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Effect of pH and phosphate supply on acid phosphatase activity in cereal roots

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Enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The literature shows that under favourable conditions microorganisms supply most of the enzyme activity. The effect of plants on soil enzymatic activity is due to changes in organic matter content and microbial populations, but is also formed by accumulated enzymes and by continuously released extracellular and endocellular enzymes; all of which originate in the plant root.

Our research studies acid phosphatase activity linked to the previous source, by which we mean cultivated plants. Evaluation was carried out on the root systems of both the chosen species and cereal varieties and also in nutrient medium on which crops were planted under conditions of changing pH and phosphorus supply.

Different varieties of winter wheat, spelt, barley and rye were used and after sterilization the seeds were sown on Murashige–Skoog nutrient medium with pH 5.6, 6.2 and 6.8 and a phosphorus supply between 30 – 160 mg P_2O_5 L^{-1} of medium. After 10 days of cultivation the plant roots were harvested, homogenized and the acid phosphatase activity was measured.

The results show that the acid phosphatase activity in the root system of various species and cereal cultivars is negatively correlated with increasing pH and available phosphorus level in the nutrient medium.

Key words: phosphatase activity, soil, roots, cereals.

Introduction

Plants meet their phosphorus requirement through the uptake of phosphate anions from the soil. To be available to plants, organic forms of soil phosphorus must be mineralized by those processes, which are mediated by phosphatase enzymes (Bieleski & Ferguson, 1983). A part of the total phosphorus in soil occurs in organic forms. The average content of organic phosphorus in soils ranges from 5 to 50% of total P (Harrison, 1987) and forest soils have a higher organic P content than arable or cultivated soils. Halstead & McKercher (1975) state that as much as 5–10% of the organic phosphorus is associated with living microbial tissue. Several re-

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searchers have described the relationship between total organic P content and other soil properties such as organic matter content, N and pH. The organic P content can also be affected by human activities. A large reservoir of organic phosphorus exists in forms, which are unavailable to plants. The microbial oxidation of organic substrates is an important source of inorganic phosphate.

Soil phosphatases play a major role in the mineralisation processes (dephosphorylation) of organic P substrates. In agricultural soils phosphatase activity is affected by soil properties, crop plants and farming systems. Relationships between phosphatase activity and soil properties have been described in numerous studies (e.g. SPEIR & ROSS, 1978; BONMATI et al, 1991; ŠARAPATKA & KRŠKOVÁ, 1997; MÄDER et al., 1993; OBERSON et al., 1993).

The enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all these organisms. The literature shows that under favourable conditions microorganisms supply most of the soil enzyme activity (SPEIR & ROSS, 1978), with their large biomass, high metabolic activity and short lifespan. Plants have a marked effect on soil enzyme activity. This effect could be due to changes in organic matter content and the microbial population, but enzymatic activity in the soil is also formed by accumulated enzymes, continuously released extracellular enzymes and by endocellular enzymes; all of which originate in the plant roots. Rhizosphere phosphatase activity tends to be higher because of increased microbial numbers in the rhizosphere and the excretion of plant root enzymes.

The phosphatase activity associated with the roots of different plants has been studied by McLACHLAN (1980), BEISNER & RÖMER (1999), GILBERT et al. (1999), RICHARDSON et al. (2000) and by other authors. Attention has also been dedicated to optimal pH, the effect of phosphorus on phosphatase activity, enzyme activities in root extracts or intact roots, activity in external root solutions, etc. BEISNER & RÖMER (1999) described that sugar beet roots with P deficiency have high potential of phosphatase activity from the acid to the neutral pH range. Under these conditions they may effectively use dissolved organic phosphorus compounds. For *Triticum aestivum* McLACHLAN (1980) described increased activity of acid phosphomonoesterase under P-deficiency in intact roots and the optimum in the range pH 5–6. There was no evidence of alkaline phosphatase activity with phosphorus deficiency. GILBERT et al. (1999) found significantly greater acid phosphatase activity associated with white lupine roots in P-deficient plants.

The aim of this work was to evaluate the activity of acid phosphatase in the root systems of both chosen cereal species and varieties, and nutrient medium on which the crops were planted under conditions of changing pH and phosphorus supply which can reflect changing soil conditions.

Material and methods

Different varieties of winter wheat, spelt, barley and rye were used. *Triticum aestivum* L. represented cultivars Astella, Hana, Samanta, Siria and Trane, *Triticum spelta* L. – Ostro, Lueg, Oberkulmer Schwarzer, Altgold, Rouquin, *Hordeum sativum* JESSEN – Norimberk, Rubín, Forum, Amulet and *Secale cereale* L. – Daňkovské nové and Rapid.

The seeds were sterilized using a solution of Savo preparation with chlorate and sown on the nutrient medium MS (MURASHIGE & SKOOG, 1962) without sacharose with pH 5.6, 6.2 and 6.8 and phosphorus supply between 30–160 mg P_2O_5 L^{-1} of medium by KH_2PO_4.

After 10 days of cultivation (2000 lx during 16 h/day, 26 °C) the plant roots were harvested and homogenized. After incubation at 30 °C, the acid phosphatase activity was measured using adjusting methods according to TABATABAI & JUMA (1988) with p-nitrophenyl phosphate as the substrate. After incubation the reaction was stopped by adding 0.5 M NaOH and the p-nitrophenol was then measured spectrophotometrically. The acid phosphatase activity was also set in the nutrient medium in which the plants were cultivated.

The results were statistically evaluated by means of linear regression analysis and analysis of variance using SPSS statistical system.

Results and discussion

The results show the effect of the concentration of phosphorus in the nutrient medium on the activity of acid phosphatases in the root system The increasing amount of available P tends toward decreasing phosphatase activity, mainly between the first (30 mg P_2O_5/L of medium) and the second level (100 mg). These levels correspond approximately to the low and optimal supplies of phosphorus in the soil. Also, pH had a statistical effect on the activity of acid phosphatase where, in more acid media, a higher activity of acid phosphatase was evaluated. The correlations are possible to outline using linear regression equations for:

P_2O_5: acid phosphatase activity $= -0.127\ P_2O_5 + 135.5$

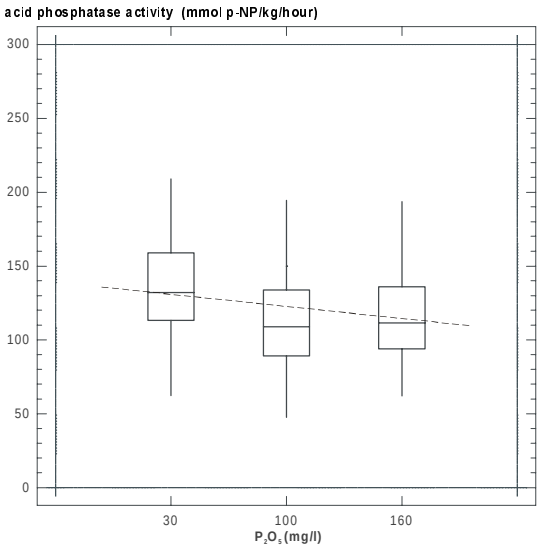

Fig. 1. Effect of phosphate level on acid phosphatase activity.

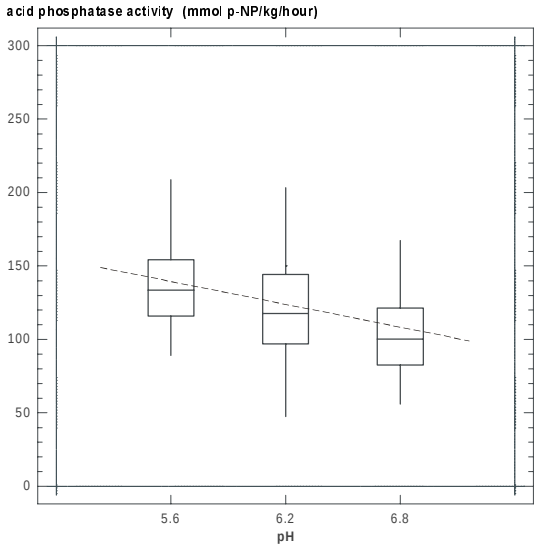

Fig. 2. Effect of pH on acid phosphatase activity.

pH: acid phosphatase activity $= -25.72$ pH $+$ 282.4

The basic statistical data (median, lower and upper quartile) of all cereals in different pH substrates and different phosphate supply, and trends are shown in Figs 1 and 2.

Differences were also found between the studied species: activity of the acid phosphatase in the root system of *Triticum spelta* differed from that of *T. aestivum* and *Hordeum sativum* differed from all other species. *Hordeum sativum* also had the highest activity of acid phosphatase, which could be caused by the size of the root system and possibly by poorer utilization of nutrients.

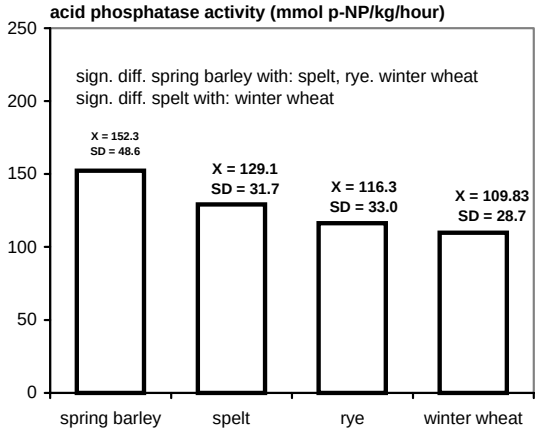

Fig. 3. Acid phosphatase activity of cereal species.

These differences were found not only between species. Statistical differences were found in the activity of the studied enzyme depending on the supplies of phosphorus and pH within an individual species:

– *Secale cereale* with pH levels of 5.6 and 6.8,

– *Hordeum sativum* also with pH levels of 5.6 and 6.8,

– *Triticum aestivum* with pH at all levels, and with 30 and 100 mg L^{-1} P_2O_5,

– *Triticum spelta* with pH levels of 5.6 and 6.8; 6.2 and 6.8, and of all levels of P_2O_5.

Differences between acid phosphatase activity of the cereals in different pH and phosphorus supply are shown in Figs 4 and 5.

The results of our research correspond with the results of Beissner & Römer (1999) who found the effect of P deficiency on phosphatase activity in sugarbeet roots from the acid to the neutral pH range. McLachlan (1980) stated that P deficient plants had greater activities than those with sufficient levels. There was no evidence of alkaline phosphatase activity with phosphorus deficiency. Similar conclusions were found by Gilbert et al. (1999) for white lupin roots and Richardson et al. (2000) for wheat. Richardson et al. (2000) also described a limited ability to obtain P from inositol hexaphosphate, whereas other monoester substrates, such as glu-

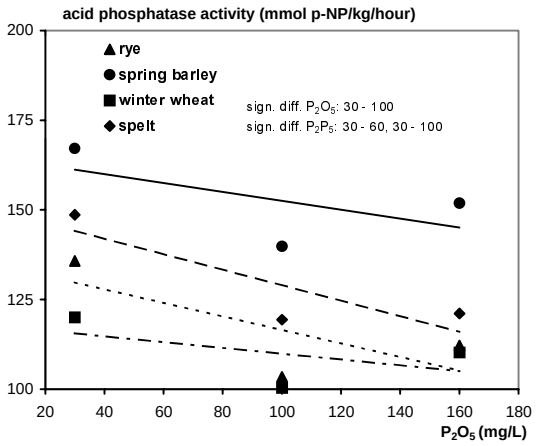

Fig. 4. Effect of phosphate level on acid phosphatase activity of the cereal species.

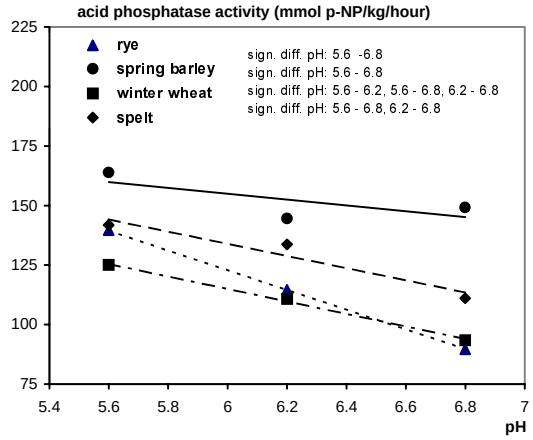

Fig. 5. Effect of pH on acid phosphatase activity of the cereal species.

cose 1-phosphate, were equivalent sources of P for plant growth when compared with inorganic phosphate. Gilbert et al. (1999) describe the development of proteoid roots when grown in phosphorus deficient conditions and these roots were adapted to increased P availability. White lupin roots from P deficient plants had significantly greater acid phosphatase activity in both the root extracts and the root exudates than comparable samples from P – sufficient plants.

In some papers (e.g. McLachlan, 1980; Beissner & Römer, 1999), the results show optimum pH to be in an acid environment. For example, McLachlan (1980) reported the greatest phosphatase activity in the acidic range – pH optima 5–6 for all species.

Our current and future research may help to indicate varieties used in cereal breeding or in low input farming with differing potentials for obtaining phosphorus from soil reserves.

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