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Variation in root hairs of barley cultivars doubled soil phosphorus uptake

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Summary

Length and density (number mm^{-1} root) of root hairs of two barley (*Hordeum vulgare* L.) cultivars Salka and Zita and their capability to absorb phosphorus (P) from nutrient solution as well as from rhizosphere soil were studied. The cultivars were chosen because they differed most among 30 cultivars in ability to absorb P from low P soil in two field conditions. In nutrient solution culture, Salka had 32±4 root hairs mm^{-1} root, 1.02±0.22 mm long. Zita had 21±3 hairs mm^{-1} root, 0.54±0.14 mm long. In soil, the root hairs of both the cultivars were slightly longer (Salka 1.10 ±0.16 mm; Zita 0.63±0.18 mm) than in solution culture but the difference was non-significant ($p<0.05$). The root hairs increased the effective root surface area of Salka by 206% and that of Zita by 81%. In solution culture, Salka produced 163±9 m g^{-1} and Zita 153±11 m g^{-1} dry roots in 21 days. Salka produced 1.65±0.22 g and Zita 1.51±0.31 g of green dry matter (DM). The cultivars did not differ in uptake of P from nutrient solution culture. The P content of DM was 0.42±0.1% in Salka and 0.41±0.08% in Zita. In soil, Salka depleted two times more P from rhizosphere than Zita. The longer root hairs of Salka increased the extension of the depletion zone for $NaHCO_3$-P_i (inorganic P extracted with 0.5 *M* $NaHCO_3$) in the rhizosphere. The cultivars also depleted NaOH-Pi (inorganic P extracted with 0.1 *M* NaOH) from the rhizosphere soil, but the difference between the cultivars was non-significant ($p<0.05$). The results suggested that the ability of Salka to absorb more inorganic soil P was due to its longer and denser root hairs.

Introduction

Genotypic variation in phosphorus (P) uptake by cereals is known from early studies (Smith, 1934) and confirmed later (Baker et al., 1970; Nielsen & Barber, 1978; Gahoonia & Nielsen, 1996). Knowledge of the extent of genotypic variation and the mechanisms causing variation in P acquisition can provide base for a genetic analysis, facilitating the use of biotechnology to make crop plants more P-efficient. Among 30 barley cultivars, Salka and Zita differed widely in ability to absorb P from a low-P soil under field conditions (Nielsen & Schjørring, 1983; Schjørring & Nielsen, 1987). The difference in P uptake was related to the variation in root length and uptake rate of P per unit root length. The reasons for the variation in P uptake rate, however, remained unclear.

Root hairs may increase the effective root surface area many-fold. Wide variation in root hairs formation exists among cereal cultivars (Gahoonia et al., 1997). The significance of root hairs in P acquisition lies in more effectively exploitation of soil close to roots, creating an uniform P-depletion zone close to the root surface (Nye, 1966; Gahoonia et al., 1994). (Misra et al., 1988) found that P uptake was a function of soil volume exploited by root hairs.

Solution-cultures provide uniform root growth conditions facilitating root hair studies, but do not alone discriminate differences in acquisition of diffusion-limited soil P. In this paper, we examine the significance of root hairs in P uptake by barley cultivars Salka and Zita grown both a) in an experimental set-up allowing investigation of rhizosphere soil with high resolution; b) in nutrient solution culture.

Table 1. The composition and concentration of Basic (Bc) and maintenance (Mt) solutions

Nutrients	mM					μM									
	NO_3	NH_4	P	S	K	Ca	Mg	Cl	Na	Fe	Mn	Zn	Cu	B	Mo
Bc	5.0	0.0	0.05	0.44	0.58	1.92	0.78	0.10	0.001	50	7.0	0.7	0.7	2.0	0.7
Mt	13	3.2	0.54	0.61	7.16	1.40	0.35	0.40	0.24	15	12	6.0	0.9	4.6	0.2

Materials and methods

Soil

The soil used in this study was collected from Ap-horizont (0-30 cm) of a field without P-fertilization for 30 years. The soil originated from glacial drift in eastern Denmark. The main properties of the soil are: Clay 15%, Silt 18%, Sand 65%; Total C = 1.15%; Total N = 0.13%. $NaHCO_3$-P_i (inorganic P extractable with 0.5 *M* $NaHCO_3$) = 0.45 mmole P kg^{-1} soil; NaOH-P_i (inorganic P extractable with 0.1 *M* NaOH) = 2.4 mmoles P kg^{-1} soil; P in soil solution = 3μ ***m;*** pH (0.01 *M* $CaCl_2$) = 5.6; CEC = 8.4 ceq. kg^{-1} soil.

Genotypes

Two barley (*Hordeum vulgare L.)* cultivars Salka (Elbo X Vada) and Zita (Vada X 203/7748) were chosen for this study under controlled conditions, because they differed widely in P-uptake in two previous field experiments (Nielsen & Schjørring, 1983; Schjørring & Nielsen, 1987).

Rhizosphere studies

The cultivars were pre-grown in vermiculite filled in PVC tubes (length 10 cm, diameter 4.4 cm) closed at the bottom by nylon cloth impervious to roots. Two ceramic fibre wicks were placed along the inner sides of the tubes to supply nutrient solution of defined composition. The tubes along with the plants having uniformly developed root mats (12 days after germination) were transfered to soil columns filled into PVC tubes (length 3 cm, diameter 5.6 cm). The soil columns were separated by a nylon screen of mesh size 53 μm into 3 cm test soil columns below and 1 cm soil layer above the screen (Gahoonia & Nielsen, 1991). The soil columns (bulk density 1.3 g cm^{-3}) were maintained at defined moisture (θ = 0.21) by placing them over small, cup-shaped sand baths each fitted with a wick dipping into a reservoir of distilled water. For some columns, six 1-mm holes were provided in the nylon screen with the aim of measuring root hairs on roots grown in soil described below. After transplantation, new root mats developed over the nylon mesh, representing a root surface area of 24.6 cm^{-2}. Due to the geotropic nature of root growth mostly the 'active' apical root zones covered the nylon mesh (open space 22%), but root hairs penetrated into the soil. The supply of external nutrient solution (Mt in Table 1) to the plant roots in the vermiculite was continued via the two wicks at 20 cm water tension. The ratio of nutrients in the supplied solution was adjusted to match the nutrient ratio in dry matter of monocotayledonous plant species. Based on previous studies (Gahoonia & Nielsen, 1991; 1992) the concentration was expected to create moderate P deficiency and to avoid deficiencies of other nutrients at a relative growth rate (RGR) of 0.15 day^{-1}. The water uptake from the external nutrient solution was expected to be almost equal, maintaining equal supply of nutrients to both the cultivars. Water uptake also occurred via the soil columns (10 $\pm$2% of the total water uptake). The percentage of total N as ammonium in the supply solution was adjusted to maintain a near-uniform rhizosphere soil pH (Gahoonia & Nielsen, 1992). The experiments were conducted under controlled conditions (light intensity 280 μE s^{-1} m^{-2}, light/dark period 16/8 h, temperature 18/15 °C, relative humidity 75%). More details of the plant growing technique and rhizosphere pH control are given in Gahoonia & Nielsen (1991; 1992). After 14 days, the soil columns were separated from the root mats, quickly frozen in liquid nitrogen and sliced with a freezing microtome to obtain rhizosphere soil samples at distances 0.2, 0.4, 0.6, 0.8, 1.0, 1.5. 2.0, 2.5, 3.5 and 4.5 mm from the mesh surface.

Determination of root hairs in soil

The plants were grown in similar way to that described above. Six holes (ca. 1 mm) were provided in the nylon screen so that roots penetrated and grew into the soil columns. After 7 days the root mats were separated from the soil columns without disturbing the penetrating roots. (After 14 days, too many roots

had penetrated making it difficult to wash them out of the soil without damaging the root hairs). The soil columns were immersed in water overnight and roots were removed carefully. The roots were treated in an Ultrasound bath (Branson 5200, 120W, 47K Hz) for about 5-10 minutes to remove remaining soil particles without damaging the root hairs. The time of the ultrasound treatment required depended on length and density of root hairs. Roots with longer and denser root hairs needed longer treatment. Root hairs and root parameters were measured on the roots using Quantimet 500_+ Image Processing and Analysis System (Leica) at 10x magnification. The roots did not show mycorrhizal hyphae.

Determination of root hairs and P uptake in nutrient solution culture

The root hairs and P-uptake was also measured in nutrient solution culture. Plants were grown in a basic nutrient solution (Bc in Table 1) under controlled conditions. The ionic strength of the solution was similar to that in soil solution (Nielsen, 1984). Its electrical conductivity was maintained at 0.63 mS cm^{-1} by addition of maintenance solution (Mt Table 1) according to a pre-determined relation between volume added and electric conductivity. The pH was maintained at 5.5 $\pm$ 0.2 by addition of ammonium or nitrate solutions (Gahoonia & Nielsen, 1992). Root systems were harvested for root hair analysis after 21 days. The roots were placed in a film of water in petri dishes. Root hair images were captured using a video camera fitted to a microscope interfaced with a computer image grabber board. The images were captured for the main root axis, first order and second order roots. Root hairs and root parameters were measured as described before. The length and density (number mm^{-1} root length) of root hairs as well as the diameter of roots (on which root hairs measured) were averaged. The total length of root system was measured using a scanner (ScanJet IIcx) and *Dt-Scan software* (Delta-T Devices, Cambridge, England).

Analytical procedures

Phosphorus analysis of rhizosphere soil

To 0.5 g of air dry soil in a centrifuge tube, 5 ml of 0.5 *M* $NaHCO_3$ (pH 8.5) was added (Hedley et al., 1982). The tubes were shaken for 2 hours and centrifuged. Inorganic P ($NaHCO_3$-P_i) in the supernatant was determined immediately. The residual soil in the centrifuge tube was dispersed in 25 ml of 0.1 ***m*** NaOH and shaken for 17 hours and centrifuged. Inorganic P in the supernatant was determined immediately. P was measured by the method of Murphy & Riley (1962). Unplanted soil samples were analyzed as controls. The quantity of $NaHCO_3$-P_i absorbed from the rhizosphere soil was calculated by integrating the area of each respective depletion profile. The distinct uniform depletion zone due to root hairs was defined as the distance from root mat where the measured P concentration points on the depletion profile did not differ significantly ($p< 0.05$).

The cross section area (A) of the root hair cylinder was calculated as

$$A = \pi[(L)^2 + 2LD_r] \quad (1)$$

where L is average root hair length and D_r is average root diameters.

The increase in 'effective root surface area' mm^{-1} root was calculated as

$$\pi D_h L_h \ / \ \pi D_r \quad (2)$$

D_h is root hair diameter of both the cultivars = 0.012 $\pm$0.001 mm, L_h is total length of root hairs mm^{-1} root = Ln, where n is root hair density (number mm^{-1} root).

Plant material (shoot) was dried at 80 °C to constant weight and analyzed for P.

Statistical analyses were performed using Statistical Analysis System (SAS Institute, 1989) and Microsoft Excel as found appropriate.

Results

The barley cultivars differed in root hair development both in nutrient solution and in soil culture (Figure 1). The root hairs of both the cultivars were longer in soil than in solution culture, but the difference between media was non-significant ($p<0.05$). Root hairs of Salka were nearly 2 times longer and 1.5 times denser than those of Zita and the average root diameters (D_r) of Salka and Zita were 0.20$\pm$0.05 and 0.17$\pm$0.06 mm respectively. The root hairs formation increased the effective root surface area for mm of root in Salka by 206% and in Zita by 81%. When grown in nutrient solution culture for 21 days, the cultivars did not differ in net uptake of P despite the variation in root hair para-

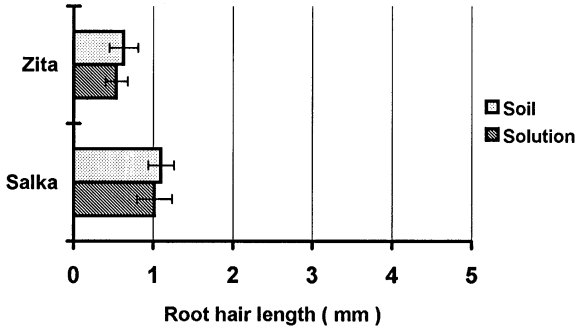

Figure 1. Root hairs lengths of barley cultivars in soil and solution culture.

meters and enhanced root surface area. The concentration of P in the green dry matter (DM) of Salka and Zita was 0.42±0.1% and 0.41±0.08% respectively. In solution culture, the cultivars did not differ significantly ($p<0.05$) in root fineness (Salka = 163±9 m g^{-1}; Zita = 153±11 m g^{-1}) and DM (Salka = 1.65±0.22 g and Zita = 1.51±0.31 g).

The cultivars differed ($p< 0.05$) in net uptake of $NaHCO_3$-P_i from rhizosphere soil (Figure 2). The concentration of $NaHCO_3$-P_i near the roots was 0.2 mmole kg^{-1} soil with Salka. It did not differ ($p<0.05$) within 1 mm, showing a distinct uniform P depletion zone near the roots. The extension of this zone was equal to the length of root hairs of Salka (1.02 ± 0.22 mm). The depletion profile of Zita (with shorter root hairs, 0.54 ±0.14 mm) was mostly steep, with distinct uniform P depletion zone extending to only 0.4 mm from the roots. If the root hair lengths are converted to cylindrical condition (Equation 1), the root hair cylinder of Salka will exploit nearly 3 times more soil in cross section than that of Zita. The quantity of $NaHCO_3$-P_i depleted from the rhizosphere of Salka was 2 times greater than by Zita.

The cultivars also depleted NaOH-P_i (inorganic P extracted with 0.1 *M* NaOH). Thus both the cultivars could mobilize P from the stable P fraction in soil within the first one mm close to roots (Figure 3). The difference between the cultivars was non-significant ($p<0.05$).

Discussion

The experimental approach here applied to study P depletion allows the root surface area, (root mat of 24.6 cm^2), rhizosphere soil pH, nutritional status of

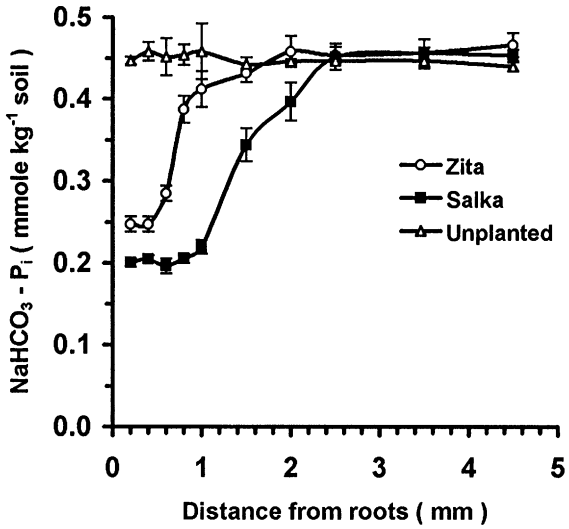

Figure 2. Depletion profiles of soil phosphorus (extracted with 0.5 *M* $NaHCO_3$) in the rhizosphere of barley cultivars.

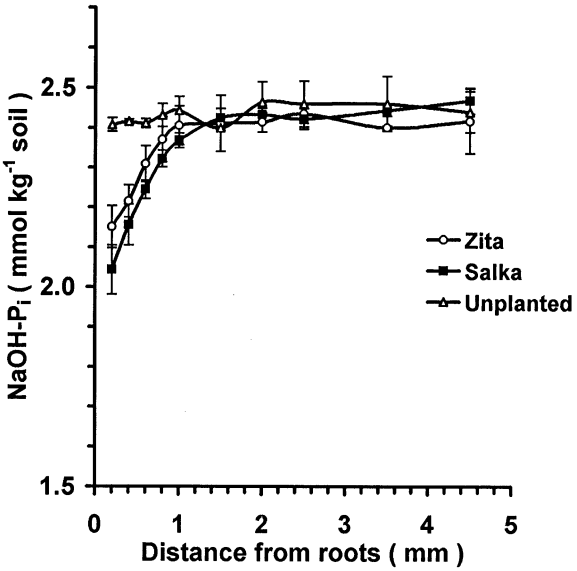

Figure 3. Depletion profiles of soil phosphorus (extracted with 0.1 *M* NaOH) in the rhizosphere of barley cultivars.

plants (Gahoonia & Nielsen, 1992, 1996) and soil moisture (Gahoonia et al., 1994), all to be kept constant, so that the role of root hairs in P-acquisition by the cultivars could be compared without confounding the effects of these factors. The cultivars (Salka & Zita) differed in net uptake of P from a soil-based system, e.g. the rhizosphere soil and in the field, but they did not differ when grown in nutrient solution culture. In solution culture, P diffuses freely to the root surface

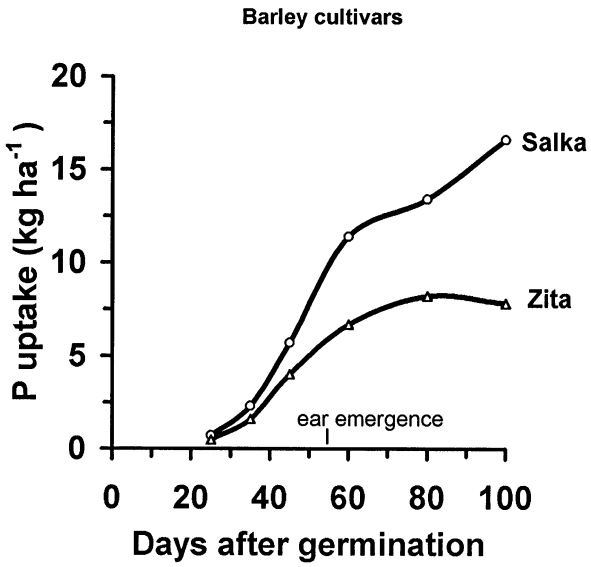

Figure 4. Phosphorus uptake of barley cultivars in field of low-P soil (from Nielsen and Schjørring, 1983)

and the added advantage of having longer root hairs is lost (Clarkson, 1991). In soil, diffusion of P to the root surface is rate limiting and the zone of soil close to the root is depleted uniformly (Figure 2), due to geometrical arrangement of root hairs on roots.

Mackey & Barber (1984) reported that root hairs may be longer in soil than in solution culture. In our study, root hairs of both cultivars were slightly longer in soil than in solution culture (Figure 1), but the difference was non-significant ($p<0.05$). The reason may be that we used a nutrient solution with ionic strength similar to that found in the soil solution. Especially long root hairs washed out of soil were curled, probably due to the growth in soil pores. This made it difficult to measure their actual length and we measured only the extent of root hair zone, which corresponds to the uniform extension of soil P depletion zone.

In previous field experiment, the cultivars differed considerably in uptake of phosphorus from a low P soil (Figure 4). Under the field conditions, Salka produced 65 m g^{-1} and Zita 57 m g^{-1} roots (Nielsen and Schjørring, 1983). Thus, both in the solution culture and in the field experiment, the root length g^{-1} roots of Salka was about 1.1 times greater than that of Zita. Nielsen & Schjørring (1983) also found that the effective minimum concentration (C_{min}) of Salka was 0.02 μmole P and that of Zita was 0.03 μmole P. This indicates that Salka possesses the ability to absorb P from lower concentrations in soil solution. A large part of plant-available P in soil may be located in small-diameter pores (Fawcett & Quick, 1962). Root hairs can penetrate these pores and act as an extension of effective root-soil contact. Curled root hairs on only soil-grown roots indicated that root hairs grew into soil pores. The present study showed that due to longer and denser root hairs, Salka will exploit nearly 3 times more soil than Zita. In addition to this, root hairs will increase the effective root surface area of Salka by 206% and that of Zita only by 81%.

The observation that there was variation in depletion of only labile P fraction ($NaHCO_3$-P_i) suggests the main importance of longer root hairs for intercepting the P diffusing towards the roots rather than dissolving P from more stable P fractions. The results showed that the ability of Salka to absorb more soil P from rhizosphere soil and in low P field conditions was due to its longer root hairs.

In white clover the heritability of root hair lengths ranged from 0.33-0.44 (Caradus, 1979). The genetic basis of the longer root hairs in Salka and their heritability is not known. Model studies indicate that root hair formation of tomato (Hochmuth et al., 1985) and *Arabidopsis thaliana* (Schiefelbein and Somerville, 1990) is controlled by single gene. Therefore, it seems worthwhile to study the genetics of root hairs development in cereals to provide knowledge for breeding genotypes that are more phosphorus-efficient and able to yield well with lower inputs of P fertilizers.

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