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A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake

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Abstract

This paper reports a new barley mutant missing root hairs. The mutant was spontaneously discovered among the population of wild type (Pallas, a spring barley cultivar), producing normal, 0.8 mm long root hairs. We have called the mutant *bald root barley* (*brb*). Root anatomical studies confirmed the lack of root hairs on mutant roots. Amplified Fragment Length Polymorphism (AFLP) analyses of the genomes of the mutant and Pallas supported that the *brb* mutant has its genetic background in Pallas. The segregation ratio of selfed F_2 plants, resulting from mutant and Pallas outcross, was 1:3 (−root hairs:+root hairs), suggesting a monogenic recessive mode of inheritance.

In rhizosphere studies, Pallas absorbed nearly two times more phosphorus (P) than the mutant. Most of available inorganic P in the root hair zone (0.8 mm) of Pallas was depleted, as indicated by the uniform P depletion profile near its roots. The acid phosphatase (Apase) activity near the roots of Pallas was higher and Pallas mobilised more organic P in the rhizosphere than the mutant. The higher Apase activity near Pallas roots also suggests a link between root hair formation and rhizosphere Apase activity. Hence, root hairs are important for increasing plant P uptake of inorganic as well as mobilisation of organic P in soils.

Laboratory, pot and field studies showed that barley cultivars with longer root hairs (1.10 mm), extracted more P from rhizosphere soil, absorbed more P in low-P field (Olsen P=14 mg P kg^{-1} soil), and produced more shoot biomass than shorter root hair cultivars (0.63 mm). Especially in low-P soil, the differences in root hair length and P uptake among the cultivars were significantly larger. Based on the results, the perspectives of genetic analysis of root hairs and their importance in P uptake and field performance of cereals are discussed.

Introduction

Root hairs are of interest in many disciplines of plant science e.g. in cell biology as single cell models (Ridge, 1996) and in plant nutrition as nutrient uptake organs (Hofer, 1996; Meharg et al., 1994; Sattelmacher et al., 1993). Root hairs develop from specialised root epidermis cells (trichoblasts) and due to their geometrical arrangement on roots, they are well suited for increasing uptake of phosphorus (P) and other nutrients from the rhizosphere soil. Cost/benefit analysis of carbon respired for P acquisition (Bates and Lynch, 2000) supports the view that the extension of the root surface area through root hairs is an efficient strategy for improving P uptake, when P supply is limited. Most of P for plant uptake is mobilised from rhizosphere soil (Gahoonia and Nielsen, 1992; Hinsinger, 1998; Jungk and Claassen, 1997). Root hairs are usually clustered within a fairly well defined cylinder around the root, which can be regarded as an effective volume of soil exploited by roots for nutrients. The beneficial effect of longer root hairs in P uptake from rhizosphere can be explained by considering a cylinder of radius equal to the radius of root plus

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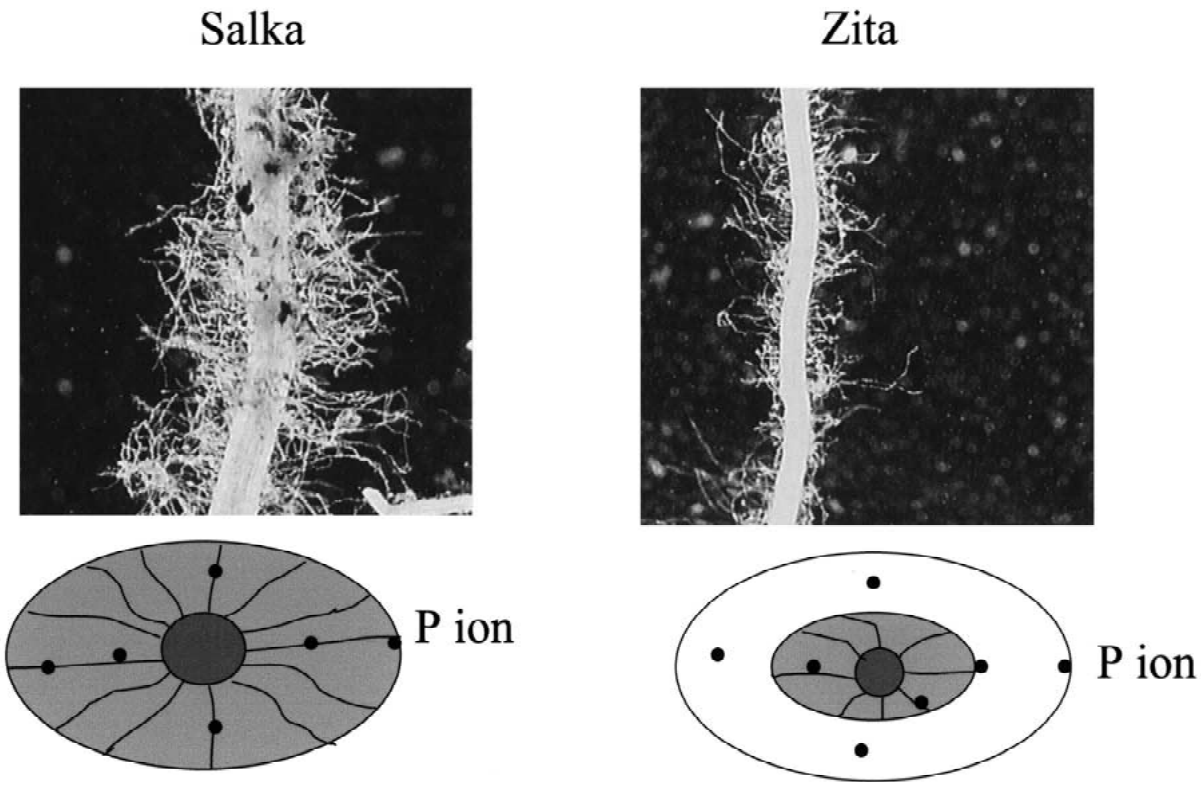

Figure 1. Illustration of P uptake by two barley cultivars, Salka and Zita, differing in root hair lengths.

length of root hairs (Figure 1). Even in a fertile soil, the P ions present on the periphery of 1 mm around the root surface will take about 10 days to reach root surface for uptake. Root hairs of, for example, barley cultivar Salka can grow 1 mm long in about 2 days and intercept the diffusing P-ion within the functional life period of root hairs (Fussender, 1987). The shorter root hairs of Zita (0.5 mm) can immediately access the P-ions only up to the periphery of 0.5 mm. The P-ions outside this periphery can only be absorbed if they move in through the slow diffusion process. When the depletion of P in the rhizosphere soil is studied using one-dimensional root–soil interface, the concentration of P within the root hair zone becomes effectively uniform (Gahoonia and Nielsen, 1997).

Many early (Cormack, 1962; Dittmar, 1949) and recent studies showed wide variation in length and density of root hairs of plant species (Föhse et al., 1991) and cereal cultivars (Gahoonia et al., 1999), supporting the view that root hair formation is under genetic control. Root hair *defective* mutants of *Arabidopsis thaliana* have been used for elucidating genetic of root hair development (Schiefelbein and Somerville, 1990) and its role in P uptake (Bates and Lynch, 2000). However, it remains to be explored, how the root hairs of agronomic important crops like cereals can be genetically manipulated for more effective exploitation of soil for nutrients and water (Gilroy and Jones, 2000). The knowledge on the genetic of cereal root hairs and their significance for P uptake and plant performance under field conditions would encourage co-operations between breeders and plant scientists for creating nutrient-efficient crop plants.

We discovered a spontaneous barley (*Hordeum vulgare* L.) mutant missing root hairs (designated as *bald root barley*, *brb*), which should be valuable for supplementing the knowledge of genetic and molecular basis of *Arabidopsis* root hairs for transferring it to agronomic important cereal plants.

In this paper, we report the discovery of *brb* mutant and discuss the perspectives of its use for elucidating the genetic background of root hair formation and P uptake under laboratory and field conditions.

Materials and methods

To facilitate root hair research and to establish their role in P uptake, several methods were developed and applied. The methods are briefly described below.

(a) Depletion of P from rhizosphere soil was studied by the method of Gahoonia and Nielsen (1991). In this method, one dimensional root-soil interface was created between root mats (surface area 24.6 cm^2) of young plants (ca. 25 days) and soil columns maintained at defined soil moisture (θ=0.20). A nylon screen (53 μm) was inserted between the root mats and the soil columns so that no roots but only root hairs penetrated the screen. After about 2 weeks the soil columns were frozen in liquid nitrogen and then sliced with freezing microtome into thin layers (0.2 mm) to obtain soil samples of known distances from the root mats. Phosphorus from the soil samples was extracted with 0.5 *M* $NaHCO_3$ and analysed for inorganic and organic P fractions as outlined in Gahoonia and Nielsen (1992).

(b) Root hair measurement, the seeds for laboratory studies were always first germinated on filter papers moisten with distilled water. The seedlings were then cultivated using solution culture or soil columns (Gahoonia and Nielsen, 1997). Root samples for root hair studies were also taken from field-grown plants. The root hairs on soil-grown roots were cleaned off soil using Ultrasound treatment (47 kHz, 120 W) and measured using Quantimet 500_+ Image Processing and Analysis System (Leica) at 10× magnification. Mutant and Pallas were crossed to get F_1 seeds. The roots of all (50) F_1 seedlings had root hairs. For investigating F_2 segregation ratio, 112 seeds were randomly taken from the stock, sterilised with 0.1% H_2O_2 solution overnight and germinated on filter papers, moisten with distilled water. Roots of the seedlings were observed under light microscope (Leica, M8). Out of 98 seeds germinated, the roots of 27 seedlings were without root hairs.

(c) Acid phosphatase (Apase) acitvity was determined as described by Dinkelaker and Marschner (1992). The method is based on enzymic hydrolysis of 1-naphtylphosphate by root-released Apase, yielding 1-naphtol as reaction product, which forms a red complex with dye Fast Red TR. Filter papers were impregnated with the reagents and placed on the roots of mutant and Pallas. The Apase activity is indicated by the intensity of red colour zone near roots.

(d) Root anatomy of Pallas and mutant was studied by growing (20 seedlings each) in sterile paper pouches covered with plastic for 7 days with nutrient supply (Gahoonia and Nielsen, 1992). Root segments of 3–4 mm length were dissected and immediately fixed in 3% Karnavsky fixative in 0.1 *M* Na-cacodylate buffer (pH 7.2) for 8 h at cool temperature. Vacuum was applied to remove air and to facilitate the penetration of the fixative into the root tissue. The tissue samples were washed with 0.1 *M* Na-cacodylate buffer, dehydrated with acetone series, infiltrated and embedded in Spurr's Epoxy Resin (Spurr, 1969) and sectioned (2 μm) using ultra-microtome (Super Nova, Reichelt-Jung). The sections were stained with Toluidine blue (pH 9.0), mounted on glass slides, coversliped with oil, examined and photographed under light microscope.

(e) Field experiment was conducted using a completely randomised design with two replicates. The plot size was 1.5×10 m. There were three P treatments. The low P plot received no P fertiliser since 1966. Nitrogen (N), phosphorus (P) and potassium (K) fertilizers applications (kg ha^{-1}) to plots were as follows:

0P plot: *60N, 0P, 60K*; **10P plot**: *60N, 10P, 60K*;
20P plot: *120N, 20P, 120K*.

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

Results and discussion

Discovery of the brb *mutant*

The discovery of the *brb* mutant was made, when large numbers of seeds of barley cultivar Pallas were being germinating on filter paper in petri dishs, actually for testing germination rate. The root of one seedling looked abnormal and it fell out of the petri dish, because due to lack of root hairs, its roots did not stick to the filter paper. The roots of the seedling were examined under light microscope and were found to lack root hairs completely. The seedling was cultivated further to get more seeds. The plants of subsequent generations were grown in various media, like on filter papers in petri dishes supplied with distilled water, in nutrient solution and in soil. In all subsequent observations, the roots of mutant were found to lack root hairs completely and that of Pallas had normal root hairs, regardless of growth media and P level. The soil-grown roots of the mutant and Pallas are shown in Figure 2a and b, respectively.

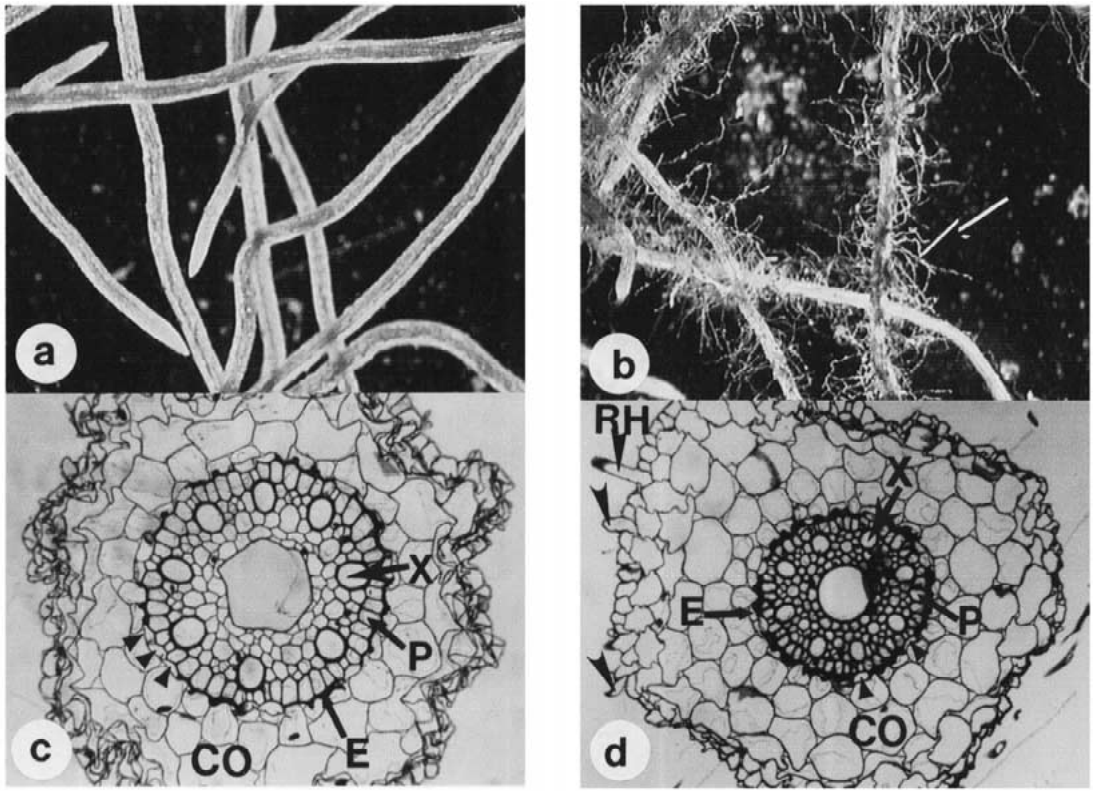

Figure 2. (**a**) Roots of barley mutant missing root hairs, (10×); (**b**) Roots of barley cv. Pallas with normal root hairs, (10×); (**c**) Transverse section (250×) of barley mutant root showing the distorted epidermis without any root hair development; (**d**) Transverse section (250×) of barley cv. Pallas showing the presence of distinct epidermis with root hairs (RH). Note the differences by comparing (**c**) with (**d**) in cortex (CO), pericycle (P), the number of passage cells (triangles) in the endodermis (E), the diameter of stele as well as individual xylem vessels (X) and the thickening of cell walls in all the elements of stele region.

Root anatomical studies also confirmed the missing root hairs as compared to Pallas (Figure 2c and d). No root hair or indication of trichoblasts differentiating into root hairs with the mutant was found when root thin sections were examined under light microscope. There seemed to be some variation in root anatomy of the mutant and Pallas. The cortex cell layers of mutant were less (2–3) and differed in shape as compared to Pallas (3–4 cell layers). The size of xylem vessels of Pallas was smaller as compared to that of mutant. Smith et al. (1994) reported that protein-phosphatase-inhibitors, okadaic acid and calyculin-A, blocked root hair growth and altered the shape of cortex cells of *Arabidopsis* roots. As shown in Figure 5, the Apase activity near the roots of mutant was indeed lower than Pallas. Whether *brb* mutant specifically produces the phosphatase inhibitors is not yet known. The root epidermis layer of the mutant appeared distorted. The normal growth and seed reproduction, at least at high P levels (50 μM in solution), suggested that the mutation is not impaired in the normal function of the epidermis for nutrients and water absorption. Unlike root hair *defective* mutants of *Arabidopsis* (Tanimoto et al., 1995), the roots of the *brb* mutant remained hairless even when treated with ethylene (data not shown).

The mutant seedling was found in the population of spring barley cultivar Pallas and repeated root morphological and anatomical investigations confirmed that root hairs are lacking. The possibility remained that it could be a seed of entirely different barley accession (or near-isogenic line) without any genetic background in Pallas. Amplified Fragment Length Polymorphism analysis (AFLP) of the genomes of the mutant and Pallas was performed according to Saghai-Maroof et al. (1984) to test this possibility. Out of the total 2376 bands tested, 18 were found polymorphic. Russell et al. (1997) compared the genetic background among 18 cultivated barley accessions and found that minimum of polymorphic bands detected between the accessions

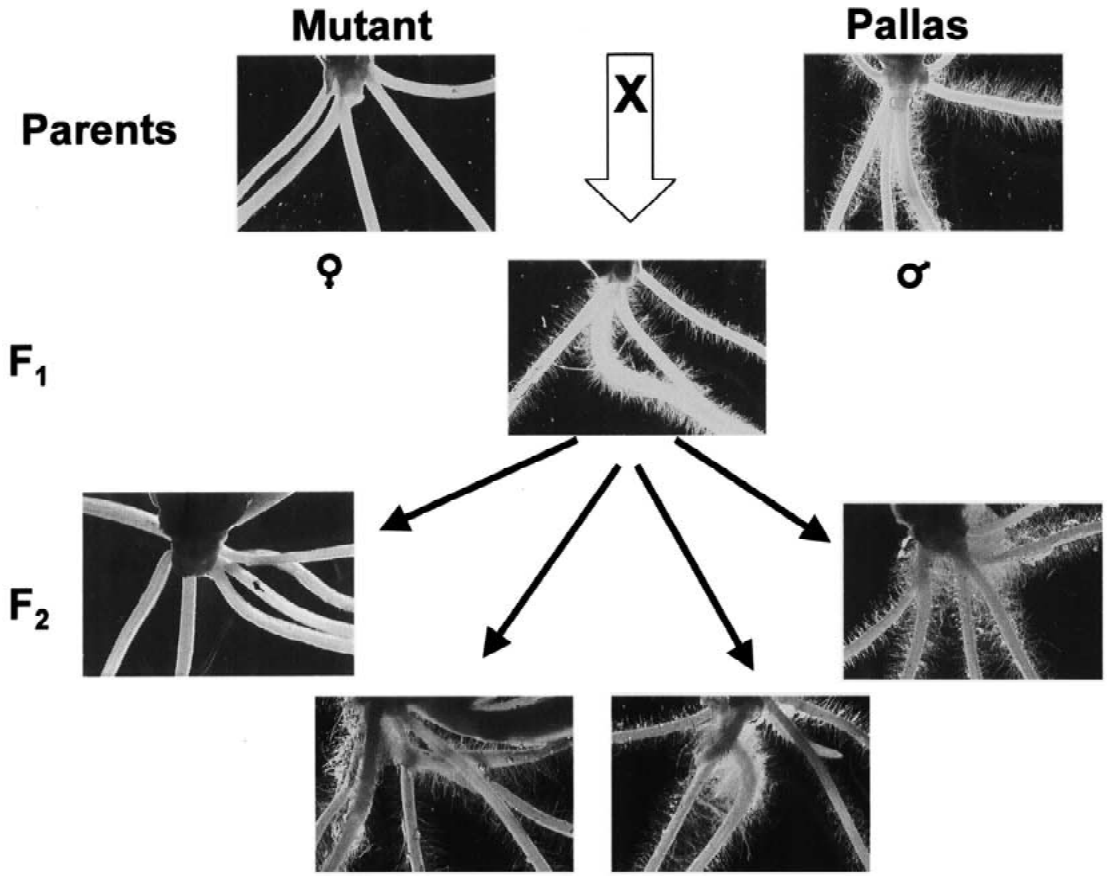

Figure 3. Segregation ratio (mutant x Pallas) of root hair formation of F_2 plants was 1:3. Out of the 98 seeds germinated, the roots of 27 seedlings were without root hairs.

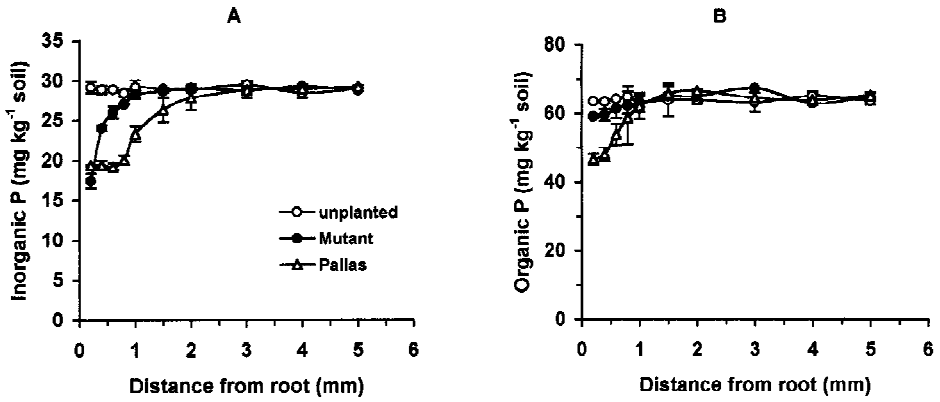

Figure 4. Depletion of inorganic P (A) and organic P (B) (extractable with 0.5 *M* $NaHCO_3$) in the rhizosphere of root hairless mutant and Pallas with root hairs (0.8 mm long).

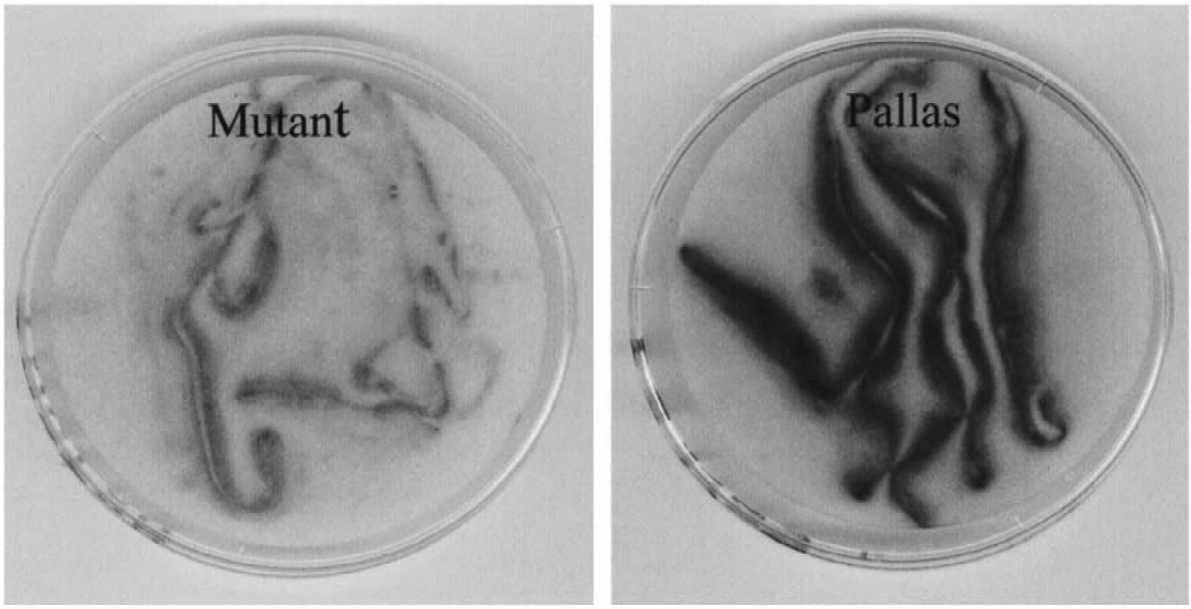

Figure 5. Acid phosphatase acitvity near the roots of Pallas is higher than mutant. The higher activity is actually indicated by greater intensity of red colour zone near roots, which is darker in the black and white print.

using AFLP was 46.8%. The small number (0.8%) of polymorph bands between the mutant and Pallas supported that the mutant has genetic background in Pallas. The existing near-isogenic lines of Pallas (Kølster et al., 1986) were tested and they all produced normal root hairs, indicating a sponteneous mutation. When mutant and Pallas were crossed, the roots of 50 seedlings of F_1 cross were tested and all had root hairs. The segregation ratio of the selfed plants in F_2 generation was 1:3 (Figure 3), indicating a monogenic recessive mode of inheritance.

The *brb* mutant has provided opportunities as well as challenges for finding root hair genes of barley, which will be explored in future through interdisciplinary co-operations. The challenges lie in relative longer life cycle of barley, the large size and repetitive elements of barley genome and yet unknown locus of the mutation. The opportunities are provided as some progress has already been made in genetic analysis of root hairs using *Arabidopsis thaliana* as a model plant. Using root hair *defective* mutants of *Arabidopsis*, Schiefelbein and Somerville (1990) reported that root hair formation is controlled by four different proteins/genes (*RHD1, RHD2, RHD3* and *RHD4*), where *RHD1* seemed necessary for proper initiation of root hairs from trichoblasts; hence, the positive regulator of root hair density. The *RHD2, RHD3* and *RHD4* are related to normal hair elongation, hence may be important for determining root hair length. Indeed, the variation in root hair length and density (number of root hairs per unit root length) exists in many agronomic crop plants and their cultivars (Föhse et al., 1991; Gahoonia et al., 1999).

Other studies (Wada et al., 1997) report that CAPRICE gene (*CPC*) determines differentiation of root epidermis cells into root hairs. The transgenic *Arabidopsis* plants (*gl2 cpc*) over expressing *CPC* had more root hairs. Grierson et al. (1997) identified two recessive mutant alleles at *CAN OF WORMS1 (COW1)*, a new locus involved in root hair morphogenesis in *Arabidopsis*, which maps to chromosome 4.

The *brb* mutant, whose root hair development is completely blocked, should be useful for providing transition of the knowledge of genetic analysis of root hairs of model plant *Arabidopsis* into agronomic important crop plants. Wen and Schnable (1994) isolated three maize mutants (*rth1, rth2* and *rth3*) with abnormal root hair morphologies, which were controlled by single recessive alleles. The *rth1, rth2* and *rth3* genes were mapped to chromosomes 1L, 5L and 1S, respectively. However, in none of the maize mutants, root hair initiation was blocked *per se*.

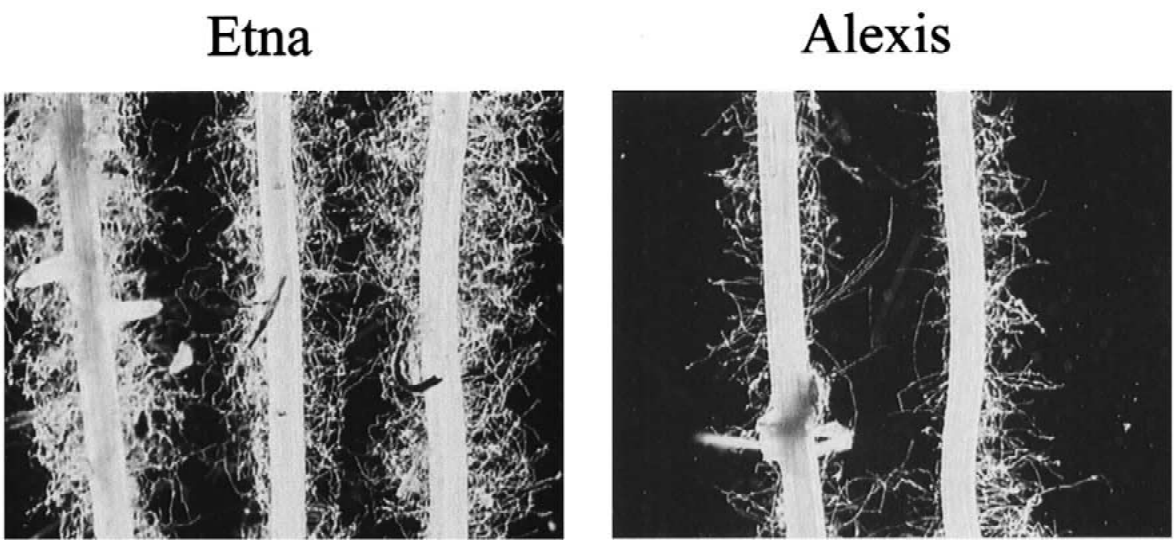

Figure 6. Root hairs on soil-grown roots of two barley cultivars Etna and Alexis. Etna had induced higher rhizosphere phosphatase activity and depleted more organic P in previous studies (Asmar et al., 1995).

Root hairs and phosphorus uptake

Phosphorus is found in soils as inorganic and organic P. Inorganic P in soil solution can be absorbed directly via root and root hairs, whereas organic P has to be converted into inorganic P before it can enter the root. We studied the role of root hairs in P uptake from soil using various approaches in the laboratory and in the field.

Laboratory experiments

The *brb* mutant provided the unique possibility of further confirming the importance of root hairs in uptake of P from rhizosphere soil. Pallas depleted nearly two times more P from inorganic (Figure 4A) as well as organic P fractions (Figure 4B) than the mutant. The uniform extension of inorganic P depletion profile of Pallas extended to 0.8 mm (Figure 4A), which was equal to its root hair length. The mutant did not show uniform extension of P depletion profile near its roots.

Pallas induced higher Apase activity near its roots as compared to that of mutant (Figure 5), suggesting a relationship between root hair formation and rhizosphere Apase activity. It has been argued for a number of years that phosphatase secreted from the roots can release P bound in organic matter (Bieleski, 1973). Apase activity is higher in root epidermis cells that eventually differentiate into root hairs (trichoblasts) than in other root cells (Avers, 1958; Dosier and Riopel, 1977). Interestingly, the higher phosphatase activity in differentiating and elongating cells does not seem restricted to root hair cells only. Joshi et al. (1985) using linted cultivar and lintless mutant of cotton showed that only linted cultivar had the enzyme activity in the differentiating cotton fibre cells in the outer integument of ovules.

The wide variation in root hairs of cereal varieties (Gahoonia et al., 1999) suggested that the varieties with greater number of root hairs may also have higher Apase activity in the rhizosphere and may mobilise more P from soil organic P fraction. To test this hypothesis, we studied the root hair formation of two barley cultivars Etna and Alexis. Etna produced more root hairs than Alexis (Figure 6). In the previous study (Asmar et al., 1995), Etna had indeed induced higher Apase activity in the rhizosphere and mobilised more organic P than Alexis. Soils often contain large amount of organic P, with values ranging from 20 to 80% of the total P (Dalal, 1977). Also the soil used in our rhizosphere studies contained twice as much organic P (60 mg kg^{-1}, Figure 4B) as compared to inorganic P (30 mg kg^{-1}, Figure 4A). Hence, it can be suggested that the selection and breeding of cultivars with longer and denser root hairs would be of dual advantage for increasing soil P use. First, the cultivars with longer root hairs will have a larger effective root surface area. Second, root-released Apase, able to catalyse hydrolysis of P esters, will liberate inorganic P in the root hair zone, where it would be immediately available for uptake by the expanded root surface. Consequently, due to the synchronised P lib-

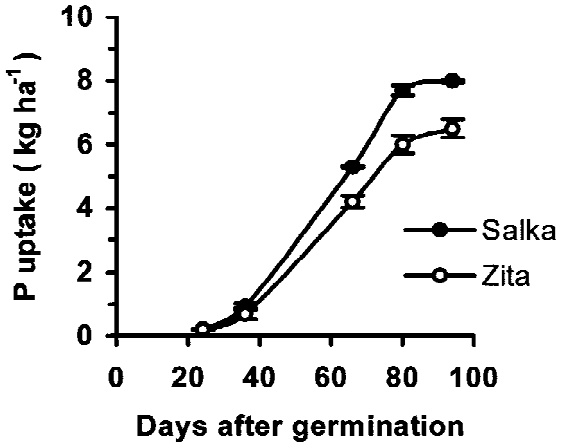

Figure 7. Phosphorus uptake of two spring barley cultivars, Salka and Zita, in the field. Root hair lengths ((Salka 1.10±0.16 mm; Zita 0.63±0.18 mm); Specific root lengths (Salka=163±9 m g^{-1}; Zita=153±11 m g^{-1}).

eration and the uptake processes, the benefit of the liberated P will be greater to plants with less proneness to runoff losses.

Field experiment

To assess the significance of root hairs for P uptake and plant performance under natural low-P conditions, the root hair formation and phosphorus uptake of the barley cultivars was studied under field conditions. We isolated the root hairs of field grown barley cultivars (e.g. Salka and Zita as shown in Figure 1) and compared them with root hairs measured under laboratory conditions. The variation in root hair formation of the cultivars was consistent under both conditions (Gahoonia et al., 1999). This indicated that field comparable ranking of cereal cultivars for root hairs could be performed by less laborious solution culture experiments with reasonable good accuracy.

The cultivars differed in P uptake in the field. For example, Salka with longer root hairs, which had depleted more P from rhizosphere soil (Gahoonia and Nielsen, 1997), also absorbed more P in the low-P plot (Figure 7). Salka also produced more shoot biomass than Zita in low-P plot (14 mg P kg^{-1} soil). When 10 and 20 kg P ha^{-1} was applied, the differences in P uptake and biomass production were reduced, because the higher P levels reduced the lengths of root hairs (Gahoonia et al., 1999). All field-grown roots had mycorrhiza, when stained with trypan blue, but it did not explain the variation in P uptake, as the cultivars did not differ in degree of mycorrhizal infection. These results show that root hairs are also important under field conditions, especially in low P soils.

Future perspectives

Both the laboratory and the field studies have confirmed the substantial role of root hairs in P uptake of cereals. This, together with the large variation in root hairs of the existing cereal cultivars, has provided a basis for increasing P efficiency of cereals, the most widely cultivated and most P fertiliser consuming food crops (Harris, 1998). The link between root hair formation and rhizosphere Apase activity suggests that root hairs have a role in mobilisation and uptake of P from inorganic as well as organic soil P, which deserves further research.

Because the P concentration in the soil solution is usually low, the membrane transport of P is mediated mainly by specific high-affinity transport proteins (Glass et al., 1992). High-affinity P transporter genes have been cloned and characterised from *Arabidopsis* and are found to be expressed mainly in the root epidermis cells and root hairs (Raghothama, 1999).

Genetic analysis of root hair formation has progressed using *Arabidopsis*. The *brb* mutant completely lacking root hair should prove useful for making transition of this knowledge to agronomic important crops such as cereals. The *brb* mutant will open new possibilities of testing root hair models of nutrient uptake (Claassen, 1990). The interactions between root hair formation and mycorrhizal colonisation have been reported (Schweiger et al., 1995). It would be informative to confirm the interactions with the use of *brb* mutant.

As outlined, the *brb* mutant would provide a number of possibilities for further progress to be made in the field of root hair research, through interdisciplinary co-operations. The use of emerging biotechnological techniques and molecular markers may facilitate cloning of barley root hair genes. This will add to the chances of making cereals more P efficient by selection and breeding for root hairs and thereby decreasing the need for P fertilisers.

Acknowledgements

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