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Changes of Available Phosphorus and phosphatase activity in the rhizosphere of some field and vegetation crops in the fast growth stage

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ABSTRACT: A large proportion of P is found in organic forms. Phosphatase, plays an essential role in the mineralization of organic phosphorus. Agronomy species can affect phosphatase activity in rhizosphere. The aim of our study was to determine the effects of some agronomy species (Gramineae, Leguminose, Solanaceae, Labiatae, Cruciferae, Umbellifera, Alliaceae) on phosphatase activity in their rhizosphere. The agronomy species were planted in a semiarid soil in the pots under greenhouse condition. Phosphatase activity in the rhizosphere of any species was studied at middle of their growth. Phosphatase activity was greatly enhanced in the rhizosphere of all species. Alkaline phosphatase activity increased 102-325% and acid phosphatase activity increased 205-455% in the rhizosphere soil compared to the non-rhizosphere soil. This suggests that agronomy species actively promote rhizosphere phosphatase activity either directly by secretion or indirectly by stimulation of microbial activity and/or by depletion of Pi. There were significant differences between phosphatase activities in rhizosphere of plant species. The highest and lowest means of alkaline phosphatase activity were found in rhizosphere of Trifolium repens and Ocimum basilicum respectively. The highest and lowest means of acid phosphatase activity were found in rhizosphere of Triticum aestium and Cicer arietinum respectively. @ JASEM

Although the total amount of P in the soil may be high, it is mainly (> 80%) present in forms unavailable to plants because of adsorption, precipitation, or conversion to organic forms. In acidic soils, P forms iron/aluminium (Fe/Al) phosphates and gets adsorbed to Fe/Al oxides or humic substances. In alkaline calcareous soils, P is often precipitated as calcium (Ca)-P. Organic P (mainly phytate) may represent more than 50% of total P in many soils (Osborne & Rengel, 2002). Plant genotypes differ in the capacity to convert nonavailable forms of nutrients to available forms and to take them up. Factors underlying the differential capacities of plant genotypes to access soil nutrients include differences in the surface area of contact between roots and soil (Rengel and Marschner, 2005) and in the composition and amount of root exudates (Rengel, 2002; Jones et al., 2004) and rhizosphere microflora (Marschner et al., 2005), resulting in differences in the chemistry and biology of the rhizosphere.

Most of the organic P mineralizing activity occurs at rhizosphere where phosphatase released from plant roots (Helal and Dressler,1989), fungi (Tarafdar et al. 1988), mycorrhizal fungi (Bower, 1973; Tarafdar and Marsshner, 1994) and bacteria (Tarafdar and Classen 1988). Organic phosphates in the rhizosphere is mineralized by acid phosphatase exuded by cluster roots at high rates (Grierson and Admas, 2000; Taranto et al., 2000). Root exudates and rhizo-deposits form the substrates for rhizosphere bacteria, and it has been recognized that composition of these substrates can differ between plant species, even between cultivars, and during plant development (Rovira, 1959; Nelson, 1990; Whipps, 2001; Rengel 2002) These differences in root-derived substrates are believed to explain the plant specific rhizosphere bacterial communities that have been observed for different plant species grown under similar conditions (Marschner et al. , 2001, 2002; Smalla et al., 2001; Kowalchak et al., 2002). Yet, the root exudate composition is also affected by the plant's growth conditions in the soil e.g. pH, nutrient limitation, moisture stress and exposure to pathogens (Yang and Crowley , 2000; Hertenberger et.al.,2002). The functional capacity of the soil microbial community, as reflected in the activities of enzymes involved in nutrient mineralization processes, varies among soils dominated by different plant species (Waldrop et al, 2000; Kourtev et al., 2003). The objective of this study was to evaluate the effect of some crop plant species on available phosphorus and phosphatase activity in the rhizosphere.

MATERIALS AND METHODS

*Material and Experimental design***:** A surface-soil sample (0-15 cm) was obtained from a field in Hamadan, Iran. Three kg of the soil was put in pots of 20 cm diameter, and arranged in a completely randomized experimental design with three replicates. The plant grew in greenhouse condition. Seven plant families were used in this study including; Gramincae (Triticum aestium, zea mays), Legominosae (Trifolium repens, Cicer arietinum), Solanaceae (Solanum tuberosum, Solanum lycopersicum), Labiatae (Ocimum basilicum, Satureja hortensis), Cruciferae (Lpidium sativum, Raphanus sativa var: radicola), Umbelliferae (Petroselinum hortanse, Anetum graveolens), Alliaceae (Allium cepa, Allium sativum). Those were planted in the same soil in a greenhouse. The

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experiment was set out as a randomized complete block. Six pots of each species were planted. Six plants-free pots were prepared as controls.

*Soil sampling***:** Three randomly selected plants of each species in each block were carefully dug from the pot. The soil strongly adhering to roots was considered as rhizosphere soil. The three plant-free pots were considered as control soil.

Soil physical and chemical analyses: Air-dry soil was subsequently crushed and sieved to pass a 2-mm mesh screen for particle-size analysis using the hydrometer method (Gee & Bauder, 1986). Calcium carbonate equivalents (CCE) were measured by back titration procedure (Leoppert and Suarez, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil: water extract after shaking for 30 min (Hesse, 1971). Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley & Black, 1934). Total nitrogen in all samples was determined by the Kjeldahl method (Hinds & Lowe, 1980). Cation-exchange capacity (CEC) and available K were measured according to Bower et al., 1952. Available phosphorus was extracted with 0.5 M NaHCO3 (pH 8.5) and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Jackson, 1958).

Microbiological and biochemical analyses: Fresh soil samples were stored at 4 ^{o}C for microbiological analyses. Spores of VAM fungi were isolated from 50 cm^3 sub-samples by wet sieving (Gerdmann & Nicolson, 1963) and sucrose gradient centrifugation (Jenkins, 1964), and counted (Sylvia, 1994). Basal respiration was measured as CO_2 evolved in 5 days (Alef & Nannipieri, 1995). Substrate induced respiration (Anderson & Domsch, 1978), was determined in 72 h. Heterotrophic bacterial and Azotobacter populations were estimated by plate count method. Soil suspension and dilutions were prepared. Media of soil extract agar (SEA) (James, 1958; Parkinson, et al., 1971), rose bengal starch casein nitrate agar (RBSCNA) and modified potato dextrose agar (MPDA) were prepared in lab and used for determination of total soil bacterial, actinomycetes and fungi populations respectively (Alef & Nannipieri, 1995). Two media were prepared in lab for study of Azotobacter population in soil samples. The first one was Ashby's mannitol agar (Subba Rao, 2001). For inhibition of the growth of gram-positive bacteria and actinomycetes it was modified by addition of 1 ml crystal violet solution (5 g L^{-1} in ethanol). The second media for Azotobacter enumeration was LG medium (Alef & Nannipieri, 1995). Colony forming units on the solid media were numbered after a week of incubation at 27 ^{o}C (Alef & Nannipieri, 1995; Subba Rao, 2001). Soil acid and alkaline phosphatases were analyzed according to the methods of Eivazi and Tabatabai (1977). Soil cellulase activity was assayed by the improved method of Schinner, & Von Mersi (1990). The source of materials and substrates used in the culture media and enzymes assessments were Merck Co.

Statistical analyses: Data statistically analyzed for standard deviation, means were calculated and Duncan's new multiple range test was made to assess the effects of some agronomy species (Gramineae, Leguminose, Solanaceae, Labiatae, Cruciferae, Umbellifera, Alliaceae) on phosphorus and phosphatase activity in their rhizosphere. The computer programs used for data analysis were Ms-Excel and SPSS 9.0 for windows (spss Inc).

RESULTS

The texture of the soil was moderate and determined as loam (Table 1). The equivalent calcium carbonate (CCE) content of the soil was considerably high (>10 %) thus the soil was calcareous. Soil pH value was greater than 7. The 2:1 extract of soil exhibited low EC (<0.3 dS m^{-1}). In this semiarid area the mean soil total nitrogen (TN) and organic carbon contents were found to be relatively as high as 0.11 and 1.02 percentage respectively. The soil available P determined by Olsen method was (39 mg kg^{-1}) higher than the critical level (10 mg kg^{-1}). Soil available K was also relatively high (394 mg kg^{-1}). Soil microbial and biochemical activities were also relatively high compared to other soil of this semiarid region.

Available P decreased significantly because of both plant uptake and immobilization in the rhizosphere of all species (Table 2). It was higher in the control soil (39 mg kg^{-1}) and decreased from 34.9 mg kg^{-1} in rhizosphere of A.graveolens to 15 mg kg^{-1} in rhizosphere of S.lycopercicum. It was Also higher than 30 mg kg^{-1} soil in the rhizosphere of S.tuberosum, O. bsilicum and L. sativum, and lower than 20 mg kg^{-1} soil in the rhizosphere of A.cepa, A.sativum and P.hotanse.

Table 1: Some soil characteristics used in this study

Parameter		*Amount*
Texture	-	Loam
CaCO3	%	10.5
pH	-	7.9
CEC	Cmolc Kg^{-1}	26.3
EC	dS m^{-1}	0.3
OC	%	1.02
TN	%	0.11
available P	mg Kg^{-1}	39.4
available K	mg Kg^{-1}	394
Cellulose act.	µmol glucose g^{-1} soil 24 h^{-1}	1.43
Alkaline phosphatase act.	µmol P.N.P g^{-1} soil h^{-1}	7.5
Acid phosphatase act.	µmol P.N.P g^{-1}soil h^{-1}	2.2
Azotobacters	N *10^4/ g^{-1} soil	25
Bacteria	N *10^7/ g^{-1} soil	1.5
Actinomycetes	N *10^4/ g^{-1} soil	70
Fungi	N *10^3/ g^{-1} soil	1.5
AM spores	N/10 g^{-1} soil	120
SIR	mg CO_2 Kg^{-1} soil day^{-1}	572
Basal respiration	mg CO_2 Kg^{-1} soil day^{-1}	20

As shown in Table 2 phosphatase activity was greatly enhanced in the rhizosphere of all species. There are high significant differences between differ agronomy species for phosphatase activity ($P<0.001$). Alkaline phosphatase activities and acid phosphatase activities increased 102-350% and 205-455% in their rhizosphere respectively compared to non-rhizosphere soil. The activity of alkaline phosphatase was much more than the activity of acid phosphatase in the rhizosphere and non-rhizosphere soil. The highest mean of alkaline phosphatase activity was found in rhizosphere of T.repens. It was 26.7 µmol PNP g^{-1} h^{-1}. The lowest mean of alkaline phosphatase activity was found in rhizosphere of O.basilicum. It was 7.81 µmol PNP $g^{-1}h^{-1}$. The highest mean of acid phosphatase activity was found in rhizosphere of T.aestivum. It was 10.43 µmol PNP $g^{-1}h^{-1}$. The lowest mean of acid phosphatase activity was found in rhizosphere of C.arietinum. It was 4.69 µmol PNP g^{-1} h^{-1}.

DISCUSSION AND CONCLUSION

As shown in above acid phosphatase activity increased more than alkaline phosphatase activity. The consensus in the literature is that acid and alkaline phosphatases are differentiated by their source of production. Plants-produced is reported to be exclusively acid phosphatase (Tarafdar and Marschner , 1994). In addition, acid phosphatase is produced by bacteria, fungi, yeasts and protozoa, so that enhanced acid phosphatase activity in the rhizosphere may produced by bacteria, fungi and earthworms (Hebrien and Neal , 1990). Previous study shown that acid phosphatase is predominant in acid soils, while alkaline phosphatase prevails in alkaline soils (Evazi et al., 1977). However the pH of soil used here (pH 7.9) was more suitable for alkaline phosphatase activity than acid phosphatase activity.

As shown in table 2 there were significant differences between phosphatase activities in the rhizosphere of plant species. Differences among plants species may be attributed to differential amount and composition of root exudates (Marschner et al, 2003; Baudion et al., 2001 a). In general the families that have a stronger than cluster roots (Graminaceae, legominosae and solanceae) have a more than phosphatase activity. Phosphorus deficiency is generally cites the reason for increased phosphatase activity in the rhizosphere with the growth of many species (Tabatabai , 1982; Tadano et al ., 1993; Tarafdar and Marschner , 1994). For example, Tadano et al. (1993) demonstrated enhanced phosphatase activity in P deficient conditions with the growth of soybean, lupine, azuki , rice , wheat , radish , cabbage , tomato and sugerbeet. In our study, we found increased alkaline phosphatase activities (102-350%) and increased acid phosphatase activities (205-455) in the rhizosphere compared to non-rhizosphere soil of 14 different agronomy species. Tarafdar (1988) found increased phosphatase activities (10-120%) in the rhizosphere compared to non-rhizosphere soil of 17 different vegetable crops. Hedley at al. (1982) reported a 10-fold increase with growth of Brassica napus.

Table 2: Means of Available P (mg kg^{-1}), acid and alkaline phosphatase activities (μmol P.N.P g^{-1}soil h^{-1}) in the rhizosphere of different agronomy species at middle of growth[a]

	Available P	*Alkaline phosphatase act.*	*Acid phosphatase act.*
Control	39 a	5.59 i	2.29 f
T. aestium	24 abcd	17.33 bc	10.43 a
Z. mays	21.5 bcd	12.09 ef	6.83 c
T. repens	20 bcd	20.25 a	8.31 b
C. arietinum	26 abcd	13.39 cde	4.69 e
S. tuberosum	32 abc	18.26 abc	5.06 de
S. lycopercicum	15 d	11.06 fg	10.02 a
A. cepa	16 cd	15.58 cd	8.45 b
A. sativum	18.5 bcd	14.05 de	9.43 ab
O.bsilicum	32.5 abc	6.26 i	6.02 cd
S. hotensis	21 bcd	17.31 bc	5.30 de
A. graveolens	34.9 ab	7.43 hi	6.27 cd
P. hotanse	17 cd	8.50 gh	8.68 b
L. sativum	30.5 abcd	19.87 ab	8.43 b
R. sativa	28 abcd	9.04 gh	7.02 c

[a] Means within columns followed by the same letter are not significantly different ($P < 0.01$).

REFERENCES

Alef, K., and P. Nannipieri. 1995. Methods in applied soil microbiology and biochemistry, Academic Press, Harcourt Brace & Company, Publishers, London.

Anderson, J.P.E., and K.H. Domsch. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:214–221.

Bower, C.A., R.F. Reitmeir, and M. Fireman. 1952. Exchangeable cation analysis of saline and alkali soils. Soil Science 73:251-261.

Bremner, J.S., and C.S. Mulvaney. 1982. Nitrogen-total. In: Page, A.l., Miller, R.H., and Keeney, D.R. (Eds), Method of soil analysis, part 2: chemical and microbiological properties. Soil Science Society of America, Inc. Publisher, Madison, Wisconsin, USA. 595-624.

Eivazi, F., and M.A. Tabatabai. 1977. Phosphatase in soils. Soil Biology and Biochemistry 9: 167-172.

Gee, G.W., and J.W. Bauder. 1986. Particle size analysis. In: Klute A. (ed),.Method of soil analysis, part 1: Physical and mineralogical methods, Soil Science Society of America, Madison, Wisconsin USA. 383-411.

Gerdmann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal endogone species extracted by wet sieving and decanting. Transaction British Mycological Society 46: 235-244.

Hebrien, S.A., Neal, J.L., 1990. Soil pH and phosphates activity. Communications in soil science and plant analysis 21, 489 – 456.

Hedley, M. J ., White , R.E ., Nye, P.H ., 1982. Plant induced changes in the rhizosphere of rape (brassica napus var emerald) seedlings. Change in 1 value, soil phosphate fractions and phosphatase activity. New phytologist 91, 45-56 .

Hertenberger, G., Zampach, P., Bachmann, G., 2002. Plant species affect the concentration of free sugers and free amino acids in different types of soils. Journal of plant nutration and soil science 165, 557–565.

Hesse, P.R. 1971. A text book of soil chemical analysis. John Murray. London.

Hinds, A., L.E. Lowe. 1980. Ammonium-N determination. Soil nitrogen. Berthelot reaction. Soil Science and Plant Analysis 11:469–475.

Jackson, M.L.. 1958. Soil Chemical Analysis. Prentice Hall, Englewood Cliffs, NJ.

James, N.. 1958. Soil extract in soil microbiology. Canadian Journal of Microbiology 4: 363-370.

Jenkins, W.R.. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Dis. Rep., 73: 288-300.

Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163: 459–480.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M.M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol and Biochem. 35, 895-905.

Leoppert, R.H. and G.L. Suarez. 1996. Carbonates and Gypsum. In: Sparks D.L. (ed.) Methods of soil analysis. Part 3, Chemical methods. Madison, Wisconsin, USA.

Marschner , P., Yang , C , H ., Lieberei , R., Crowley, D .E ., D.E ., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil biology and biochemistry 33, 1437 – 1445.

Marschner P, Solaiman Z, Rengel Z. 2005. Rhizosphere properties of Poaceae genotypes under P-limiting conditions. Plant and Soil. (In press.)

Osborne LD, Rengel Z. 2002. Growth and P uptake by wheat genotypes supplied with phytate as the only P source. Australian Journal of Agricultural Research 53: 845–850.

Nelson, E. B., 1990. Exudate molecules initing fungal responses to seeds and roots. Plant and Soil. 129, 61–73.

Rengel Z. 2002. Genetic control of root exudation. Plant and Soil 245: 59–70.

Rengel Z. and Marschner P. 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytologist, 168: 305–312.

Rovira, A. D. 1959. Root excretions in relation to the rhizosphere effect. IV. Influence of plant, light, temperature, and calcium nutrition on exuation. Plant and soil, 53 – 64

Schinner, F., and W. Von Mersi. 1990. Xylanase, CM-cellulase and invertase activity in soil: an improved method. Soil Biology and Biochemistry 22: 511 –515.

Small , K ., Wieland , G ., Buchner , A., Zock , A ., Parzy , J ., Kaiser , S., Roskot, N., Heuer, H ., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant- dependant enrichment and seasonal shifts reveared. Applied and Environmental Microbiology 67, 4742 – 4751.

Subba Rao N.S. 2001.Soil microbiology (Forth edition of soil microorganisms and plant growth). SciencePublishers, Inc. Enfield (NH). USA.

Sylvia, D.M.. 1994. Vesicular-Arbuscular Mycorrhizal Fungi.Methods of soil Analysis, Part 2: Microbiological and Biochemical properties. SSSA, Book series, no. 5. Pp 351-378.

Tabatabai, M.A., 1982. Soil enzymes. In : page, A.L ., Miller , R .H ., Keeney , D.R .(Eds) , Methodes of soil analysis . 2. Chemical and microbiological properties, second ed , Agronomy Monogragh no. 9, ASa- SSSA , Madison , WI , PP 903-947.

Tadano, T., Ozawa , K., Sakai , H ., Osaki . M., Matsui, H., 1993. Secretion of acid phosphatase by roots of crop plants under phosphorus deficient conditions and some properties of the enzyme secreted by lupin roots. Plant and soil 155/ 156, 95- 98.

Tarafdar, J .C ., 1988. Activity of urease and phosphatase in the root – soil interface of vegetable crops. Journal of horticultural science 63, 605- 608.

Tarafdar, J .C, Marschner, H., 1994. phosphatase activity in the rhizosphere and hyphosphere of

VA mycorrhizal wheat supplied with inorganic and organic phosphorous. Soil Biology and Biochemistry 26, 387- 395.

Tarafdar, J. C., rao, A. V., Bala , K ., 1988 . Production of phosphatase by fungi isolated from desert soils. Folia microbiologia 33, 453- 457.

Tarafdar, J.C., Claassen, N., 1988. Organic phosphrous compounds as a phosphrous source for higher plants through the activity of phosphatase produced by plant roots and microorganisms. Biology and fertility of soils 5, 308 – 312.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in tropical soil. Soil boil. Biochem. 32, 1837- 1846.

Walkley, A., and I.A. Black. 1934. An examination of the Degtareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37: 29–38.

Whipps, J. M., 2001. Microbial interactions and biocontrol in the rhizosphere. Experimental Botany 52, 487 – 511.

Yang, C.H., Crowley. D.E., 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Applied and Environmental microbiology 66, 345 – 351.