids	8.86^a	8.71^a
CK	Malic acid	224.5^a	156.8^b
	Succinic acid	8.60^a	8.23^b
	Citric acid	58.16^a	45.12^b
	Acetate acid	35.48^a	25.71^b
	Total organic acids	326.74^a	235.86^b

Mean values; n = 3. Means with the same letter are not statistically different at $P = 0.05$. Capital letters refer to differences between P treatments. Small letters refer to differences between genotypes (read horizontally). Means of 3 pots each with 60 plants (3 L pot).

genotype rapes have obvious differences. With normal P supply treatment, LG's total root length, root number, root volume and root surface area were similar to HG. But when supplied with sparingly soluble phosphate, HG's root length, root surface area, root number were significantly higher than the LG. Compared with the control treatment (inorganic phosphate as phosphorus source), Al-P treatment reduces root length and the number of lateral roots of rice, but increases root volume. The other three indicators have no big difference with normal P supply, and the difference among varieties was mainly caused by phosphorus deficiency.

Mobilization of root exudates on Al-P and Fe-P

Table 3 shows whether with normal P supply or P deficiency treatment, the root exudates of rape has certain ability to activate Al-P and Fe-P, and activation amount of Al-P was higher than Fe-P. In contrast, with phosphorus deficiency treatment, the root exudates have significantly higher Al-P and Fe-P activation than normal P supply. Root exudates of HG genotypes showed higher P-solubilizing activity (defined as the difference in P release between root exudates and distilled water) than those of LG genotypes. For example, P release from Al-P by P-deficient root exudates of HG genotypes was 166% of that by LG. P release from Fe-P by P-deficient root exudates of HG genotypes was 122% of that by LG. The two genotypes showed similar response to Al-P and Fe-P, though Al-P is more easily solubilized than Fe-P.

Exudation of organic acids

P-starved rape exuded different types of organic acids. Four kinds of organic acid, namely malic, succinic, citric and acetate acid were detected in the rape root exudates (Table 4). The amount of malic acid was the highest of the four. Malic acid was the major component, followed by citric acid, acetate acid and succinic acid. The total organic acids (malic acid, acetate acid, succinic acid and

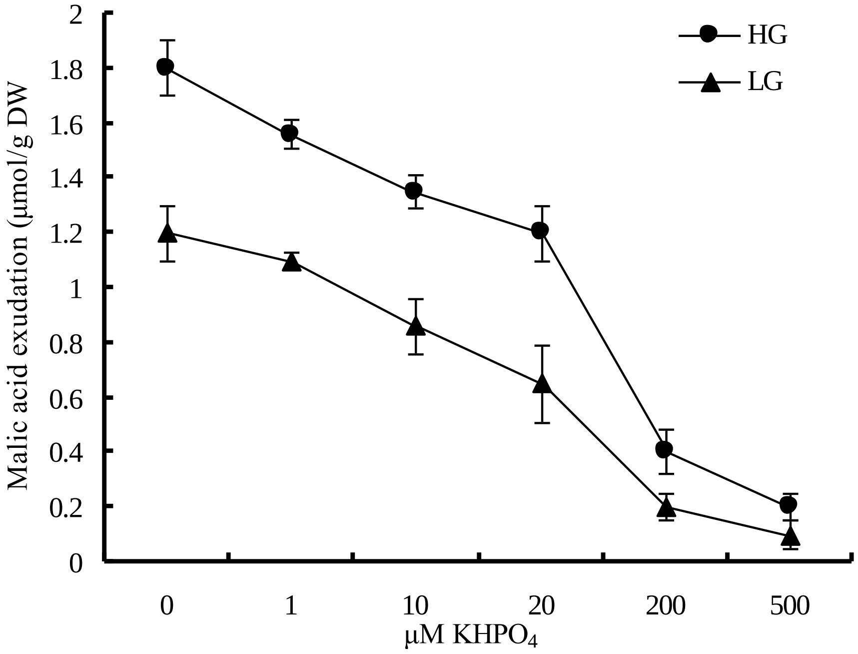

Figure 1. Malic acid exudation induced by different phosphate levels for two genotypes of rape. Malic acid was analyzed with ion chromatography. Means of 3 pots each with 60 plants (3 L pot). Vertical bar represents ± S.D. (n = 3).

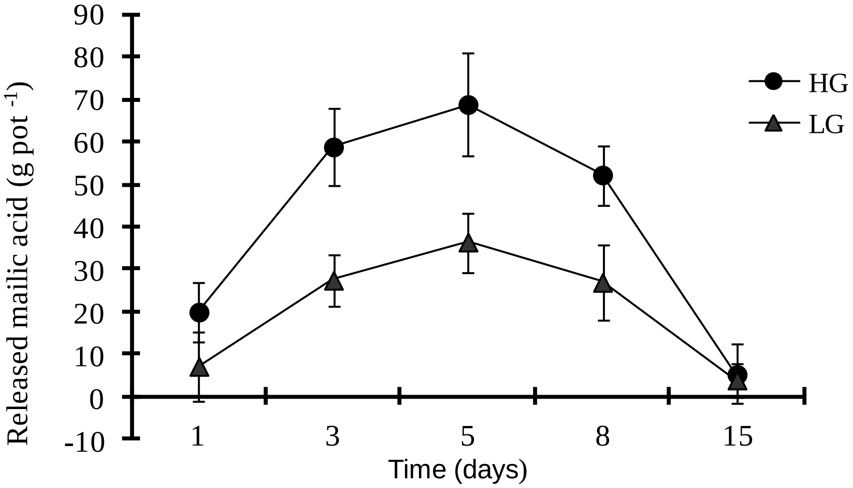

Figure 2. The dynamics of malic acid exudation from P-starved rape genotypes. Vertical bar represents ± S.D. (n = 5).

citric acid) secreted in HG was significantly higher than those of LG genotypes. P starvation caused a 68.4-fold increase in the amount of malic acid produced.

Malic acid exudation was greatly affected by P treatments (Figure 1). Malic acid exudation at low P was higher than that at high P concentration. HG exuded more malic acid rate than LG at all P concentration. The dynamics of malic acid exudation from P-starved rape genotypes is shown in Figure 2. Malic acid exudation of two genotypes attained a maximum after the rape genotypes were transferred to the P-deficient nutrient solution for 5 days. Thereafter malic acid exudation decreased sharply, dropping to a minimum at day 15 after treatment. Different genotypes showed similar responses in malic acid exudation to P-deficient stress over time. Under P deficiency, malic acid exudation in HG was higher than that in LG, particularly at day 5. Malic acid secretion of the two varieties reduces almost to zero in the 15th day.

DISCUSSION

Phosphorus is usually the most limiting nutrient for growth of rape crops. P deficiency is mostly caused by the fixation of P in the soil due to physical or chemical processes and large amount of sparingly soluble phosphate usually spread in the soil. For this reason, it would be effective to screen or to breed up crop varieties with high ability to acquire P from sparingly soluble phosphate. Genotypic variation in P acquisition from sparingly soluble phosphate such as Al-P or Fe-P has been reported for pigeon pea (Subbarao, 1997), rape (Hoffland et al., 1992), wheat (Osborne, 2002), and common bean (Shen, 2002). The results of this present study also showed significant genotypic variations in plant dry weight and P uptake by two genotypes rape plants supplied with K-P, Fe-P and Al-P, thus indicating the possibility to identify or to breed up rape cultivars capable of an effective absorbing P from sparingly soluble phosphate. Root morphology and properties of root exudates are two important factors that affect P absorption capacity of plants (Marschner, 1994).

Research and clarifying the traits relevant to rape's capacity of absorbing and use of sparingly soluble phosphate means a lot to select rape genotype with strong ability to absorb sparingly soluble phosphate. It is generally accepted that plant nutrition, especially for phosphorus, is closely associated with root morphological parameters. For example, Tinker and Nye reported that net P uptake per unit weight of a plant is determined by root length, uptake kinetics and the root length per unit weight of the plant and P movement by diffusion to the root cylinder (Tinker and Nye, 2000). Root hair exploitation of the soil increases the quantity of bio-available P, because plant-available P in the root hair cylinder is very close to P-adsorbing root hair cell membranes.

The P uptake per plant from Fe-P by different rice was significantly ($P<0.05$) correlated with root surface area and root volume as well as with the number of lateral roots, suggesting that the ability of rice to absorb P from Fe-P was closely related to root morphology (Li et al., 2007). Borkert and Barber (1983) reported that P uptake by soybean plants grown with low P supply in the solution culture experiment was highly correlated with total root surface area, but not with the root length, root biomass, or average root diameter. By comparison, cv. HG showed most extensive root surface area, greatest root volume, and most lateral roots than LG when exposed to Al-P and Fe–P. There was significantly genotypic variation in each root morphological parameter with either sufficient P supply, Al-P or Fe–P treatment. Similarly, Li et al. (2007) also reports that with Al-P and Fe-P treatment, the P uptake of rice were in significant positive correlation with root surface area, root volume, number of lateral roots ($P < 0.05$), indicating that the ability of rice absorbing sparingly soluble phosphate had close relationship with root morphology. It would be feasible to screen or to breed rape genotypes with high P uptake ability from Al-P and Fe-P according to the root morphological parameters. However, other reported that root hairs increase the effect of organic acid exudation (Hoffland, 1992).

Among the crop species, rape is reported to be very efficient in utilizing P not only from the reactive rock phosphate but also from the un-reactive phosphate rock sources (Bekele and Hofner, 1993). Exudation of organic acids is indicated as the mechanism responsible of the phosphate rock solubilizing capacity of rape (Hoffland et al., 1989b). Higher phosphorus efficiency showed better ability to mobilize P from Al- and Fe-bound phosphates in comparison with those of low phosphorus efficiency rape (Table 3).

Many studies have shown that plant root exudates are of great importance to activate insoluble soil phosphate (Hinsinger et al., 2001). Organic acid exudation experiment provided clear evidence that the efficient utilization of Al-P and Fe-P by HG genotypes may be attributed to the high P-solubilizing ability of the root exudates. Subbarao et al. (1997) found that the Fe-P or Al-P solubilizing activities of pigeon pea root exudates were presumably due to the chelating ability of the organic compounds released, rather than to their acidifying ability on the rhizosphere. Our results indicate that the amount of P release by root exudates of HG was significantly higher than that of LG. Further analysis of P-solubilizing ability of the root exudates revealed that more citrate, tartrate and acetate were exuded from the root of HG than from those of LG.

Therefore, the higher P uptake in HG may be largely explained by the higher exudation of organic acids. These results are in agreement with some previous reports (Grierson, 1992; Jones and Darrah, 1994; Johnson et al., 1996; Otani et al., 1996), which suggested that root-exuded organic acids could increase phosphate availability from sparingly soluble inorganic phosphate compounds.

Plant traits related to P acquisition are largely influenced by culture conditions or P nutritional status. A modified sand culture system was used because morphology of rape roots grown in sand was supposed to be much closer to that of plants grown in the soil as compared with roots grown in solution, and iron or aluminum oxides were dominant form of P in tropical and subtropical regions. As compared to the sufficient P supply, the dry weight, P uptake, and P concentration per dry weight of rape plants exposed to Fe-P and Al-P were significantly ($P<0.05$) lower (Table 1), indicating rape plants treated with Fe-P and Al-P as the source of P experienced as low-P stress.

There is no evident genotypic variation between two rape cultivars where two rape cultivars were cultivated in Fe-P. Root exudates consist of very complex composition, and organic acids plays a particularly important role in the activation of sparingly soluble phosphate (Hocking, 2001). The results showed that rape root exudates with P deficiency treatment had certain activation capacity of Al-P and Fe-P. In comparison, the exudates' activation of P efficient rape was higher than phosphorus inefficient rape. Ae et al. (1990) showed that pigeon pea has more efficient at utilizing iron-bound phosphorus (Fe-P) than several other crop species.

This ability is attributed to root exudates, in particular piscidic acid and its derivative. Shen et al. (2002) showed that large-seeded Andean genotypes (G19833 and G19839) exuded higher amounts of citrate, tartrate and acetate and mobilized more P from Al-P and Fe-P than small-seeded Mesoamerican genotypes (DOR364 and G21212). They showed that the active component of this organic acid was the hydroxyl and carboxyl groups of the tartaric portion, rather than the phenolic group. Phenolicas have also been reported as important root exudates that might affect the speciation of both Fe (Marschner and Römheld, 1994) and Al (Heim et al., 1999) via complexation reactions. Solubilization of P compounds from sparingly soluble phosphate by organic acids is achieved by complex formation between organic acids/anions and metal ions such as Fe, Al, and Ca. Complex formation depends on the number and position of carboxyl (-COOH) and hydroxyl (-OH) functional groups in the organic acids (Bolan et al., 1994). Solubilization of P by several organic acids corresponded to the stability constant for Al (log K_{Al}; 12.26 for citric acid, 6.21 for tartaric acid, 6.00 for malic acid, and 4.62 for succinic acid (Hue et al., 1986).

The more stability constant of Al, the more the aluminum dissolutions are, and the dissolution of iron also follows this rule (Ae et al., 1990).

However, there are some studies, such as Subbarao et al. (1997), showing that different Fe-P activation capacity of different genotype pigeon pea root exudates cannot well explain the genotype difference of pigeon pea's ability to absorb Fe-P. Li et al. (2007) also shows different sparingly soluble phosphate activation capacity of different genotype rice root exudates cannot well explain the genotype difference of rice's ability to absorb sparingly soluble phosphate.

This may be because plant's absorption and utilization of insoluble phosphorus is a complex process, including at least two processes: (1) plant roots' absorption of soluble phosphate, which is largely affected by other factors, For example, root growth and architecture, development of root hairs and of symbiotic, mycorrhizal associations (Raghothama, 1999).

Two above processes are crucial for an efficient acquisition of poorly mobile nutrients such as P. Study of Zhao et al. (2002) also reveals that root exudates and organic acids amount of bean is not consistent with the sparingly soluble phosphate activation capacity of root exudates. This also illustrates that the activation of insoluble P, in addition to detected organic acids, is also affected by some other material, such as amino acids, phenolic acids and other organic acids etc. In addition, P absorption of roots is a comprehensive process, including root morphology, root exudates, root architecture and other mechanisms, and plants' responses were not the same at different growth stages, further study is necessary to evaluate the role of the organic acids in Al-P and Fe-P activation and absorption.

Acknowledgement

This project was supported by the National Natural Science Foundation of China (No. 31101610), The Open Fund of State Key Laboratory of Soil and Sustainable Agriculture (0812000030; Y052010014), The Fundamental Research Funds for Central Universities (XDJK2011C012), The Open Fund of Ministry of Agriculture Key Laboratory of Crop Nutrition and Fertilization (20090401).

REFERENCES

Ae N, Arihara J, Okada K, Yoshihara T, Johansen C (1990). Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. Science, 248: 477-480.

Batten GD (1992). A review of phosphorus efficiency in wheat. Plant Soil, 146: 163-168.

Bekele T, Hofner W (1993). Effects of different phosphate fertilizers on yield of barley and rape seed on reddish brown soils of Ethiopian highlands. Fertil. Res. 34: 243-250.

Bolan NS, Raidu R, Mahimairaja S, Baskaran S (1994). Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biol. Fertil. Soils, 18: 311-319.

Borkert CM, Barber SA (1983). Effect of supplying P to a portion of the soybean root system on root growth and P uptake kinetics. J. Plant Nutr. 6: 895-910.

Fita A, Nuez F, Pico B (2011). Diversity in root architecture and response to P deficiency in seedlings of *Cucumis melo* L. Euphytica, 181(3): 323-339.

Gahoonia TS, Asmar F, Giese H, Nielsen GG, Nielsen NE (2000). Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. Eur. J. Agron. 12: 281-289.

Grierson PF (1992). Organic acids in the rhizosphere of *Banksia integrifolia* L. Plant Soil, 144: 259-265.

Heim A, Luster J, Brunner I, Frey B, Frossard E (1999). Effects of aluminium treatment on Norway spruce roots: aluminium binding froms, element distribution, and release of organic substances. Plant Soil, 216: 103-116.

Hingsinger P (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil, 237: 173-195.

Hocking PJ (2001). Organic acids exuded from root in phosphorus uptake and aluminum tolereance of plants in acid soils. Adv. Agron. 74: 63-97.

Hoffland E, Boogaard R, Nelemans JA, Findenegg GR (1992). Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. New Phytol. 122: 675-680.

Hoffland E, Findenegg GR, Nelemans JA (1989). Solubilization of rock phosphate by rape. local root exudation of organic acids as a response to P-starvation. Plant Soil, 113: 161-165.

Hoffland E (1992). Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant Soil, 140: 279-289.

Hue NV, Craddock GR, Adams F (1986). Effect of organic acids on aluminum toxicity in subsoils. Soil Sci. Soc. Am. J. 50: 28-34.

Johnson JF, Allan DL, Vance CP, Weiblen G (1996). Root carbon dioxide fixation by phosphorus-deficient *Lupinus albus*: contribution to organic acid exudation by proteoid roots. Plant Physiol. 112: 19-30.

Jones DL, Darrah PR (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil, 166: 247-257.

Krasilnikoff G, Gahoonia T, Nielsen NE (2003). Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. Plant Soil, 251: 83-91.

Li YF, Luo AC, Wei XH, Yao XG (2007). Genotypic variation of rice in phosphorus acquisition from iron phosphate: contributions of root morphology and phosphorus uptake kinetics. Russ. J. Plant Phys. 54(2): 230-236.

Lin ZH, Chen LS, Chen RB, Zhang FZ, Jiang HX, Tang N, Smith BR (2011). Root release and metabolism of organic acids in tea plants in reponse to phosphorus supply. J. Plant Physiol. 168: 644-652.

Liu GD, Li JY, Li ZS (1996). The genotypic differences in response of wheat root system to low-phosphorus stress. Plant Nutr. Fertil. Sci. 3(2): 212-218. (In Chinese).

Marschner H, Römheld V (1994). Strategies of plants for acquisition of iron. Plant Soil. 165: 261-274.

Marschner H, Romheld V, Cakmak I (1987). Root-induced changes of nutritient availability in the rhuzosphere. J. Plant Nutr. 10(9-16): 1175-1184.

Murphy J, Riley JP (1962). A modified single solution method for determination of phosphate in natural water. Anal. Chim. Acta. 27: 31-36.

Osborne LD, Rengel Z (2002). Genotypic differences in wheat for uptake and utilization of P from iron phosphate. Aust. J. Agric. Res. 53: 837-844.

Otani T, Ae N (1996). Phosphorus (P) uptake mechanisms of crops grown in soils with low P status. I. Screening of crops for efficient P uptake. Soil Sci. Plant Nutr. 42: 155-163.

Raghothama KG (1999). Phosphate acquisition. Annu. Rev. Plant Physiol. Mol. Biol. 50: 665-693.

Ryan PR, Delhaize E, Jones DL (2001). Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Mol. Biol. 52: 527-560.

Sample EC, Soper RJ Racz GJ (1980). Reactions of phosphate fertilizers in soils. In The role of phosphorus in agriculture. Eds. FE Khasawneh, EC Sample, EJ Kamprath. 263-310. ASA, CSSA, SSSA. Madison, WI.

Shen H, Yan XL, Zhao M, Zheng SL, Wang XR (2002). Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. Environ. Exp. Bot. 48: 1-9.

Subbarao GV, Ae N, Otani T (1997). Genetic variation in acquisition, and utilization of phosphorus from iron bound phosphorus in pigeonpea. Soil Sci. Plant Nutr. 43: 511-519.

Tinker PB, Nye PH (2000). Solute Movement in the Rhizosphere. Oxford University Press, New York.

Wang WH, Fan TT, Luo LY, Jin KX, Huang WQ, Chen X P, Zhou XB (2011). Study on the morphological and physiological characteristic of roots with different P efficiency. Soil Fertil. Sci. China, 4: 26-29 (In Chinese).

Zhang FS, Ma J, Cao YP (1997). Phosphorus deficiency enhances root exudation of low-molecular weight organic acids and utilization of sparingly soluble inorganic phosphates by radish (*Raghanus satiuvs* L.)and rape (*Brassica napus* L.) plants. Plant Soil, 196: 261-264.

Zhao M, Shen H, Yan XL (2002). Mobilization and uptake of insoluble phosphorus by different common bean genotypes. Plant Nutri. Fertil. Sci. 8(4): 435-440 (In Chinese).