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Changes in acid and alkaline phosphatase enzyme activity in rhizosphere ash *Fraxinus rotundifolia* and its correlation with soil and plant phosporus

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Abstract

We investigated in field experiments seasonal dynamics of acid and alkaline phosphatase activity. Alkaline and acid phosphatase activity showed a seasonal pattern. Alkaline phosphatase activity was significantly higher in springandacid phosphatase activity significantly higherin autumn.Soil phosphorus activity in spring is considerably higher than soil phosphorus activity in the autumn.The results showed that average plant phosphorusin spring higher than the autumn but difference between average plant phosphorus on the treatments (spring and autumn) was not significant. Average plant phosphorusin spring higher than the autumn but difference between average plant phosphorus on the treatments (spring and autumn) was not significant. The results of correlation analysis indicated that there wassignificant correlation among alkaline phosphatase with available phosphorus.

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Introduction

Arbuscular mycorrhizalfungi (AMF) are major component of rhizospheremicro florain natural ecosystems. Accumulating evidence indicates that mycorrhizal association plays a significant role in decomposition of soil organic matter, mineralization of plant nutrients and nutrient cycling.Mycorrhizalplants have greater ability to absorb nutrients, soil water increased plant fitness, which may lead to better survival under stressed environmental conditions (Sylvia and Williams, 1992).

Phosphorus (P) is an essential element for plant growth, since it isinvolved in the most important plant biochemical processes. While many soils have high organic and inorganic phosphorus(P) content (Sanyal and De Datta, 1991), only a small proportion(generally <1%) is immediately available to plants (Richardson*et al*., 2009). Although P is critical for plant growth, its availabilityto plants is limited (Smith *et al*., 2011). Plants have thus evolved arange of strategies to increase either P uptake capacity or P availabilityin the soil (Marschner, 1995; Van Aarle and Olsson, 2008). The most widespread strategy to increase P uptake is the associationof plants to arbuscular mycorrhizal fungi (AMF) (Smith *et al*., 2011).Arbuscularmycorrhizalfungi colonization can significantly promote plant P uptake fromthe soil, so that other functions are often inextricably linked withthe improvement of P nutrition status (Cozzolino *et al*., 2010). Themechanisms involved include extended extra radical hyphae pass through the P depletion zone and expand the absorption area ofthe host plant root (Li *et al*., 1991); strengthened P uptake kineticparameters as P uptake rate of mycorrhizae is six times of theroot hair (Sanders and Tinker, 1973); accelerated P transfer ratewhich is ten times faster in AMF than in the root (Smith *et al*., 1994); and improved rhizosphere environment in which P solubilization and availability are strengthened. Arbuscular mycorrhizalfungi also can provide other macro- and micro-nutrients to plantssuch as N, K, Mg, Cu, and Zn usually present in soil in solubleform, especially in low concentrations (Clark and Zeto, 2000; Smithand Read, 2008). In addition, AMF can improve the resistance andsurvival ability of plant to adverse environment, and thus play acritical role in vegetation recovery and reconstruction process inseverely disturbed sites (Bedini *et al*., 2010; Miller and Jastrow, 1990).

Soil acid phosphatase (ACP) and alkaline phosphatase (ALP) areof particular importance in the enzyme system participated in Pabsorption, assimilation and metabolism. They can mediate therelease of inorganic P (Pi) from organically bound P, and facilitatethe transportation of P by AMF to mycorrhizal plant. Former reportshave confirmed that AMF could increase soil phosphatase activities (Dodd *et al*., 1987; Kothari *et al*., 1990; Mar Vázquez *et al*.,2000). The AMF hyphae could also excrete organic acids, whichchanged the rhizosphere pH and dissolved the insoluble phosphatein the soil (Bolan, 1991). Moreover, AMF infection could stimulateplant phosphatase secretion and activities, so that more Pi could be released (Javot *et al*., 2007).

Our primary objectives were to (1) examine seasonaldynamics of acid/alkaline phosphatase activity (2) analyze the correlation among acid/alkaline phosphatase activity withsoil/plant phosphorus activity.

Materials and methods

Study area

The experimental site is located in western Iran, at Deldarcity in the Chaharmahal-Bakhtiari province of Iran.The area situated between 31°49'29"N and50°51'33"E, and 2400 m above the sea level. The area has an annual mean air temperature of 12.4°C and precipitation of 530 mm y^{-1}.

The site is dominated by *Fraxinus rotundifolia Crataegus aronia* stands. Canopy cover, mean diameter, mean height and stand density were 18.66%, 20 cm, 3 m and 36 trees ha^{-1}, respectively.

Sampling

Sampling was done in at the outset and end of vegetation growth period. In each time, five soil samples were taken from the upper 20 cm of soil. Samples were put in polyethylene bags and chest, and then brought to the laboratory from the research area.Soil samples were put in an icebox under 4˚C.

The samples were air dried in the shade at laboratory temperature for the assay of phosphatase activities.Samples were passed through 2 mm sieve, and then phosphatase activities were determinedby measuring the p-nitrophenol (PNP) (mg PNP $g^{-1}h^{-1}$) releasedby phosphatase activity using p-nitrophenyl phosphate disodium(PNPP) as substrates. The soil was incubated with buffered (pH 6.5for ACP, pH 11 for ALP) sodium p-nitrophenyl phosphate solutionand toluene at 37˚C for 24 h, which was modified according mainlyto the method proposed by Tabatabai and Bremner (1969).

Available phosphorus was analyzed using the Olsen method (Olsen and Sommers, 1982).For estimation of phosphorus in aerial organ, aerial organic separated and thensamples were dried in an oven under 60 ˚C for 72 h.Then samples crushed with mill and conversion into1-cm long segments.In order to obtain the ashes from samples, 2 g of samples were cast in porcelain and were placed at 550 C. after burning by 10 ml of hydrochloric acid 15% extraction was performed (Waling *et al*., 1989).

Particle size analyses were done using the Hydrometer method (Gee and Bauder, 1982).

Statistical analysis

All the data were analyzed using the SPSS 16.0 software package. The root colonization data in two seasonscompared with T-test at the 5% level.Spearman correlation analysis was used to determine whether there is linear correlation among variables.

Results

Soil texture was found Clay-Loam along the general research area.

Soil acid phosphatase and alkaline phosphatase activity was significantly ($P < 0.05$) different between two seasons.Average acid phosphatase values ranged from 74.65µgρNP $g^{-1}h^{-1}$(in spring) to 126.92µgρNP $g^{-1}h^{-1}$(in autumn). The results showed that the acid phosphatase was affected by season (Fig. 1).

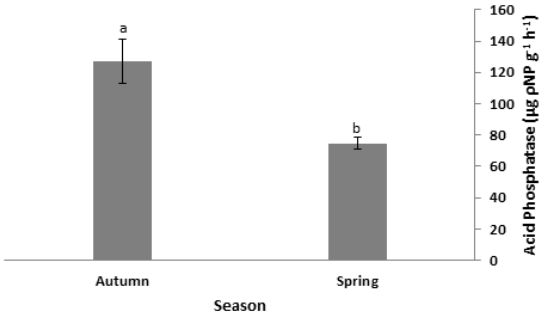

Fig. 1. Effects ofSeason on Acid Phosphatase.

Alkaline phosphatase in spring treatment(154.09) was higher than those of autumn treatment (81.49µgρNP $g^{-1}h^{-1}$) (Fig.2).

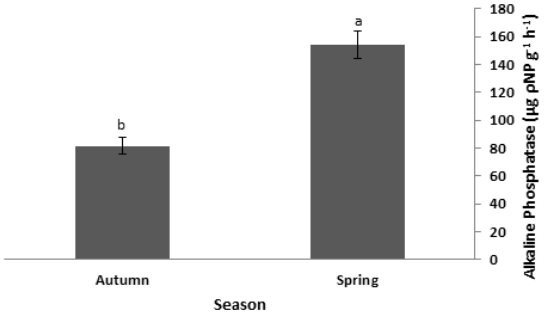

Fig. 2. Effects ofSeason on AlkalinePhosphatase.

The results of correlation analysis indicated that there was no significant correlation among acid phosphatase with available phosphorus andwith plant phosphorus.Also, the results showed that there was no significant correlation among alkaline phosphatase with plant phosphorus, but there was significant correlation among alkaline phosphatase with available phosphorus (Table 1).

Table 1. Correlation analysis among acid phosphatase and plant phosphorus with available phosphorus and with plant phosphorus.

	Available phosphorus	Plant phosphorus
Acid Phosphatase	-0.685	0.7
Alkaline Phosphatase	-0.995**	0.76

** Correlation is significant at a p=0.01.

Soil phosphorusactivity was influenced significantly by season. Soil phosphorus activity in spring is considerably higher than soil phosphorus activity in the autumn(Fig.3).

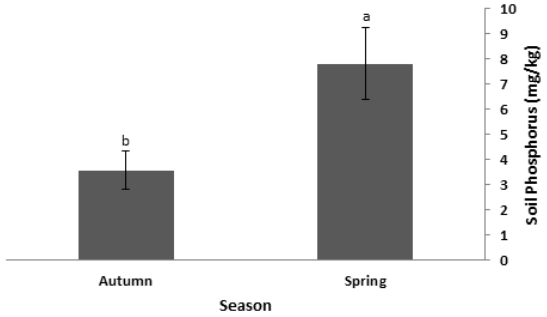

Fig. 3. Effect of Season on Soil Phosphorus Activity.

The results showed that average plant phosphorusin spring higher than theautumnbut difference between average plant phosphorus on the treatments (spring and autumn) was not significant (p>0.05) (Fig.4).

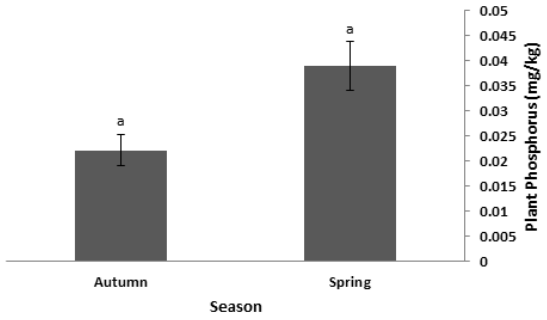

Fig. 4. Effect of Season on Plant Phosphorus.

Discussion

Plant roots are major producers of acid phosphatase (Speir and Cowling, 1991), but do not produce alkaline phosphatase (Nakas *et al.*, 1987; Tarafdar and Claassen, 1988). Alkaline phosphatase originates from soil bacteria, fungi and fauna (Nakas *et al.*, 1987; Tarafdar and Claassen, 1988).Microbes can produce and release large amounts of extracellular phosphatase due to their large combined biomass, high metabolic activityand short lifecycles (Speir and Ross, 1978).

In this study soil acid phosphotase activity was significantly higher than alkaline phosphotase activity in autumn treatment, but in spring treatment acid phosphotase activity was significantly lower than alkaline phosphotase activity. This may be due to a larger fungal community under trees in spring. Soil fungi are effective producers of alkaline phosphatase (Tarafdar and Chhonkar, 1979).

Alkaline and acid phosphatase activity showed a seasonal pattern (Krämer and Green 2000). The results showed that soil acid phosphatase and alkaline phosphatase activity was significantly ($P < 0.05$) different between two seasons. This observation is consistent with Krämer and Green (2000). But Skujins (1976) noted that seasonal variations in phosphatase activity are generally small. Our data suggestthat the decrease in alkaline phosphatase activity in autumn.Alkaline phosphatase activity decreased due to unfavorable environmental conditions such as low soil moisture and high temperaturethat reduces the activity of soil micro-organisms and fauna.Significant correlations of phosphatase activity with soil moisture have beenreported (Speir and Cowling, 1991).Krämer and Green (2000) reported that soil moisture and temperature had a limitedinfluence on phosphatase activities.

The attractive result in this study was negative correlation amongalkaline phosphatase with soil phosphorus (Huang *et al.*, 2011). Since phosphatase enzymes are produced in low available phosphorus condition, this correlation can be reasonably assessed.

Since the amount of alkaline phosphatase in spring higher than autumn, and the effect of alkaline phosphatase enzymes in converting non-absorbed phosphorus to available phosphorus for plant, these results seem reasonable.

In conclusion, results from this study indicate that:

(1) Acid/alkaline phosphataseactivity was significantly different between two seasons.
(2) Soil available phosphorus was influenced significantly by season.
(3) Plant available phosphorus was not influenced significantly by season.

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