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**The Proceedings of the International Plant Nutrition
Colloquium XVI**

Title

The Expression of P-Responsive Genes is Related to Root Hair Growth

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Publication Date

2009-04-15

Peer reviewed

Introduction

Phosphorus is an essential macronutrient for plant growth and development. However, it is one of the least available nutrients in the soil because of the binding to the soil matrix. Plants developed morphological, physiological adaptations to overcome limited phosphorus supply to enhance the acquisition of phosphorus in the soil. A morphological response to P starvation is the formation of longer root hairs which has been well studied, but the molecular mechanisms that sense and trigger the P signal remains largely unknown. The possibility of surveying a whole genome expression of an organism with microarray technologies identified a number of involved genes in the P response (Hammond et al. 2003; Wu et al. 2003; Misson et al. 2005; Morcuende et al. 2007, Müller et al. 2007). However, the gene chip technology has some limitations regarding non-sequenced organisms and least abundant gene transcripts. In this case, suppression subtractive hybridization (SSH) is an efficient PCR-based approach to identify differentially expressed genes and to isolate novel genes in plants. In the present study, an attempt was made to identify phosphorus responsive genes that are up- and downregulated in Ethiopian mustard (*Brassica carinata*) resulting in enhanced root hair length elicited by the lack of phosphorus.

Materials and Methods

Ethiopian mustard (*Brassica carinata*) seedlings were cultivated in nutrient solution with complete nutrition or without phosphorus, nitrogen or potassium for 5 days, respectively. Lateral root tips were harvested for RNA extraction. For the transfer experiment, plants grown 5 days in nutrient solution (complete or free of P) were transferred to the contrary P-supply (-P > +P; +P > -P) or remained in the original nutrient solution as control. Root tips were harvested after 0.5, 1, 1.5, 2, 3, 6, and 24h in transfer conditions or after 0 and 24h in control conditions. Additionally, sections of lateral root tips (0-1, 1-2, 2-3 cm, Fig.1) grown in complete or free of P nutrient solution were harvested for RNA isolation. cDNA synthesis of 1000 ng total RNA was carried out. Gene expression was investigated by semi-quantitative RT-PCR with gene-specific primers based on the sequenced SSH ESTs. PCR with actin primers served for normalization.

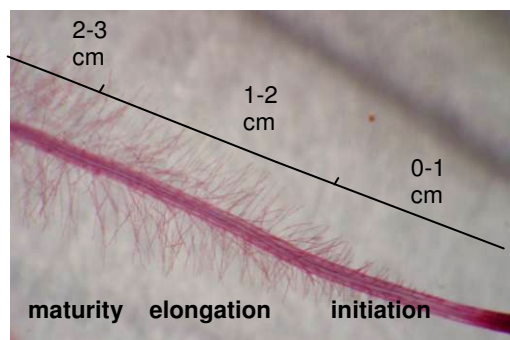


Fig 1. Sections of 1 cm length were harvested to characterize the expression pattern along the root hair differentiation stages.

Results and Discussion

Root hair length in *Brassica carinata* was considerably increased with phosphate and nitrogen depletion but not with potassium deprivation. As there were similar phenotypic changes, the responses to P and N deprivation may share some common features. Accordingly, the expression of LRR and PRP was induced in response to P and N starvation, but not along with potassium deprivation. The transcription level of the putative transcription factor 14-3-3 was enhanced only weakly in P- starved roots compared to control and -N conditions. This

suggested a function of the LRR receptor-like protein kinase and the proline rich protein in root hair growth induced by P- and N-deficiency whereas 14-3-3 presumably does not play a direct role. However, 14-3-3 may be part of an unspecific early stress response. To characterize the gene expression pattern along the root hair differentiation stages, 1cm sections were harvested for PCR analysis representing initiation, elongation and maturity zone of root hairs (from tip to the root base, Fig.1). The level of LRR, PRP and 14-3-3 gene transcript abundance decreased in P-starved roots from the tip to the mature zones of the root. Under P-sufficient conditions the expression showed the same trend but was weaker compared to -P (Fig.2). The results suggest for all investigated genes a role in the initiation and elongation process of root hairs since transcription was most pronounced in the 0-2cm sections of the root tip.

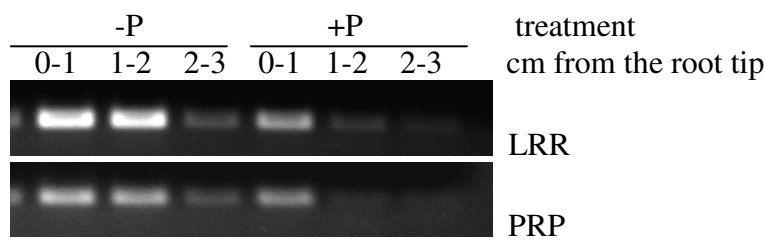


Fig 2. Expression profile of leucine rich repeat receptor like protein kinase (LRR) and proline rich protein (PRP) along the root of *Brassica carinata* cv. Bale seedlings grown 5 days in -P and +P nutrient solution.

The velocity of changing gene expression in response to altered environmental conditions may predict the position in the signalling pathway that leads to phenotypic adaptations. Discontinuing P-supply resulted already in longer root hairs within one day. Accordingly, resupply of P decreased root hair length (Fig.3). The expression of the LRR receptor like protein kinase was induced 24 hours after interruption of P nutrition and was reduced within 2 hours after resupply of P. The proline-rich protein responded similarly, but more delayed compared to the LRR protein kinase (Fig.4). However, the activity of 14-3-3 reacted in both transfer experiments with an upregulation after 6 hours in the -P > +P situation and a weak induction after 24 hours in the +P > -P treatment. However, in this treatment a short term repression between 3 and 6 hours was observed.

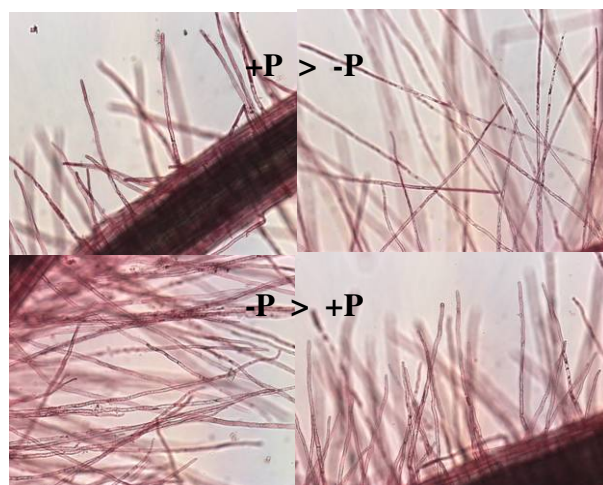


Fig. 3 Root hair phenotype of *Brassica carinata* cv. Bale, before and one day after changing nutrient solution.

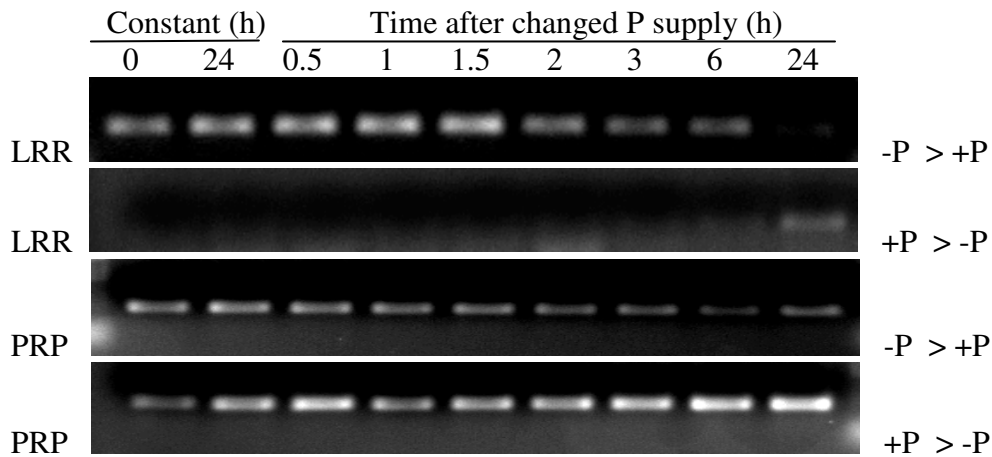


Fig 4. Semi-quantitative PCR of the response of LRR and PRP transcript level in roots of *B. carinata* cv. Bale after changing P-supply (-P > +P, +P > -P).

In summary, the expression pattern of the leucine rich repeat receptor like kinase and the proline rich protein suggests a function in root hair growth that is common in -P and -N conditions. The more rapid induction of the LRR compared to PRP suggests an involvement in the early signal transduction pathway, whereas the PRP may be a part of more downstream steps leading to increased root hair length. For the putative transcription factor 14-3-3 no clear indication was obtained.

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