Pak. J. Bot., 41(1): 109-119, 2009.

RELATIONSHIP BETWEEN LEAF ACID PHOSPHATASE ACTIVITY AND EITHER P NUTRITIONAL STATUS OR P EFFICIENCY IN RICE

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Abstract

To elucidate relationship between leaf acid phosphatase activity (APA) with either P nutritional status or P efficiency in rice, a hydroponic culture experiment supplied with either sufficient P (10 mg P L^{-1}) or deficient P (0.5 mg P L^{-1}) was conducted by using 8 rice genotypes different in their response to low P stress. Plants were sampled at 5, 10, 15 and 20 days after treatments (DAT). Leaf APA, leaf inorganic P concentration, total P concentration and total dry weight of rice plants were determined. Results showed that there were significantly (p<0.05) genotypic variations in P acquisition efficiency (PAE), P use efficiency (PUE) and leaf APA at different plant ages under either sufficient P treatment or deficient P treatment. The response of leaf APA to P deficiency varied significantly (p<0.05) 8 rice genotypes. Correlation analysis showed that relative leaf APA of rice plants was inversely correlated to relative PAE (p<0.01) and was positively correlated to relative PUE (p<0.01), suggesting that leaf APA was closely associated with P efficiency for rice plants. Under deficient P treatment, leaf APA was inversely correlated (p<0.05) to plant P concentration, including leaf inorganic P concentration and plant total P concentration, for rice plants sampled at 10 DAT and 15 DAT, respectively, whereas there was no significant correlation between leaf APA and plant P concentration for rice plants sampled at 5 DAT and 20 DAT, respectively. There date indicated that the extent of P deficiency for rice plants showed whether there was a significant correlation between leaf APA and plant P concentration.

Introduction

Phosphorus (P) deficiency is one of the major limiting factors to the crop production in most of the soils throughout the world (Hinsinger, 2001). Screening or breeding of crop varieties with high P efficiency would be one of the effective alternatives to alleviate the problem of P deficiency in the soil. It has been reported that there are significantly genotypic variation in P efficiency, including P acquisition efficiency (PAE) and P use efficiency (PUE), among different plant species or among different plant genotypes within a given species (Raghothama, 1999; Zaheer *et al.*, 2001; Vance *et al.*, 2003). Under P-deficient conditions, plants would develop numerous morphological, physiological, biochemical and molecular adaptations to increase PAE or PUE (Snapp & Hynch, 1996; Halsted & Lynch, 1996; Raghothana, 1999; Zaheer *et al.*, 2001). Enhancement of intracellular or extracellular acid phosphatase activity (APA) induced by P deficiency is one of plants' responses to low P stress that has been paid much attention in the recent two decades (Duff *et al.*, 1994; Vance *et al.*, 2003).

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It is generally accepted that acid phosphatase (APase) plays an important role in regulating P nutrition of plants (Duff *et al.*, 1994). The functions of intracellular APase related to P nutrition of plants are mainly including (1) hydrolyzing organic P into inorganic P (P_i), (2) releasing P from senescent tissue for remobilization and (3) bypassing the P-requiring steps in C metabolism (Duff *et al.*, 1989; Vincent *et al.*, 1992; Plaxton & Carswell, 1999). Therefore, it was assumed that intracellular APA was related to PUE (Duff *et al.*, 1994). However, results of Yan *et al.*, (2001) showed that there was no significant correlation between leaf APA and PUE in common bean and they concluded that the response of leaf APA to P deficiency was not an adaptive mechanism to low P stress in common bean. In addition, it has been suggested that leaf APA could be used as an indicator for P deficiency of plants because leaf APA is generally related to P concentration in some plants (Besford, 1980; McLachlan *et al.*, 1987). However, McLachlan (1984) suggested that P nutritional status of plants cannot be diagnosed merely by a single 'critical value' of leaf APA. Therefore, more investigations are needed to study the feasibility of diagnosing P nutritional status by intracellular APA.

In the present study, a hydroponic culture experiment was conducted to elucidate relationship between leaf APA with either P nutritional status or P efficiency by using eight rice genotypes different in their response to low P stress in a preliminary experiment with 90 rice genotypes (Li *et al.*, 2005).

Materials and Methods

Plant materials and plant culture: A hydroponic culture experiment was carried out in a glasshouse from 10 May to 20 June, 2005 at Zhejiang University, Huajiachi Campus, Hangzhou, China. Eight genotypes of rice viz., Zhenongda 454, Zaoniqiudao, Zhongbu 51, Pembe, Azucena, 31079, Xiqixuan and Hongmixian were used. Rice seeds were sterilized with 0.1% $HgCl_2$ solution for 1 min and then thoroughly washed with distilled water. Seeds were soaked in the distilled water for 36 h (30°C) and then sown on a nylon net supplied with half strength of standard rice nutrient solution (Yoshida *et al.*, 1976). The standard rice nutrient solution had the composition of the macronutrients NH_4NO_3 (1.4 mM), NaH_2PO_4 (0.32 mM), K_2SO_4 (0.5 mM), $CaCl_2$ (1.0 mM) and $MgSO_4$ (1.6 mM) and the micronutrients $MnCl_2$ (9.5 μM), $(NH_4)_6Mo_7O_{24}$ (0.01 μM), H_3BO_3 (20 μM), $ZnSO_4$ (0.15 μM), $CuSO_4$ (0.15 μM) and $FeCl_3$ (36 μM). At 2-leaf stage (about 10 days after germination), seedlings of each rice genotype uniform in size and vigor were transplanted into 60-L vessels containing standard rice nutrient solution. After 4-days growth, the nutrient solution was changed. Two P treatments were applied in the following period of the present experiment: (1) Control (sufficient P supply, 10 mg P L^{-1}); (2) Low P (deficient P supply, 0.5 mg P L^{-1}). Each treatment had three replicates. The pH of nutrient solution was adjusted to 5.0 by adding 1 M HCl or NaOH daily and the nutrient solution was renewed every 5 days. Twelve plants of each rice genotype were sampled at 5, 10, 15, 20 days after treatment (DAT). Three plants were used for determination of leaf inorganic P concentration, 3 plants were used for measurement of leaf APA and the remaining 6 plants were used for determination of total dry weight and total P concentration.

Leaf inorganic P concentration determination: A 0.2 g of fresh samples taken from the youngest fully expanded leaf was ground in ice-cooled 0.2 M perchloric acid (Jungk & Barber, 1975). The extract was transferred into 10-mL plastic centrifuge tube, and then centrifuged at 16000 × *g* for 10 min at 25°C. The P concentration in the supernatant was colorimetrically determined using molybdate-blue method (Murphy & Riley, 1962).

Leaf APA measurement: Leaf APA was determined according to the method of McLachlan *et al.,* (1987) with small modifications. A 0.2 g of fresh samples taken from youngest fully expanded leaves were frozen in liquid nitrogen immediately, ground in a cold mortar and macerated in 5 mL of 0.2 M acetic acid-sodium acetate buffer (pH 5.8). The extract was then transferred into 10-mL plastic centrifuge tube, followed by centrifuged at 27000 × *g* for 10 min at 4°C. Acid phosphatase activity was assayed using *p*-nitrophenyl phosphate (*p*-NPP) (Sigma, USA) as substrate and the reaction mixture consisted of 0.05-mL crude enzyme extract, 0.45-mL 0.2 M acetic acid-sodium acetate buffer (pH 5.8) and 4.5-mL 5mM *p*-nitrophenyl phosphate. After incubation for 30 min., at 30°C in darkness, the reaction was stopped by adding 2 mL of 1 M NaOH. The reaction solution was then centrifuged at 3000 × *g* for 2 min., and absorbance of supernatant was measured at 405 nm. The control tubes were added by 2 mL of 1 M NaOH at time zero of reaction. Leaf APA was expressed as micromole of *p*-nitrophenyl produced per gram leaf fresh weight per minute.

Total dry weight and total P concentration determination: After harvest, plant samples were divided into shoots and roots. Shoot and root samples were dried at 105°C for 30 min., and then oven-dried at 70°C to a constant weight. Dry weights (DW) of shoot and root samples were recorded. Shoot and root samples were ground with stainless steel mill and passed through 0.25 mm sieve for chemical analysis. The ground plant samples were digested in concentrated H_2SO_4 and H_2O_2 and P concentration in the digestion was colorimetrically determined using molybdate-blue method (Murphy & Riley, 1962).

Statistical analysis: A one-way analysis of variation (ANOVA) was carried out on the data obtained from the present study, and means were compared using least significant difference (LSD) test. The statistical analyses were performed according to the procedure of the SAS system (Anon., 1989).

Results

Genotypic variation of rice in P efficiency: There was a significant (p<0.05) genotypic variation in P acquisition efficiency (PAE), calculated as the total amount of phosphorus in the plant, among 8 rice genotypes regardless of plant ages or P treatments (Table 1). P acquisition efficiency of plants was significantly (p<0.05) decreased with deficient P supply in comparison to sufficient P supply (Table 1). There was a significant (p<0.01) genotypic variation in P use efficiency (PUE), calculated as the plant dry weight produced by per unit of phosphorus, among 8 rice genotypes regardless of plant ages or P treatments. P use efficiency of rice plants was significantly (p<0.05) increased with deficient P treatment compared with sufficient P treatment (Table 2).

Plant P nutritional status: There were significant (p<0.05) genotypic variations in both of two P forms, leaf inorganic P concentration and total P concentration, at different plant ages under sufficient P treatment and deficient P treatment (Table 3). Both of the two P forms were gradually decreased as the plants aged for all eight rice genotypes regardless of P treatments. Low P treatment significantly (p<0.05) decreased both of the P forms for all 8 rice genotypes compared to sufficient P treatment (Table 3).

Table 1. Genotypic variation in P acquisition efficiency (PAE) calculated as the total amount of phosphorus in the plant of 8 genotypes at different plant ages under sufficient P treatment (Control) and deficient P treatment (Low P).

Rice genotype	Days after treatment							
	5		10		15		20	
	Control	Low P	Control	Low P	Control	Low P	Control	Low P
	P acquisition efficiency (mg P plant^{-1})							
Zhenongda 454	0.807	0.354	1.632	0.546	2.375	0.683	4.497	0.658
Zaoniqiudao	0.856	0.359	1.854	0.544	2.849	0.698	5.420	0.741
Zhongbu 51	0.722	0.338	1.338	0.462	2.053	0.550	3.318	0.520
Pembe	1.129	0.546	2.319	0.670	3.666	0.891	6.738	0.729
Azucena	1.035	0.458	2.105	0.623	3.262	0.704	5.398	0.822
31079	0.951	0.456	1.857	0.570	2.644	0.720	5.518	0.702
Xiqixuan	0.824	0.417	1.925	0.525	2.596	0.620	5.490	0.723
Hongmixian	0.947	0.518	2.206	0.608	3.000	0.713	5.551	0.725
ANOVA F	8.8**	8.4**	4.7**	3.7*	7.6**	7.1**	6.8**	4.7**
$LSD_{0.05}$	0.134	0.080	0.440	0.101	0.555	0.109	1.130	0.125

ANOVA represents analysis for variation. NS: Not significant; *: Significant at p=0.05; **: Significant at p=0.01.

Table 2. Genotypic variation in P use efficiency calculated as the plant dry weight produced by per unit of phosphorus of 8 rice genotypes at different growth stage under sufficient P treatment (Control) and deficient P treatment (Low P).

Rice genotype	Days after treatment							
	5		10		15		20	
	Control	Low P	Control	Low P	Control	Low P	Control	Low P
	P use efficiency (g DW mg^{-1} P)							
Zhenongda 454	0.090	0.211	0.113	0.334	0.121	0.425	0.159	0.913
Zaoniqiudao	0.136	0.280	0.176	0.514	0.195	0.650	0.265	1.298
Zhongbu 51	0.102	0.185	0.127	0.384	0.145	0.477	0.191	1.024
Pembe	0.099	0.207	0.122	0.432	0.137	0.540	0.213	1.202
Azucena	0.092	0.201	0.121	0.408	0.133	0.536	0.176	0.997
31079	0.117	0.233	0.163	0.482	0.187	0.581	0.214	1.123
Xiqixuan	0.086	0.165	0.101	0.338	0.121	0.483	0.132	0.855
Hongmixian	0.106	0.174	0.141	0.422	0.170	0.600	0.234	1.222
ANOVA F	11.6**	18.1**	15.1**	17.8**	19.5**	10.9**	12.0**	8.2**
$LSD_{0.05}$	0.014	0.026	0.019	0.045	0.020	0.067	0.037	0.165

ANOVA represents analysis for variation. NS: Not significant; *: Significant at p=0.05; **: Significant at p=0.01.

Leaf APA: There was significantly (p<0.05) genotypic variation in leaf APA at different plant ages under sufficient P treatment and deficient P treatment (Fig. 1). The response of leaf APA to low P treatment was significantly affected by rice genotypes and time of low P treatment. At 5 DAT, leaf APA of P-deficient plants showed no significant difference to that of P-sufficient plants for all of eight rice genotypes. At 10 DAT, for some of 8 rice genotypes such as Zaoniqiudao, Pembe, Azucena and 31079, leaf APA of P-deficient plants showed significant (p<0.05) increase compared to that of P-sufficient plants (Fig. 1B, D-F). At 15 DAT, leaf APA of three rice genotypes including Zhenongda 454, Zhongbu 51, Xiqixuan were still not affected by low P treatment (Fig. 1A, C, G). At 20 DAT, low P treatment significantly (p<0.05) increased leaf APA of plants for all of eight rice genotypes (Fig. 1).

Table 3. Chronological changes in leaf inorganic P concentration and total P concentration of 8 rice genotypes under sufficient P treatment (Control) and deficient P treatment (Low P).

Rice genotype	Days after treatment							
	5		10		15		20	
	Control	Low P	Control	Low P	Control	Low P	Control	Low P
	Leaf inorganic P concentration (μg g^{-1} FW)							
Zhenongda 454	2172±95	282±14	1122±114	114±17	814±34	106±7	726±67	54±4
Zaoniqiudao	630±52	133±9	413±6	68±3	3044±18	37±1	329±22	26±4
Zhongbu 51	1206±52	313±27	931±73	95±5	4124±30	88±8	557±27	32±3
Pembe	1058±111	205±12	708±24	115±115	6024±22	46±5	637±98	25±2
Azucena	1452±761	304±332	976±60	97±6	536±87	72±7	621±22	35±5
31079	963±128	245±24	493±46	73±10	331±22	59±4	530±35	265±2
Xiqixuan	1324±34	275±20	1187±82	106±6	761±37	81±12	686±76	335±1
Hongmixian	1191±87	263±40	788±56	92±8	415±37	61±3	422±36	165±13
	Total P concentration (%)							
Zhenongda 454	1.12±0.06	0.47±0.02	0.88±0.03	0.30±0.02	0.84±0.09	0.24±0.02	0.63±0.01	0.11±0.01
Zaoniqiudao	0.74±0.06	0.36±0.03	0.57±0.03	0.20±0.02	0.52±0.05	0.16±0.02	0.38±0.02	0.08±0.01
Zhongbu 51	0.98±0.10	0.54±0.04	0.79±0.05	0.26±0.01	0.69±0.03	0.21±0.02	0.54±0.10	0.10±0.01
Pembe	1.02±0.09	0.48±0.03	0.83±0.13	0.23±0.01	0.73±0.03	0.19±0.01	0.47±0.05	0.08±0.01
Azucena	1.08±0.07	0.50±0.02	0.83±0.08	0.25±0.02	0.75±0.05	0.19±0.01	0.59±0.02	0.10±0.01
31079	0.88±0.03	0.43±0.03	0.62±0.05	0.21±0.01	0.54±0.02	0.17±0.00	0.47±0.04	0.09±0.00
Xiqixuan	1.17±0.08	0.61±0.04	1.00±0.10	0.30±0.01	0.83±0.10	0.21±0.02	0.77±0.11	0.12±0.00
Hongmixian	0.95±0.11	0.58±0.04	0.71±0.05	0.24±0.02	0.59±0.02	0.17±0.01	0.43±0.05	0.08±0.01

Each value represents mean ± SD (n = 3)

Relationship between Leaf APA and P concentration: Relationship between leaf APA and leaf inorganic P concentration showed that under sufficient P treatment, Leaf APA had no significant correlation to leaf inorganic P concentration regardless of plant ages (Fig. 2A-E). Under deficient P treatment, leaf APA seemed to be inversely correlated to leaf inorganic P concentration (Fig. 2F), however, the relationship between leaf APA and leaf inorganic P concentration was different at different plant ages (Fig. 2G-J). Leaf APA was inversely correlated to leaf inorganic P concentration significantly at p=0.05 and p=0.01 for rice plants sampled at 10 DAT and 15 DAT, respectively (Fig. 2H and I), whereas there was no significant correlation between leaf APA and leaf inorganic P concentration for rice plants sampled at 5 DAT and 20 DAT, respectively (Fig. 2G and J). Relationship between leaf APA and total P concentration was generally similar to the relationship between leaf APA and leaf inorganic P concentration (Figs. 2 & 3).

Relationship between leaf APA and P efficiency: Relative leaf APA was inversely correlated to relative PAE (p<0.01) and was positively correlated to relative PUE (p<0.01) (Fig. 4).

Discussion

Screening or breeding of crop varieties with high P efficiency may be one of the effective alternatives to alleviate P deficiency and to increase the utilization efficiency of P fertilizer. It is generally accepted that plants exhibit inter- and intra-specific variations in P uptake efficiency and P use efficiency (Raghothama, 1999; Zaheer *et al.*, 2001; Vance *et al.*, 2003). Our results also showed that PAE and PUE varied significantly (p<0.05) among

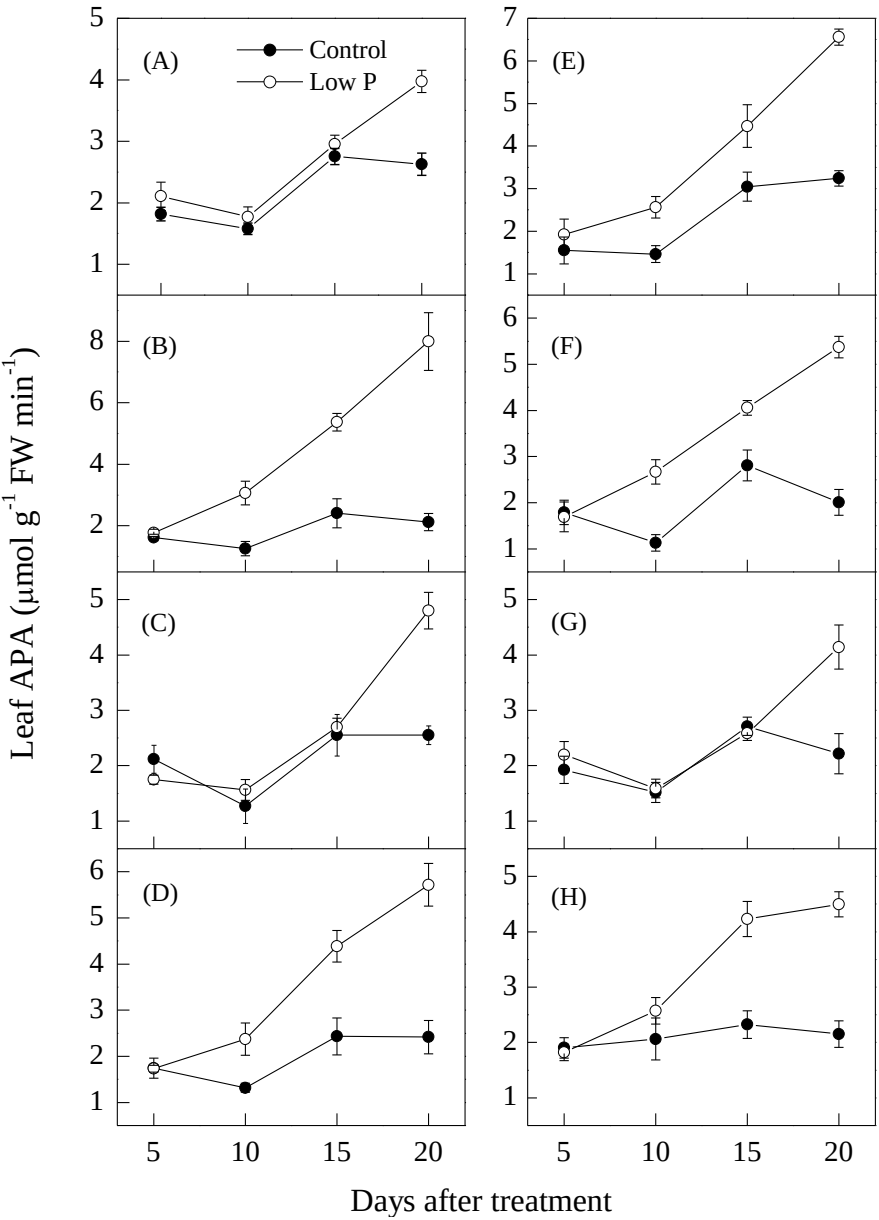

Fig. 1. Chronological changes in leaf APA of 8 rice genotypes, including Zhenongda 454 (A), Zaoniqiudao (B), Zhongbu 51 (C), Pembe (D), Azucena (E), 31079 (F), Xiqixuan (G) and Hongmixian (H).

plants of eight rice genotypes supplied with either sufficient P nutrient solution (Control) or deficient P nutrient solution (Low P) were sampled at 5, 10, 15, 20 days after treatments. Each point represents the mean of three replicates. Error bars show standard deviation (n=3); those

smaller than the symbols are not indicated. Eight rice genotypes at different plant ages with either sufficient P supply or deficient P supply, which confirmed the fact that these 8 rice genotypes were different in P efficiency screened from 90 rice genotype (Li *et al.*, 2005). P efficiency of plants would be affected by low P availability. Our results showed that low P treatment significantly ($p<0.05$) increased PUE, but significantly ($p<0.05$) decreased PAE for all 8 rice genotypes (Table1&2), which was consistent with other reports for the plants concerning the effect of low P treatment on the P efficiency of plants (Yan *et al*., 2001; Dechassa *et al*., 2003).

In the present study, leaf APA of rice was significantly ($p<0.05$) increased by low P treatment (Fig. 1), which was consistent with studies for other plants such as maize (George & Läuchli, 1986; Yun & Kaeppler, 2001), wheat (McLachlan, 1984), common bean (Yan *et al.*, 2001), soybean (Jiang *et al.*, 2003). It has been found that the response of leaf APA to P starvation varied among different rice genotypes (Fig. 1). For example, leaf APA of several rice genotypes such as Zaoniqiudao, Pembe, Azucena, and 31079 was significantly ($p<0.05$) increased at 10 days after low P treatment (Fig. 1B, D-F), however, leaf APA of Zhenongda 454, Zhongbu 51 and Xiqixuan was not significantly ($p<0.05$) increased until 20 days after low P treatment (Fig. 1A, C, G). Considering these 8 rice genotypes different in P efficiency screened from 90 rice genotypes, it seems to be logical to assume that variation in the response of leaf APA to P starvation might be related to variation in P efficiency among eight rice genotypes. This hypothesis was confirmed by the fact that relative leaf APA was significantly ($p<0.01$) correlated to either relative PAE or relative PUE of rice plants (Fig. 4). However, contrary results reported by Yan *et al.*, (2001) showed that there was no significant correlation between leaf APA and either PAE or PUE of common bean. The difference between this and other studies may be attributed to different materials used, different plant growth environments, or time of low P treatment.

Due to the fact that leaf APA was inversely related to P concentration for some plants, it has been suggested by some researchers that leaf APA could be used as an index to diagnose P deficiency of plants (Besford, 1980; McLachlan *et al.*, 1987). Our results showed that relationship between leaf APA and P concentration (including leaf inorganic P concentration and total P concentration) was dependent on the extent of P deficiency for rice plants (Figs. 2 & 3). There was inverse relationship between leaf APA and P concentration when rice plants were in the status of P deficiency moderately (Fig. 2H & I; Fig. 3H & I). However, there was no significant relationship between leaf APA and P concentration when rice plants were in the status of P deficiency slightly (Fig. 2G; Fig. 3G) or severely (Fig. 2J; Fig. 3J). This result suggested that theoretically it would be possible that leaf APA was used as an index to diagnose the P deficiency only when rice plants were in the status of P deficiency moderately. However, due to the fact that leaf APA was significantly affected by rice genotypes and plant ages (Fig. 2), it would be difficult to diagnose P deficiency merely by the value of leaf APA. McLachlan (1984) suggested that P deficiency in field grown wheat plants could be diagnosed by developing phosphatase zymograms technique. It would need investigation in further study about whether P deficiency of rice plants could be diagnosed by phosphatase zymograms technique.

In the present study demonstrated that leaf APA was closely associated to P efficiency in rice. Additionally, the extent of P deficiency dictated whether there was a significant correlation between leaf APA and plant P concentration in rice.

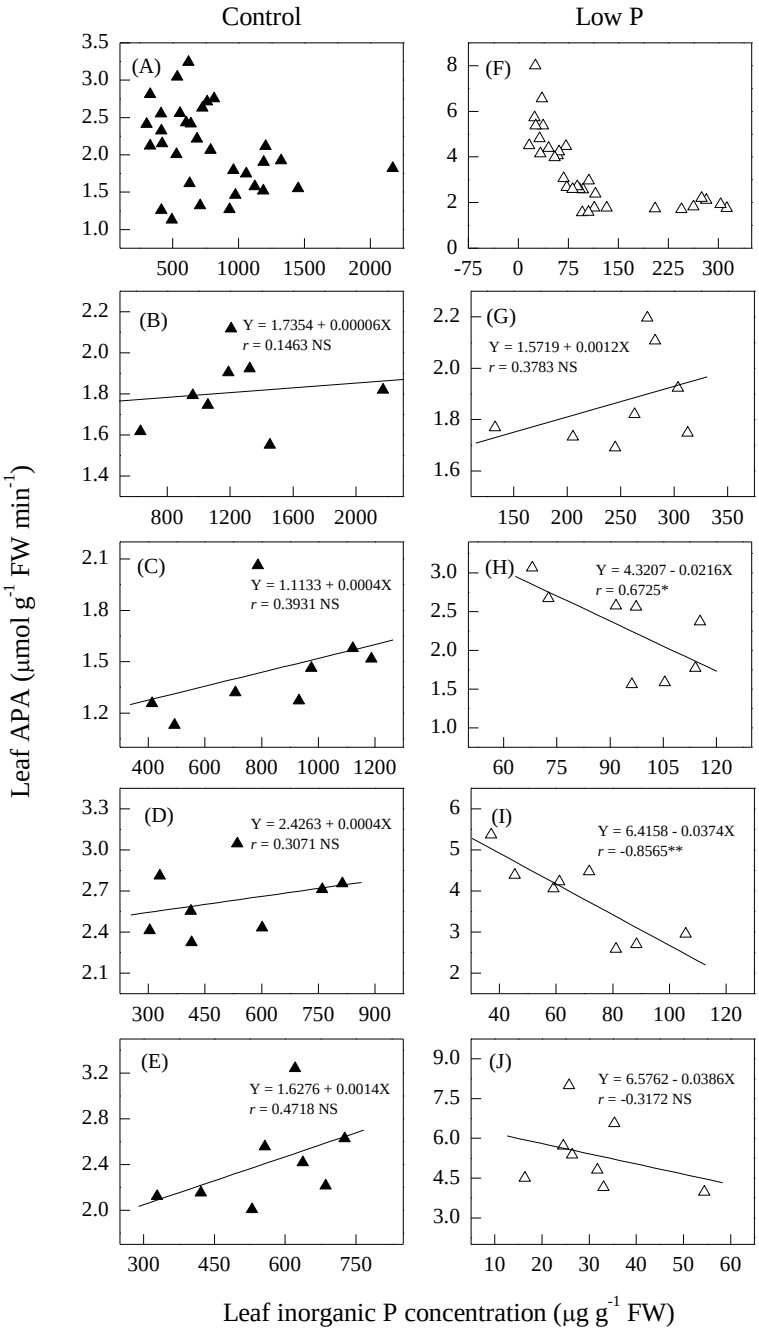

Fig. 2. Correlation between leaf APA and leaf inorganic phosphorus concentration at different plant ages under sufficient P treatment (Control) and deficient P treatment (Low P). Age of plants: (A) and (F): all different plant ages; (B) and (G): 5 DAT; (C) and (H): 10 DAT; (D) and (I): 15 DAT; (E) and (J): 20 DAT. NS: not significant; *: Significant at $P = 0.05$; **: Significant at $p=0.01$.

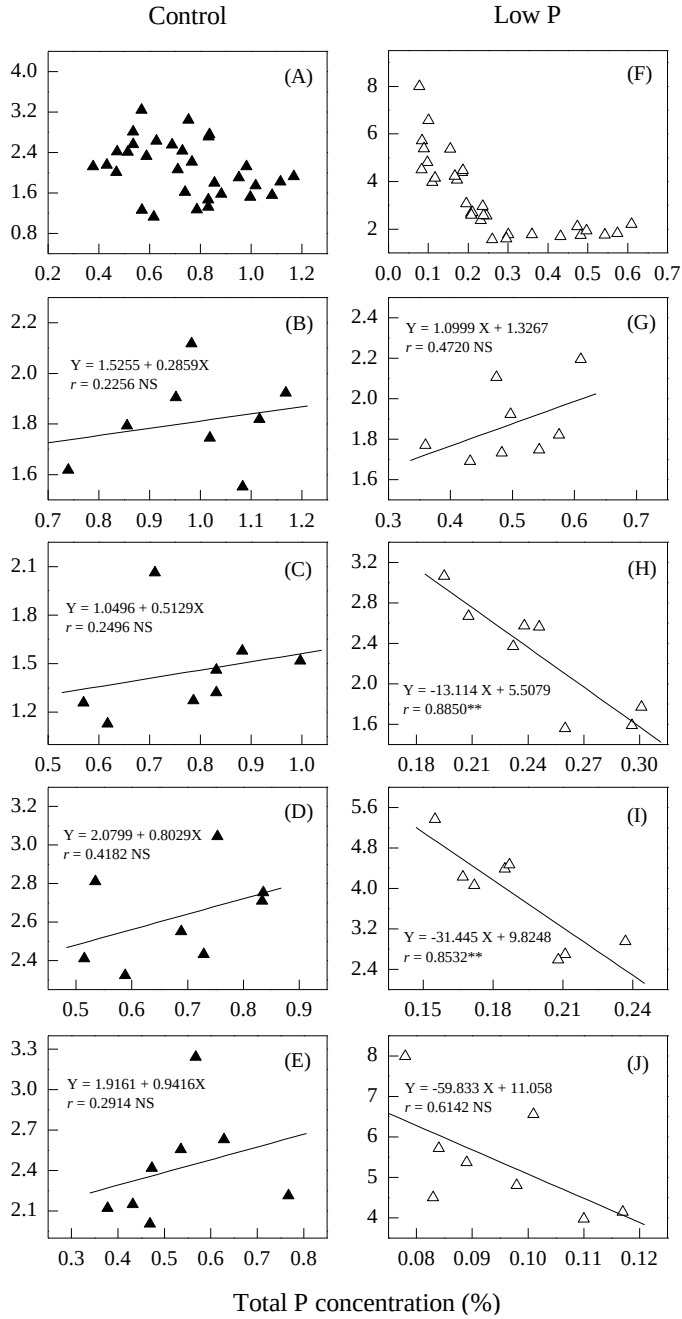

Fig. 3. Correlation between leaf APA and total P concentration at different plant ages under sufficient P treatment (Control) and deficient P treatment (Low P).
Age of plants: (A) and (F): all plant ages; (B) and (G): 5 DAT; (C) and (H): 10 DAT; (D) and (I): 15 DAT; (E) and (J): 20 DAT. NS: not significant; *: Significant at $P = 0.05$; **: Significant at $P = 0.01$.

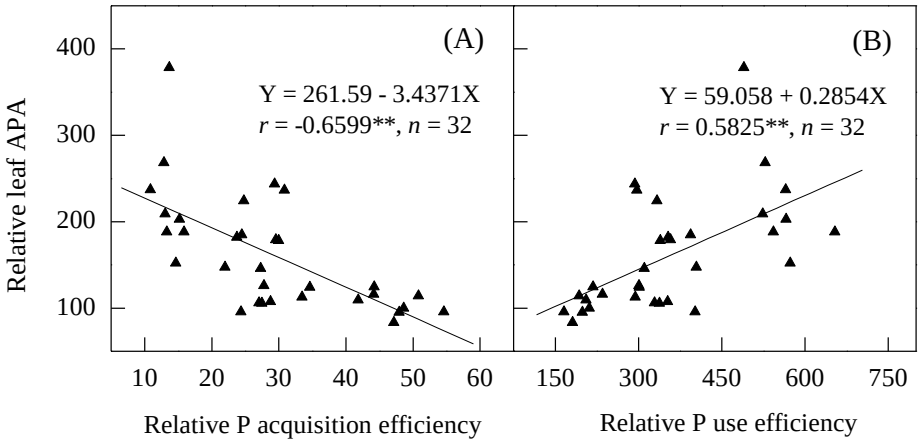

Fig. 4. Correlation between relative leaf APA and either relative P acquisition efficiency (A) or relative P use efficiency (B).
Relative=Value under deficient P treatment/ value under sufficient P treatment. **: Significant at p=0.01.

Acknowledgements

This work was supported by a grant from the National High Technology Research Development Program of China (863 Program) (No. 2001AA241025-1). The authors are grateful to the China National Rice Research Institute for kindly providing the rice seeds.

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(Received for publication 13 November 2006)