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Adaptability of rubber (*Hevea brasiliensis*) trees to low soil phosphorus : Some mechanisms involved

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Abstract

The study was conducted in a rubber plantation, planted in 1984 which received five levels of P (0,10, 20, 30, 40 kg P_2O_5 ha^{-1} $year^{-1}$) from 1998 onwards. After five years, trees in the control plots maintained leaf P status at comparable levels with the P fertilized treatments. Growth, dry matter production and total P up take were not influenced by P application. Root surface acid phosphatase activity and rhizosphere acid phosphatase activity were higher in control plots. PEP carboxylase and malate dehydrogenase activities were also higher in the roots of the trees which were not supplied with P. Rhizosphere pH was generally higher than the non-rhizosphere soil pH and was lower in the control plots. The results indicated that when soil available P status is low, rubber trees enhance P uptake by metabolic adaptations.

Keywords: acid phosphatase, *Hevea brasiliensis*, malate dehydrogenase, phosphoenolpyruvate carboxylase, rhizosphere pH

Introduction

In plants, phosphate plays a pivotal structural and regulatory role in photosynthesis, energy conservation and carbon metabolism. However, it is frequently a major or prime limiting factor for plant growth. Among macronutrients, phosphorus (P) is the least mobile and least available to plants in most soil conditions. The poor mobility of the soil inorganic P is due to the large reactivity of phosphate ions with the numerous soil constituents and their consequent strong retention on those constituents. Because of the unique interaction of P with other elements, upto 80 per cent applied P may get fixed in the soil (Raghothama, 1999). Therefore, only a marginal proportion of the soil phosphorus is present as P ions in the soil solution. The mineralogy of tropical soils with high sesquioxide content favour strong retention of P ions, thus limiting P availability to crop plants.

To adapt with low phosphate availability, plants have evolved metabolic and developmental strategies to enhance phosphate acquisition and remobilization. Development of extensive root system, in association with or without mycorrhizal fungi, specialized roots like proteoid roots and changes in root physiology allowing the uptake of P at lower concentrations in the soil solution and /or uptake of P from insoluble inorganic or organic forms are some of the mechanisms by which plants enhance their P acquisition (Gaume *et al.*, 2001).

Although organic P often accounts for more than 80 per cent of total soil P, it is considered to be unavailable to plants unless first mineralized by the action of phosphatases. According to Tarafdar and Jungk (1987), rhizosphere phosphatases play an important role in the release of Pi from organic soil P, for subsequent uptake by plants.

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Increased organic acid exudation in response to P deficiency has been frequently reported (Nagarajah *et al.*, 1970; Jones, 1998; Radersma and Grierson, 2004). Hocking (2001) suggested that an increase in the exudation of citrate increased soil solution P by solubilising calcium phosphates due to decrease in pH in the rhizosphere and desorption reactions in acid soils. Gaume *et al.*, (2001) also observed that increased content of organic acids in the root tissues under P deficiency were related to increased phosphonol pyruvate carboxylase activity.

Results from fertilizer experiments in India and other rubber growing countries have established that balanced application of nitrogen, phosphorus and potassium improves the growth of rubber seedlings (Pushparajah, 1969; Punnoose *et al.*, 1975; Pushparajah *et al.*, 1975). Five to six years after planting, annual defoliation of the trees adds about five to six tonnes of leaf litter in one hectare and the trees cease to respond to application of fertilizers consistently. However, results in majority of the experiments revealed that application of nitrogen and potassium fertilizers improved the yield, whereas, phosphorus had no effect (Pushparajah, 1969; Lim 1977).

Rubber trees have the ability to adapt to low soil P situations. Even under low soil P conditions, trees acquire sufficient P to maintain leaf status at comparable levels with trees growing in P rich soil. Pushpadas *et al.*,(1979) observed a deficiency of available P in soils which did not receive P fertilizers, but no deficiency in leaves. It is possible that trees could be meeting their P requirement from the organic forms resulting from cover crop residues and leaf litter. The present study investigated some of the mechanisms by which rubber trees acquire P in low soil P situations.

Materials and Methods

The experimental treatments were initially imposed in 1998 in a low soil P field planted with clone PB 217 in 1984 at Malankara estate, Thodupuzha in Central Kerala (9° 50′ N, 76°48′ E and 115 m above mean sea level). The experiment area receives a warm humid tropical climate. Trees were planted at a spacing of 4.6 x 4.6 m. The soil of the experiment site is sandy clay loam and classified as Ustic Kandihumults. The total P status of the soil before commencement of experiment was 740 kg ha^{-1} and available P (Bray II) status was 18.80 kg ha^{-1}. Till the commencement of the experiment, all the trees received nitrogen, phosphorus and potassium uniformly. From 1998 onwards, trees were supplied with phosphorus at five levels (0,10, 20,30 and 40 kg P_2O_5 ha^{-1} y^{-1}). Rock phosphate (Rajphos- 18.15 per cent P_2O_5) was applied as the source of P. All the trees received nitrogen and potassium uniformly at the rate of 30 kg ha^{-1} y^{-1} as urea and muriate of potash. The experimental design was RBD with four replications. There were 36 trees in one plot and observations were recorded from eight net trees excluding the border trees. Fertilizers were applied in two equal splits during April- May and September- October. Observations were recorded during 2002 and 2003.

Growth, dry matter accumulation and P uptake

Growth of the trees was measured at 150 cm from ground level during January 2004. The total dry matter accumulation of trees was determined by destructive sampling of a sample tree of representative growth in each treatment. The weight of leaves, branches, main stem and roots were recorded. Sub samples were dried in hot air oven at 80 ° C, and dry weights were recorded. The total dry matter accumulated was estimated. The content of P in each part was determined by dry ashing as described by Piper (1966). Four samples of each part per treatment were analysed. Total P uptake was computed based on the dry weight of each tree part and P content.

Soil and leaf P content

Available soil P content was determined before second fertilizer application following Bray II extraction procedure (Bray and Kurtz, 1945). Leaf samples were also collected before second fertilizer application from all plots. P content was determined by dry ashing followed by estimation with an autoanalyser (AA 3-Brant Luebbe, Germany).

Root surface acid phosphatase activity

Root surface acid phosphatase activity was measured using p-nitrophenyl phosphate as artificial substrate as described by Antibus and Lesica (1990). Fresh roots were collected from the field during May, July, September and November (corresponding to before and after first and second fertilizer application) in ice buckets and stored at –80 °C overnight for enzyme assay. Roots were washed gently under a stream of running water to remove soil and adherent organic materials. The terminal 3 cm portion of undamaged roots were removed by scissors. Roots were kept moist with 0.50 mM $CaCl_2$. Four replicates were assayed for each treatment. Root samples were incubated at room temperature for two hours in vials containing 2.0 ml of 50.0 mM citrate buffer

(pH 5.0), artificial substrate p-nitrophenyl phosphate (SISCO Research Laboratories, Mumbai) and substrate solution (pH 5.0) containing EDTA (1mM), citric acid and p-nitrophenyl phosphate. The final concentration of p-nitrophenyl phosphate was 5.0 mM. Reaction blanks received substrate immediately prior to the termination of the incubation. At the end of the incubation period, 1.0 ml of the reaction mix was added to 4 ml of 0.50 M NaOH and the amount of p-nitrophenol released was measured at 410 *nm* against standard solutions. Root samples were washed in distilled water and dried overnight at 105 ^{0}C. Phosphatase activity was expressed on dry weight basis.

Rhizosphere acid phosphatase activity

Rhizosphere soil samples (soil adhering to the fresh roots) were collected during May, July, September and November (corresponding to before and after first and second fertilizer application) and acid phosphatase activity was measured as described by Tabatabai (1994) using artificial subtrate p-nitrophenyl phosphate at pH 6.5.

Phosphoenol pyruvate carboxylase (PEPC) and malate dehydrogenase activities (MDH)

Determination of *in vitro* activities of PEPC and MDH were carried out as described by Sadasivam and Manickam (1996) before and after second fertilizer application (September and November). Four replicates were assayed for each treatment. Frozen samples of fresh root tissue was homogenized in a pre-chilled mortar and pestle in cold grinding medium containing 50.0 mM Tris-HCl (pH 8.0), 50.0 mM $MgCl_2$, 5.0 mM 2-mercaptoethanol and 1.0 mM EDTA,1 ml/250 mg tissue (SRL, Mumbai) and centrifuged for 20 min at 3000 rpm. Activity of PEPC in the supernatant solution was determined spectrophotometrically following the disappearance of NADH at 340 nm for 3 min and expressed as nmol NADH oxidised ml^{-1} enzyme extract min^{-1} g^{-1} FW. MDH was also assayed using the same extract spectrophotomerically and expressed in the same unit.

Rhizosphere and non-rhizosphere soil pH

Soil samples were collected during May, July, September and November 2003 (corresponding to before and after first and second fertilizer application). Soil adhering to the fresh roots was collected as the rhizosphere soil and soil without root concentration was collected as the non-rhizosphere soil. Determination of pH was done at a soil water ratio of 1:2.5.

Statistical analyses

The data were subjected to analysis of variance for comparison.

Results and Discussion

Soil and leaf P status

Soil available P content increased with P application (Table 1). In earlier studies, Pushparajah (1969) and Lim (1977) also reported that application of P fertilizers increased soil available P status. Compared to nitrogen and potassium, loss of phosphorus from the soil is low and a part of the applied phosphorus gets accumulated in the soil leading to higher soil P levels. Soil P status of treatments which received P at 10 and 20 kg ha^{-1} were comparable with control and were significantly lower than that of other treatments. Application of phosphorus did not influence the leaf P status (Table 1) as observed in many earlier experiments also (Kalam *et al.*, 1979; Pushpadas *et al.*, 1979). The trees which did not receive P fertilizer and which received the highest level (40 kg ha^{-1}) had the same leaf P status (0.23%) in the present experiment. Pushpadas *et al.* (1979) suggested that trees could be meeting their P requirement from organic forms resulting from the

Table 1. Influence of P levels on growth, soil and leaf P status, dry matter accumulation and P uptake in a 17 year old *Hevea brasiliensis* plantation

P levels (kg ha^{-1})	Soil P status (kg ha^{-1})	Leaf P content (g kg^{-1})	Girth(cm)	Total P uptake (g)	Dry matter accumulation (kg $tree^{-1}$)					
					Roots	Main trunk	Branches	Twigs	Leaves	Total
0	14.1^{a}	2.3^{ns}	72.20^{ns}	807.6	218.0	342.3	417.6	29.0	43.1	1049.9
10	24.2^{a}	2.1^{ns}	72.85^{ns}	850.2	220.0	360.5	405.2	42.6	50.5	1078.8
20	28.5^{a}	2.0^{ns}	72.25^{ns}	756.0	232.1	330.9	418.4	23.0	40.2	1044.6
30	52.8^{b}	2.0^{ns}	73.70^{ns}	864.0	212.5	370.6	422.5	48.0	48.4	1102.0
40	69.6^{b}	2.3^{ns}	72.28^{ns}	847.9	216.2	340.4	436.9	20.2	41.2	1054.9
SE	5.6	0.01	2.07	-	-	-	-	-	-	-

SE- Standard error of the difference between the means ns – not significant
Means with the same letters are not statistically different at $P = 0.05$

cover crop residues and leaf litter. In our study also, the leaf status indicated that trees which were not supplied with P acquired sufficient P. Statistical analysis also indicated lack of correlation between leaf P status and soil P content (r = 0.115).

Growth, dry matter accumulation and P content in tree parts

Growth of the trees was not influenced by P application. The highest dry matter accumulation was observed in the branches followed by main trunk and roots (Table1). Of the total dry matter, 39.40 per cent was accumulated in the branches, 32.70 per cent in the main trunk and 20.60 per cent in the roots. The dry matter accumulation in different parts of the tree showed a variation of less than 6 per cent. This might be due to the lack of influence of applied P on the growth or dry matter partitioning of trees. Rate of growth decreases after the initial three to four years and the trees might be able to maintain growth with less nutrients after this period. Moreover, the roots explore a large volume of soil to access nutrients. The P uptake was also not influenced by the different treatments.

Root surface acid phosphatase activity

Trees in the control plots had a higher root surface acid phosphatase activity than trees which were supplied with P (Table 2). During the first sampling (May), root surface acid phosphatase activity in the control (2.30 µg nitrophenol mg^{-1} dry root), was on par with trees which received P at 10 kg ha^{-1}, but significantly higher compared to other treatments. During July, a decrease was noticed in the root surface acid phosphatase activity and the activity was significantly higher in the control plots. During September also, the highest activity was observed in the control (1.80 µg nitrophenol mg^{-1} dry root). All the trees which were supplied with P at various levels had comparable levels of activity. During November, the same trend was observed, but there was no significant difference between treatments.

Phosphatases are capable of hydrolyzing organic phosphate esters and are very important for the P nutrition of plants (Antibus and Lesica, 1990). Large increase in root phosphatase activity under P deficient conditions was reported earlier (Ascencio, 1997). Higher acid phosphatase activity in the control plots observed (Table 2) might be an adaptive mechanism of trees to utilize the organically bound P in soil. Pushpadas *et al.* (1979) also suggested that trees could be meeting their P requirement from the organic P in soil and the lack of response of rubber trees to applied phosphorus was attributed to this ability. Significant differences between P applied treatments were observed only during the first sampling. Significant negative correlation was also observed between soil P status and acid phosphatase activity (r = -0.366).

Rubber is a deciduous tree and adds about 5-6 tonnes of organic matter annually through litter fall (Krishnakumar and Potty, 1992). The quantity of P recycled through litter was reported to be 5 to 6 kg in one hectare of a 14 year old RRIM plantation (Varghese *et al.*, 2001). The trees shed their leaves during December-January and refoliate during January-February. Pre-monsoon showers are received during April and plants resume active growth during this period after the summer and refoliation. During this period, release of P from decomposing litter will be less since litter decomposition might have just commenced only and the trees will not be able to meet the increased demand for P caused by increased growth and hence might secrete more acid phosphatase to increase P availability. During the subsequent period P from the litter will become gradually available. In deciduous forests, most of the P taken up by trees was found to be

Table 2. Influence of P levels on root and rhizosphere acid phosphatase activity in *Hevea brasiliensis*

P levels (kg ha^{-1})	Root surface acid phosphatase activity (µg nitrophenol mg^{-1}dry root)				Rhizosphere acid phosphatase activity (µg nitrophenol g^{-1} soil h^{-1})			
	May	July	Sep.	Nov.	May	July	Sep.	Nov.
0	2.30^{a}	1.78^{a}	1.80^{a}	2.05^{ns}	58.00^{ns}	73.00^{ns}	71.67^{a}	84.25^{ns}
10	2.05^{ab}	1.48^{b}	1.57^{ab}	1.70^{ns}	59.25^{ns}	71.50^{ns}	56.25^{b}	63.25^{ns}
20	1.70^{bc}	1.48^{b}	1.45^{b}	1.46^{ns}	56.00^{ns}	69.50^{ns}	59.00^{b}	75.50^{ns}
30	1.75^{bc}	1.45^{b}	1.35^{b}	1.48^{ns}	54.50^{ns}	64.00^{ns}	58.50^{b}	55.75^{ns}
40	1.58^{c}	1.40^{b}	1.45^{b}	1.49^{ns}	57.00^{ns}	65.50^{ns}	57.80^{b}	66.25^{ns}
SE	0.12	0.04	0.09	0.27	2.52	2.93	2.74	8.18

SE- Standard error of the difference between the means ns – not significant
Means with the same letters are not statistically different at P = 0.05

mineralized from organic matter in the forest floor (Yanai, 1992).

Rhizosphere acid phosphatase activity

In general rhizosphere acid phosphatase activity was higher in the control, but the difference was statistically significant during the third sampling only (Table 2). Enzyme activity was generally lower during the first sampling compared to the subsequent samplings.

Plants can utilize only inorganic P and since a large proportion of the total soil P is organically bound, the mineralization of organic fraction is an important factor in P nutrition. During the first sampling, the highest soil phosphatase activity was noticed in the treatment which received P at the lowest level (10 kg ha^{-1}). The stimulation of soil phosphatase activity by low levels of fertilizer phosphorus was earlier reported (Speir and Ross 1978; Attiwill and Adams, 1993). In the treatments which received P at higher levels, the higher soil P status might have caused a decline in phosphatase activity as reported earlier by Spiers and McGill (1979). During the subsequent samplings, higher enzyme activity was observed in the control plots. The higher root surface acid phosphatase activity in this treatment also might have contributed to the higher rhizosphere phosphatase activity.

Phosphatase activity showed seasonal variation. The higher activity observed during the second sampling might be due to the higher microbial activity associated with the decomposing rubber litter during this period and favourable moisture conditions. Enzyme activity decreased during the third sampling and showed an increase in most cases during the fourth sampling. The fluctuations in soil moisture content and microbial population might have resulted in this variation. Breakwell and Turco (1989) also observed higher phosphatase activity under field conditions at the beginning of the decomposition period. The activity then decreased and later showed an increase near the end of the decomposition period which was attributed to the rupturing of cells and release of cell constituents.

Secretion of acid phosphatase by plant roots into the rhizosphere form a source of phosphatase in soil. In general, acid phosphatase on roots and soil phosphatase activity were higher in the control and lower in P applied treatments. However, no definite trend was observed in the seasonal variations in enzyme activity. The acid phosphatase activity on roots was the highest during the first sampling, and then showed a declining trend during the next two samplings, whereas, the soil phosphatase activity was the lowest during this period and then showed an increasing trend.

Phosphoenol pyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) activities in roots

Higher activities of PEPC and MDH were found in the root tissues of control trees (Table 3). During the first sampling, no significant difference was noticed between treatments in the PEPC activity. However, the activity was the highest in the control (9.35 nmol NADH oxidised ml^{-1} enzyme extract min^{-1} g^{-1} FW^{-}). During the next sampling, there was a remarkable increase in the enzyme activity in the roots of trees in the control plots. Contrary to this, the activity of the enzyme decreased in the roots of trees which received P fertilizers.

In the case of MDH also, the highest activity was observed in the control during both the samplings. During the first sampling, MDH activity in the control was comparable with trees which received P at 20 and 30 kg ha^{-1}. During the next sampling, MDH activity in the control plots (7.26 nmol NADH oxidised ml^{-1} enzyme extract min^{-1} g^{-1} FW^{-}) was significantly higher than that of P supplied treatments.

Increased exudation of organic acids from roots of many plants under conditions of P stress was reported earlier (Bar-Yosef, 1991 and Jones, 1998). Because of the low pk values of many organic acids compared with the neutral cytosol pH, organic acids are dissociated in the cytosol of root cells and are released as organic anions along with H^{+} ions leading to some acidification of the rhizosphere (Hinsinger, 2001).

In our study also, higher PEPC and MDH activities were observed in the control plots (Table 3). Significant negative correlation was also observed between soil P status and root PEPC and MDH activities ($r = -0.519$ and -0.513, respectively). This might be the biochemical response of plants to limited soil P availability. Johnson *et al.* (1996) suggested transcriptional regulation of PEPC in response to P limitation. According to Bar-Yosef (1991) PEPC in roots was involved in the extra citric acid production under P stress. Neumann and Romheld (1999) observed increased PEPC activities in the roots of P deficient white lupin, chickpea and tomato. The higher PEPC and MDH activities in the control treatment indicate a possibility for exudation of organic anions and H^{+} ions in to the rhizosphere.

Table 3. Phosphoenol pyruvate carboxylase and malate dehydrogenase activities in the roots of *Hevea brasiliensis* (nmol NADH oxidised ml^{-1} enzyme extract min^{-1} g^{-1} FW)

P levels (kg ha^{-1})	PEPC		MDH	
	Sep.	Nov.	Sep.	Nov.
0	9.35^{ns}	19.6^{a}	5.95^{a}	7.26^{a}
10	8.78^{ns}	5.21^{b}	3.90^{b}	4.24^{ab}
20	8.93^{ns}	2.18^{b}	5.80^{ab}	4.03^{ab}
30	8.91^{ns}	2.76^{b}	5.42^{ab}	1.45^{c}
40	6.23^{ns}	3.20^{b}	3.30^{b}	2.18^{bc}
SE	1.31	1.44	0.64	0.78

SE- Standard error of the difference between the means
ns – not significant
Means with the same letters are not statistically different at *P = 0.05*

Non-rhizosphere and rhizosphere pH

Though not statistically significant, non-rhizosphere soil pH was generally higher in the P applied treatments (Table 4). In general, rhizosphere pH was also lower in the control plots. Significant differences between treatments were observed during May and September when samples were collected before fertilizer application. After fertilizer application (July and November), the differences were not statistically significant.

Table 4. Influence of P levels on non-rhizosphere and rhizosphere pH

P levels (kg ha^{-1})	Non-rhizosphere pH				Rhizosphere pH			
	May	July	Sep.	Nov.	May	July	Sep.	Nov.
0	4.64^{ns}	4.41^{ns}	4.80^{ns}	4.84^{ns}	4.53^{a}	4.73^{ns}	4.80^{a}	4.70^{ns}
10	4.79^{ns}	4.44^{ns}	4.93^{ns}	4.88^{ns}	4.72^{b}	4.72^{ns}	4.98^{b}	4.80^{ns}
20	4.71^{ns}	4.46^{ns}	4.82^{ns}	4.79^{ns}	4.58^{ab}	4.69^{ns}	4.94^{b}	4.80^{ns}
30	4.71^{ns}	4.46^{ns}	4.83^{ns}	4.80^{ns}	4.66^{ab}	4.73^{ns}	4.97^{b}	4.80^{ns}
40	4.81^{ns}	4.57^{ns}	4.86^{ns}	4.78^{ns}	4.68^{b}	4.85^{ns}	5.01^{b}	4.96^{ns}
SE	0.04	0.05	0.03	0.04	0.04	0.05	0.03	0.06

SE- Standard error of the difference between the means
ns – not significant
Means with the same letters are not statistically different at *P = 0.05*

Rhizosphere pH has a strong influence on the availability of P to plants. Enhanced H^+ release by plants under conditions of P deficiency has been reported (Imas *et al.*, 1997; Bertrand *et al.*, 1999). Studies with phosphate rocks indicated that H^+ release by plant roots considerably increased the dissolution of phosphate rocks and availability of P to plants.

Though rhizosphere pH was lower in the control plots, it was higher than non-rhizosphere soil pH in many cases. According to Hinsinger and Gilkes (1996) root induced acidification of the rhizosphere increased the bio-availability of P whenever calcium phosphates were present *i.e.*, in alkaline to mildly acidic soil, but its effect was not clear in acidic soils. The dominant forms of P minerals in acidic soil are Fe and Al phosphates and their solubility decreases with decreasing pH. Moreover the positive surface charge of Fe and Al oxides and hence their P adsorption capacity increases with decreasing pH. Hence increasing the rhizosphere pH might be more effective in increasing the availability of P in acidic soils (Hinsinger, 1998).

In the present study, the soil of the experiment area was acidic, but still a reduction in rhizosphere pH in the control plot was observed in the treatments. Reduction in rhizosphere pH might increase the solubilization of calcium phosphates in the soil. Higher levels of PEPC and MDH in the roots of trees which did not receive any P fertilizer indicate a possible exudation of H^+ ions along with organic anions from the roots and this might have contributed to this reduction in rhizosphere pH. However, the slightly higher pH in the rhizosphere when compared with non-rhizosphere soil pH indicate a general rhizosphere alkalinization by trees to increase the availability of phosphorus by anion exchange from iron and aluminium phosphates.

Significant differences between treatments were observed during first and third sampling (before fertilizer application). Samples collected after fertilizer application (second and fourth sampling) did not indicate any significant difference among treatments. Rock phosphate was applied as the source of P and rhizosphere acidification might have occurred in the fertilizer applied plots for the solubilization of calcium phosphate.

The present study indicates that under low soil P conditions, uptake of P by rubber trees is maintained by metabolic adaptations. Higher activities of acid phosphatase on roots and rhizosphere and higher PEPC and MDH activities in the root tissue and lower rhizosphere pH might have a role in improving uptake of P by rubber trees when soil is deficient in P.

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