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Phosphatase and Phytase Secreting Efficiency of Some Multipurpose Trees of Arid Region

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Abstract: Eight tree species were compared for the activity of root associated and root released acid phosphatase and phytase to select the best tree species to exploit native unavailable P for better nutrition. The activity of root associated acid phosphatase and phytase of all genotypes was about 7 to 10 times higher than that of root released acid phosphatase and phytase and the tree species performed differently with regard to the activity of the enzymes. P deficiency increased significantly ($P<0.01$) both acid phosphatase and phytase release. There was a significant correlation ($P<0.01$) between the activities of root associated and root released acid phosphatase and phytase. A significantly higher ($P<0.05$) phosphatase and phytase activity was observed with the introduction of plants in soil. The results suggested that low phosphorus supply enhanced secretion efficiency of phosphatase and phytase and *Acacia nilotica* was found to be best tree species to exploit native unavailable phosphorus for their nutrition.

Key words: Phosphorus deficiency, acid phosphatase, alkaline phosphatase, phytase.

The most common uses of trees are wood (fuel and timber) and foliage as fodder, green manuring, fruits as food, medicine etc. The trees have very deep and developed extensive root system, therefore, if planted on the soil, will act as very good sand binder. Normally the trees are planted under virgin and degraded lands without addition of any P fertilizer. The available P content of the arid and semi-arid areas is much below the required amount. Therefore, there is a need to explore unavailable native phosphorus to meet the requirements of trees for nutrition. Phytic acid (salt of myo-inositol hexaphosphate) and their derivatives may comprise 50% of total organic P present in soil. The abundance of myo-inositol hexaphosphate in the soil seems to be due to their low solubility, firm association with solid phase, and high stability (Anderson, 1980). Phosphatase and phytase are the enzyme, which may help to hydrolyze the phytin and release of P as inorganic phosphate ($H_2PO_4^-$, $HPO_4^=$, $PO_4^=$) so that they may be available to plants for their nutrition. These enzymes are normally present in soils, where they originate from both plant roots and microorganisms (Li *et al.*, 1997; Tarafdar *et al.*, 2001). The enzymes released by the roots could be important for hydrolysis of soil bound phytate in the rhizosphere and the root associated enzymes might be important for the mineralization of soluble phytate found in very low concentrations in a soil solution. The root associated enzymes may also reach soil bound phytate by interception of growing roots and may increase the rate of hydrolysis of organic P compounds in the root surface (Boutin *et al.*, 1981). So far, information about the activity of phosphatase and phytase associated and released by the roots and variation in the activity among tree species is scarce. This information is needed to improve our knowledge of the rhizosphere process affecting P mobilization and acquisition. Here, we have compared the activities of root associated and root released extracellular phosphatase and phytase so that we may select the best tree species to exploit native unavailable P for better nutrition.

Materials and Methods

Healthy and viable seeds of eight tree species (*Acacia ampliceps*, *Acacia nilotica*, *Acacia senegal*, *Acacia tortilis*, *Colophospermum mopane*, *Hardwickia binata*, *Inga dulce* and *Prosopis cineraria*) were surface sterilized and placed in a sterilized beaker of 500 mL capacity containing sterilized vermiculite.

To study the secretion of acid phosphatase and phytase eight tree species grown in solution culture at two P regimes (applied as KH_2PO_4): P sufficient (250 mg P L^{-1}) and P deficient (5 g P L^{-1}). The treatment without any P was excluded because growth of plants was severely affected. Five 45-day-old seedlings raised in vermiculite were placed in a sterilized flask containing 100 mL sterilized nutrient solution in triplicate. After 24 h uncontaminated samples were used for analysis. Enzymes released in nutrient solution were termed as extracellular enzymes or root released enzymes. Plant roots rinsed 10-15 times with double distilled water were ground with quartz sand in deionized water and used for enzyme assay.

A pot experiment having loamy sand soil (hypothermic typic haplocambids) with eight trees was undertaken screen to the plants for phosphatase and phytase released by the roots. The characteristics of experimental soil are: sand-85%, silt-9.5%, clay-4.5%, pH-8.2, EC-0.18, total P-1261 mg kg^{-1}, organic P-367 mg kg^{-1}, available P-10 mg kg^{-1}. About 69% of organic P was present as phytin. Surface soil (0-20 cm) was collected from fallow land and after removing root pieces were air-dried for 48 h at 30±1°C, sieved (<2 mm) and thoroughly mixed. The experiment was carried out with two treatments; soil with plants and soil without plants. Twelve pots were used for each tree, out of which three pots were harvested after 45, 55, 65 and 75 days of sowing. Ten seeds of each tree were sown in each pot and three seedlings were maintained after germination. The water was added at 50% water holding capacity (WHC) of the soil and watered every second day. The pots were completely randomized and re-positioned weekly to minimize any effect of uneven environmental factors.

Acid and alkaline phosphatase were assayed by adopting the standard method of Tabatabai and Bremnar (1969). Phytase activity was assayed by measuring the inorganic phosphate (Pi) released by hydrolysis of sodium phytate (Ames, 1966). Microsoft Excel 2000 was used for statistical analysis of data (standard error and correlation analysis).

Results and Discussion

Root released acid phosphatase and phytase

Release of acid phosphatase and phytase (Table 1) by different trees showed a wide variation and was consistently higher under P deficient condition. The release of acid phosphatase and phytase was high in *Acacia nilotica* followed by *Acacia tortilis* and was lowest in *Hardwickia binata* under both the conditions. The increase of the enzymes activities ranged from 11.6% to 51.6% in the P deficient compared with that of P sufficient treatment.

Low phosphorus supply enhances the secretion of acid phosphatase and phytase from roots may be a widespread adaptive function for trees to grow in P-deficient soil. Phosphorus deficiency induced root secretion of acid phosphatase is probably regulated at the transcriptional level (Neumann *et al.*, 2000), and may involve sensing P concentrations in the growth medium (Wasaki *et al.*, 1999) and differential induction of isoenzymes (Gilbert *et al.*, 1999). P deficiency can significantly alter the composition of root exudates in a way that is, at least in some plant species, related to an increased ability for the mobilization of sparingly soluble P sources (Jones, 1998). Increased exudation of carboxylates (citrate, malate, oxalate) is a P deficiency response (Neumann *et al.*, 2000).

Enhanced secretion of acid phosphatase (Tarafdar and Jungk, 1987) and phytase (Li *et al.*, 1997) by plant roots under P-deficient conditions may contribute to inorganic P acquisition by hydrolysis of organic P esters in the rhizosphere. Tarafdar and Jungk (1987) showed that the acid phosphatase excreted by the roots of nine crop species increased under P deficiency. Differences in the release of acid phosphatase and phytase by tree species (Table 1), which could be related to rhizosphere chemical mobilization, root excretion or root hair density (Tarafdar and Kumar, 2003).

Root associated versus root released enzymes

The result (Fig. 1) showed that the trees responded differently in activity of root associated and root released acid phosphatase and phytase to P level. The activity of root-associated phosphatase (Fig. 1A) was 7.4 times higher than root-released phosphatase under P deficient condition and 9.3 times higher under P sufficient condition. A similar response was found between root associated and root released phytase (Fig. 1B). There was a significant ($P<0.01$) correlation between the activity of root

Table 1. Acid phosphatase and phytase released by different tree species grown under P-deficient and P-sufficient conditions (±standard errors of mean)

Tree species	Acid phosphatase (EU x 10^{-6})		Phytase (EU x 10^{-6})	
	P-deficiency	P-sufficiency	P-deficiency	P-sufficiency
Acacia ampliceps	13.5±0.5	9.0±0.3	11.2±0.4	8.5±0.3
Acacia nilotica	33.1±1.4	29.3±0.9	29.5±1.1	26.5±0.9
Acacia senegal	13.4±0.7	9.1±0.3	11.8±0.5	8.1±0.3
Acacia tortilis	27.3±1.1	22.8±0.9	25.3±0.8	21.0±0.7
Colophospermum mopane	16.2±0.7	10.6±0.4	14.8±0.7	10.0±0.4
Hardwickia binata	10.1±0.5	7.8±0.3	10.3±0.4	6.8±0.2
Inga dulce	10.9±0.4	9.5±0.3	10.6±0.4	9.0±0.3
Prosopis cineraria	15.0±0.7	12.9±0.5	13.1±0.5	11.1±0.4

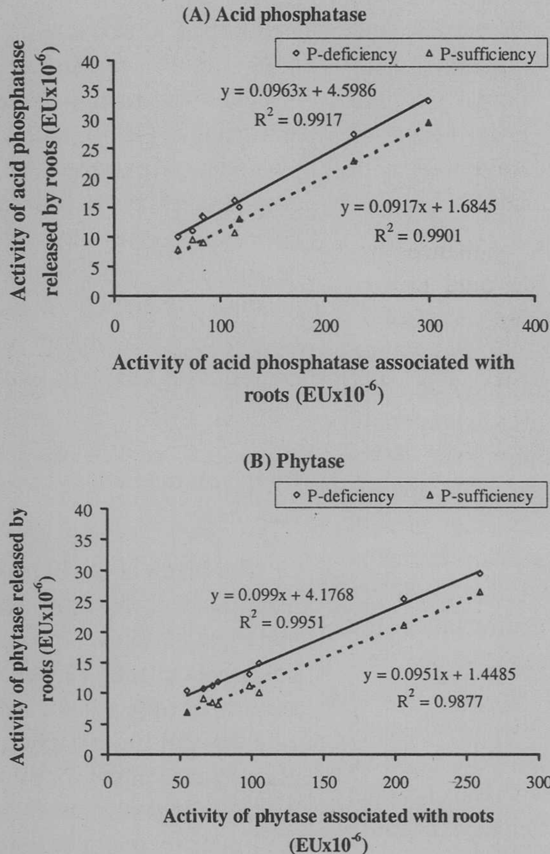

Fig. 1. Relationship between the activity of root associated and root released enzymes of different tree species.

associated and root released phytase and acid phosphatase both under deficient and sufficient P condition.

The higher activity of root associated compared to root released acid phosphatase and phytase (Fig. 1) is in agreements with the results (Asmar and Gissel-Nielsen, 1996), where the activity of extracellular phosphomono and phosphodiesterase was mainly associated with roots and correlated significantly with the activity of root released phosphatase. In accordance, a positive trend was found between the roots associated and root released phosphatase and phytase. The present results indicate that acid phosphatase and phytase activities were mainly associated with the roots and the root-released acid phosphatase and phytase activities had only 7 to 9% contribution to the root total associated acid phosphatase and phytase activities. However, root released enzymes might be more important for hydrolysis of soil bound organic P, because the root associated

Table 2. Acid phosphatase, alkaline phosphatase and phytase released in different tree rhizosphere at different growth periods

Tree species	Acid phosphatase (EU x 10^{-5})				Alkaline phosphatase (EU x 10^{-5})				Phytase (EU x 10^{-5})			
	Days after sowing				Days after sowing				Days after sowing			
	45	55	65	75	45	55	65	75	45	55	65	75
Control (without plant)	1.7	1.5	1.5	1.4	2.5	2.5	2.3	2.3	4.9	4.6	4.6	4.4
Acacia spp.*	7.8	7.2	7.0	6.2	9.9	9.5	8.9	8.2	10.6	9.1	6.9	6.2
C. mopane	6.7	6.3	5.9	5.5	8.4	8.4	7.8	7.2	9.5	8.9	6.7	6.4
H. binata	4.3	3.9	3.8	3.3	6.9	6.5	6.1	5.9	10.2	9.3	6.0	5.8
Inga dulce	5.2	5.0	4.7	4.6	7.2	6.9	6.5	6.2	8.1	6.6	5.8	5.5
P. cinerria	6.1	5.8	5.5	5.2	7.8	7.2	6.9	6.5	8.9	7.6	6.3	5.8
LSD (P=0.05)	0.15	0.13	0.14	0.14	0.08	0.11	0.10	0.09	0.28	0.19	0.12	0.11

* average of four *Acacia* species.

enzymes may not reach P beyond rhizosphere (Helal and Sauerbeck, 1984). A depletion of organic P, which correlated with increase in activity of root-released extracellular phosphatase, has been measured up to 1-2 mm in the rhizosphere (Tarafdar and Jungk, 1987; Asmar *et al.*, 1995). The phytase released by the roots could be important for hydrolysis of soil bound phytate in the rhizosphere and the root associated phytase might be important for the mineralization of soluble phytate found in very low concentrations in a soil solution. The root associated phytase may also reach soil bound phytate by interception of growing roots and may increase the rate of hydrolysis of organic P compounds on the root surface (Boutin *et al.*, 1981).

Enzyme activities in the rhizosphere

The acid phosphatase activity of different plant species (Table 2) at different ages showed 2.7 to 4.8 times increase in activity, after introduction of plants as compared to control (soil without plant). Higher improvement in phytase activity at 45 days in all the tree rhizosphere was observed, but thereafter activity marginally declined. *Acacia* sp. released maximum acid phosphatase followed by *C. mopane,* whereas *H. binata* secreted the minimum amount of acid phosphatase.

In general, introduction of different trees increased alkaline phosphatase activity (Table 2). The alkaline phosphatase activity significantly increase with increase in crop age up to 45 days increased in all tree species. This increase was maximum in *Acacia* sp. (292%) followed by *C. mopane* (234%) and was least in *H. binata* (175%).

A significantly higher (P<0.05) phytase activity was observed when trees were grown in soil as compare to control soil during the entire growth period tested (Table 2). Maximum phytase was released by *Acacia* sp. followed by *H. binata* and *C. mopane*. The per cent increase varied from 96-119% over control (without plant) after 45 days. In general, there was marginal change in phytase activity in blank soil up to 75 days, but phytase release progressively declined, except in few cases, was observed after 45 days in all tree species tested.

The increase in phosphatase and phytase activity in tree rhizosphere may be attributed to the development of the root system with age and increase in total root surface area. The roots of many, if not all, plant species are able to exude substances that may effectively increase the solubility of soil P and other nutrients (Greke *et al.*, 1994). Increased phosphatase activity in the rhizosphere soil seems to increase the hydrolysis of soil phosphate esters (Bielski and Johnson, 1972), releasing inorganic P for plant uptake (Tarafdar and Jungk, 1987). Therefore, there is a need to enhance the ability of plant roots to secrete phosphatase and phytase to increase plant availability of P from soil. Although the relationship between rhizosphere phosphatase activity and depletion of rhizosphere organic P is often reported (Asmer *et al.*, 1995; Tarafdar and Jungk, 1987), any evidence, that the level of acid phosphatase or phytase are limiting the mobilization of P from soil organic P pools, is yet to be obtained. The extent of intraspecific variation in rhizosphere phosphatase and phytase activity is yet unexplored and the analysis of acid phosphatase and phytase activity is only at the initial stage.

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