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EFFECT OF PHOSPHORUS DEFICIENCY ON PMEase ACTIVITY IN DIFFERENT SPECIES OF HIGHER PLANTS

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Abstract

Effect of phosphorus deficiency on PMEase activity in cotton, wheat and rice was investigated. Seedlings grown with and without phosphorus added to the growth medium using pNPP substrates at different pH levels showed that PMEase activity in cotton seedlings was 68-69% higher in without and 55-56% with phosphorus added to growth medium than rice and wheat seedlings in pNPP substrate, whereas in 4-MUP substrate PMEase activity in cotton seedlings was 68-70% higher than those of rice and 61-62% in wheat seedlings. All plant species displayed higher PMEase activity when grown in growth medium without phosphorous than the plants grown in growth medium with phosphorus.

Introduction

When plant is grown in a P- deficient substrate the intracellular and cell wall phosphatase activities increase as the tissue phosphorus concentration decrease (Bieleski, 1973). Surface phosphatase activity increase in phosphorus deficient plant (Boutin *et al.*, 1981; Baloch *et al.*, 1998; Mclachlan & DeMarco, 1982; Dracup *et al.*, 1984). Besford (1979) interpreted a strong relationship between the intracellular phosphatase activity and the phosphorus status of the tomato leaf and suggested that the enzyme activity could be used for an early detection of P-deficiency in tomato plants. P-deficiency causes a 4-12 fold increase in phosphatase activity in *Escherchia coli* (Torriani, 1960), *Saccharomyces* sp., (Suomalainan *et al.*, 1960), *Euglena gracilis* (Blum, 1965) and *Neurospora crassa* (Nye, 1967). Reid & Bieleski (1970) reported that P-deficiency caused a 10-50 fold increase in phosphatase activity and the appearance of a new isoenzyme in the higher plant *Spirodela oligorhiza*. The phosphatase activity of cell walls from *Agrostis tenus* was two-fold higher where plants were grown with 1.0 μM phosphate than in plants grown with 100 μM phosphate (Woolhouse, 1969). Strother (1980) observed that the increased phosphatase activity in P-deficiency is possibly a response favouring phosphate homeostasis. Acid phosphatase activity of the intact roots of the young plants is influenced by the phosphorus status of the growth medium (Bieleski, 1973). Kummerova (1986) stated that starvation induced an increase in the activity of phosphatases on the root surface of *Zea mays* and that this enhancement was specially pronounced in new roots. Mclachlan (1976) noted that increases in inorganic phosphorus in the resulting medium reduced the phosphatase activity of the plant roots. The amount of organic phosphorus hydrolyzed by clover root phosphatases surpassed the amount of phosphorus taken up by the plant by a factor of 20 (Tarafdar & Classen, 1988). Besford (1979 b) observed that phosphatase activities

also increased with P-deficiency in the leaves of wheat. P-deficiency in higher plants has been shown to increase the activity of acid phosphatase in homogenized leaves (Besford, 1978). Phosphatase activities were influenced by water deficit and leaf age as well as by P-deficiency (Barrett -Lennard *et al.*, 1982). The present paper describes the effect of P-deficiency on PMEase activity in different species of higher plants viz., cotton, wheat and rice for the selection of crop for enhanced ability to mobilize organic phosphate for zoning of specific crop for particular phosphate deficient area according to the phosphatase capacities of the crop plants.

Materials and Methods

The experiment was carried out at the University of Durham, England. Cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L) and rice (*Oryza sativa* L.) seeds were sterilized with 5% sodium hypochlorite for 10 min, washed 6 times in distilled water and planted on vermiculite in small plastic trays incubated at 25°C with 100% R.H in continuous darkness under automatic water sprinklers until germination which took place usually after 5 days. After germination, uniform seedlings were transferred to aerated Hoagland & Arnon (1938) solution culture containing KCl 1.02, Ca $(NO_3)2$ 0.492, NH_4 H_2 PO_4 0.23, Mg SO_4 $7H_2O$ 0.49 g^{-1} H_3 BO_3 2.86 $MnCl_2$ $4H_2O$ 1.81, $CuSO_4$ $5H_2O$ 0.08, $ZnSO_4$ $7H_2O$ 0.22 and H_2 Mo O_4 H_2O 0.092 mg^{-1} Eight seedlings were placed in each jar containing 500 ml nutrient solution adjusted at pH 6.0 using NaOH. The nutrient solution was changed weekly. Seedlings were grown with and without P added to the growth medium and Phosphomonoeasterase (PMEase) activity in roots was assayed in pNPP and 4-MUP substrate (Jansson *et al.*, 1988) using 5 M NaOH, 100 mM NaOH plus 100 mM K_2 HPO_4 and 2.5 mM EDTA as terminator. All colorimetric analyses on phosphatase activity were carried out on Shimadzu Digital Double beam spectrophotometer (model 150-2) using cuvettes with path length of 1.0 cm at 405 nm. A Baird-Atomic Flouripoint spectrofluorimeter was used for fluorescence measurements of phosphatase activity at wave length of 444 nm for emission, and 356 nm for excitation. Weighing measurements were carried out on Electronic ER-182 A Balance (A&D Co. Ltd. Japan). For pH measurements an Ingold combination WTW E50 Electrode and EIL meter (Model 7050) was used.

After recording the phosphatase data, the roots were washed with distilled water and transferred to 5x2 cm glass vials. The vials were kept in the oven at 105°C over night and then cooled in the dessicator for at least 30 min. Dry weights (μ mol mg-1 d.wt) depending on the incubation time were converted into the rate of enzymic activity per hour (μ mol mg d.wt-1 h-1). Buffers were prepared in the Chu 10-D assay medium to a final concentration of 50 mM and stored in the refrigerator at 4°C until required.

Results and Discussion

Cotton: Plants grown with and without phosphorus added to the growth medium produced highly significant ($P \leq 0.001$) differences in PMEase activity (Table 1). When pNPP and 4-MUP substrates were used the peak activity of 0.195 and 0.160 μ mol mg d.wt^{-1} h^{-1} was recorded at pH 6.0. (Table 2,3). PMEase activity displayed consistent

Table 1. Analysis of variance of PMEase activity of 19 days different seedlings.

Source	F-value	Probability
pH	1249 (II)	** 0.001
Condition	908 (1)	** 0.001
Species	8814 (2)	** 0.001
Substrate	892 (1)	** 0.001
pH x condition	9 (II)	** 0.001
pH x Species	301 (22)	** 0.001
pH x Substrate	32 (1)	** 0.001
Condition x Species	188 (2)	** 0.001
Condition x substrate	32 (1)	** 0.001
Species x substrate	17 (2)	** 0.001
pH x species x condition	4 (22)	** 0.001
Condition x species x substrate	16 (2)	** 0.001

increase from pH 4.0 reached to the maximum at pH 6.0 and there after declined from pH 6.5 to pH 10.0. Phosphorus deficient plants exhibited higher PMEase activity at all pH levels ranging from 0.011 (pH 10.0) to 0.195 μ mol mg d.wt^{-1} h^{-1} (pH 6.0) under pNPP substrate and 0.004 (pH 10.0) to 0.153 μ mol mg d. wt^{-1} h^{-1} (pH 5.5) under 4-MUP substrate. On the other hand plants grown with phosphorus added to the growth medium, PMEase activity ranged from 0.005 (pH 10.0) to 0.160 μ mol mg d. wt^{-1} h^{-1} (pH 5.5) under pNPP substrate and from 0.003 (pH 10.0) to 0.132 μ mol mg d. wt^{-1} h^{-1} (pH 5.5) under 4-MUP substrate.

Wheat: Plants grown with and without phosphorus added to the growth medium produced highly significant ($P \leq 0.001$) differences in PMEase activity (Table 1). When pNPP and 4-MUP substrates were used (Table 2,3) the peak activity (0.087 and 0.071 μ mol mg d. wt^{-1} h^{-1}) respectively was recorded at pH 6.0. PMEase activity showed consistent increase from pH 3.5 reached to the maximum at pH 6.0 and thereafter declined from pH 6.5 to pH 10.0. Phosphorus deficient plants yielded more PMEase activity at all pH levels ranging from 0.087 (pH 6.0 to 0.006 μ mol mg d. wt^{-1} h^{-1} (pH 10.0) under pNPP substrate and from 0.060 (pH 5.0) to 0.002 μ mol mg d. wt-1 h-1 (pH 10.0) under 4-MUP substrate. On the contrary, plants grown with phosphorus added to the growth medium, PMEase activity ranged from 0.071 (pH 6.0) to 0.003 μ mol mg d. wt^{-1} h^{-1} (pH 10.0) under pNPP substrate and from 0.050 (pH 5.0) to 0.004 μ mol mg d. wt^{-1} h^{-1} (pH 10.0) under 4-MUP substrate.

Rice: Plants grown with and without phosphorus added to the growth medium showed highly significant ($P \leq 0.001$) differences for PMEase activity between pH, conditions, species, substrates and their interactions (Table 1). When pNPP and 4-MUP substrates were used (Table 2,3) maximum activity (0.063 and 0.049 μ mol mg d. wt^{-1} h^{-1}) was recorded at pH 5.0 and pH 4.5, respectively. PMEase activity revealed consistent increase from pH 3.5 reached to maximum at pH 5.0 and thereafter declined from pH 5.5 to pH 10.0 under pNPP substrate. While in case of 4-MUP substrate maximum

Table 2. Comparison of phosphatase activity (μ mol mg d.wt-1 h-1) in seedlings of different species of higher plants grown without and with phosphorus added to the growth medium, pNPP used as substrates (n=4, (SD)

pH	-P Cotton	+P	-P Wheat	+P	-P Rice	+P
3.0	0.101±0.000	0.073±0.001	0.040±0.001	0.031±0.002	0.031±0.000	0.020±0.000
3.5	0.082±0.001	0.059±0.003	0.042±0.001	0.033±0.002	0.035±0.000	0.023±0.001
4.0	0.117±0.001	0.083±0.001	0.044±0.002	0.036±0.001	0.038±0.001	0.026±0.001
4.5	0.122±0.003	0.095±0.001	0.046±0.002	0.041±0.001	0.040±0.001	0.028±0.001
5.0	0.148±0.001	0.113±0.002	0.049±0.000	0.044±0.000	0.063±0.002	0.050±0.001
5.5	0.156±0.001	0.128±0.002	0.058±0.001	0.050±0.001	0.040±0.001	0.030±0.002
6.0	0.195±0.003	0.160±0.002	0.087±0.002	0.071±0.001	0.027±0.001	0.024±0.002
6.5	0.103±0.002	0.067±0.001	0.050±0.001	0.043±0.001	0.025±0.001	0.022±0.002
7.0	0.058±0.001	0.051±0.003	0.040±0.002	0.035±0.001	0.022±0.001	0.019±0.000
8.0	0.056±0.001	0.028±0.002	0.027±0.002	0.017±0.001	0.020±0.000	0.014±0.001
9.0	0.032±0.003	0.014±0.001	0.011±0.001	0.010±0.001	0.006±0.000	0.004±0.001
10.0	0.011±0.001	0.005±0.000	0.006±0.000	0.003±0.000	0.003±0.000	0.001±0.000

-P = Without Phosphorus, +P = With Phosphorus

Table 3. Comparison of phosphatase activity (μ mol mg d. wt-1 h-1) in seedling of different species of higher plants grown without and with phosphorus added to the growth medium, 4-MUP used as substrates (n=4, (SD)

pH	-P Cotton	+P	-P Wheat	+P	-P Rice	+P
3.0	0.092±0.002	0.074±0.003	0.034±0.002	0.028±0.005	0.027±0.001	0.019±0.002
3.5	0.077±0.003	0.063±0.000	0.037±0.002	0.033±0.000	0.031±0.001	0.023±0.003
4.0	0.098±0.002	0.081±0.001	0.040±0.004	0.036±0.003	0.036±0.002	0.026±0.001
4.5	0.107±0.002	0.090±0.002	0.041±0.005	0.040±0.001	0.049±0.002	0.040±0.001
5.0	0.118±0.001	0.093±0.003	0.060±0.001	0.050±0.002	0.027±0.001	0.017±0.000
5.5	0.153±0.002	0.132±0.002	0.034±0.000	0.032±0.000	0.023±0.002	0.013±0.003
6.0	0.117±0.001	0.091±0.001	0.031±0.000	0.028±0.000	0.017±0.001	0.009±0.001
6.5	0.094±0.001	0.074±0.000	0.028±0.002	0.022±0.003	0.012±0.000	0.006±0.001
7.0	0.050±0.002	0.047±0.001	0.025±0.003	0.016±0.000	0.008±0.000	0.004±0.001
8.0	0.025±0.002	0.014±0.000	0.019±0.002	0.011±0.001	0.005±0.001	0.003±0.001
9.0	0.009±0.000	0.005±0.000	0.009±0.001	0.007±0.000	0.004±0.000	0.002±0.001
10.0	0.004±0.000	0.003±0.001	0.002±0.001	0.004±0.000	0.002±0.000	0.001±0.000

-P = Without Phosphorus, +P = With Phosphorus

PMEase activity was recorded at pH 4.5 and declined consistently from pH 5.0 to pH 10.0. Phosphorus deficient plants showed higher PMEase activity at all pH levels ranging from 0.003 (pH 10.0) to 0.063 μ mol mg d. wt^{-1} h^{-1} (pH 5.0) under pNPP substrate and from 0.049 (pH 4.5) to 0.002 μ mol mg d. wt^{-1} h^{-1} (pH 10.0) under 4-MUP substrate. On the contrary, plants grown with phosphorus added to the growth medium maximum activity of 0.05 (pH 5.0) and lower activity 0.001 μ mol mg d. wt^{-1} h^{-1} was recorded at pH 10.0 in pNPP substrate and from 0.040 (pH 4.5) to 0.001 μ mol mg d. wt^{-1} h^{-1} (pH 10.0) in 4-MUP substrate.

In general, higher PMEase activity was recorded in cotton roots than in wheat and rice roots at all pH levels, which may be due to nutrient uptake capacity of the cotton plant roots. Plant species displayed different PMEase activity rates, this could be due to different phosphate content in the plant tissues of these species. Plant tissues often contain high activity non-specific phosphatases (Bieleski, 1973) and the activity of the enzymes has been shown to be related to the phosphate nutrition of the organism. These results are in agreement with those of Pakarinen & Tolonent (1977) who concluded that the concentration of elements in the plants tissue provide an accurate record of the nutritional status of the environment and hence acid phosphatase activity may be related to the tissue phosphorus concentration. Similar results were also reported by men *et al.*, (1960), Heredia *et al.*, (1963) and Nye, (1967) where phosphorus deficiency in the growth medium have also been found to produce increased acid phosphatase activity.

It would suggest that the differences in PMEase activity in different crops was related to their P absorption. The higher PMEase activity in cotton roots could be due to lower uptake of P as compared to wheat and rice PMEase activity. These results are in agreement with Mclachlan (1980) who reported that plants with lower activity use P more readily that those with higher activity. Acid phosphatase produced by plant roots and microorganisms, which cleave phosphate-ester bonds are enzymes of wide specificity and their presence in root surfaces has been associated with the ability of each plant or cultivar to grow in low P medium levels. It has been hypothesized that wheat and rice roots are capable of taking up P at this stage better than cotton roots, which would be expected to have lower PMEase activity.

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