

Molecular characterization of the effects of phosphorus on the development of root hairs in *Arabidopsis*

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Abstract

Root hair development is influenced by nutrient availability. In particular, it has been described that nitrogen and phosphorus concentrations influence secondary root formation and root hair development. In *Arabidopsis* it has been reported that low P concentrations stimulate root hair formation and/or elongation. To determine at which level P interferes with root hair development, we have analyzed the effect of different concentrations of P in different *Arabidopsis* genetic backgrounds. Using root hair defective *Arabidopsis* mutants, we determined that P interferes with root hair elongation but not with root hair formation. We also found that P is able to suppress root hair elongation in mutants that form ectopic root hairs and that auxins and the ethylene precursor ACC are not able to suppress the root hair elongation inhibitory effect of high P concentrations.

Introduction

Plants incorporate environmental information into many developmental decisions. This includes information about the availability of nutrients and water. Root hairs are an ideal system for studying the environmental regulation of root development because it is easy to watch them develop, and a large number of genes that control and take part in root hair development have been identified. Phosphate is a nutrient that has a strong effect on root development. Bates and Lynch have reported that the development of root hairs responds strongly to changes in local phosphate concentration (Bates, 1996) and that this increases the efficiency of phosphorous uptake (Bates, 2000). It is not clear which stages of root hair development are affected by phosphate, or whether any of the many genes already known to control hair formation are important for the phosphate response. This information would help us to understand how plants use environmental cues during development. We performed an extensive survey of the effects of a range of phosphate concentrations on the root hair phenotypes of wild type plants and of plants with mutations in a series of genes affecting different stages of root hair development.

Materials and methods

Arabidopsis wild type and different mutants affected in root hair development were grown in media containing concentrations of PO₄ ranging from 1 μM to 5 mM and 1x and 0.1x micronutrients. Root hair development was determined using a video stereomicroscope. Root exudates were prepared by centrifuging solid media in which wild type *Arabidopsis* seedling were grown, followed by extraction with ethyl acetate.

Results

When wild type *Arabidopsis* seedlings were grown at different concentrations of PO₄, it was found that root hair elongation is stimulated at low PO₄ concentrations and totally inhibited at high concentrations. It was also observed that the effect of PO₄ on root hair development changes depending upon the concentration of micronutrients. When the root of seedlings were examined at high magnification, it was found that root hair elongation is inhibited by high PO₄ concentrations, but root hair formation, at the bulge stage, occurs even at 10mM PO₄. To determine whether auxins or ethylene are involved in the effect of PO₄ on root hair development, *Arabidopsis* mutants affected in the perception/signal transduction pathway of these plant hormones, such as *axr1*, *axr2*, *axr4*, *ctr1*, *ein2* and *etr1*, were grown at different PO₄ concentrations. It was found root hair elongation was inhibited in all these mutants. Moreover, treatments with auxins and the ethylene precursor ACC were not able to revert the inhibitory effect of high PO₄ concentrations.

The effect of PO₄ was also examined in *Arabidopsis* mutants (*gl2*, *wer* and *ttg*) affected in genes encoding proteins that prevent root hair development, and as a consequence produce ectopic root hairs. It was found that high PO concentrations inhibit root hair elongation in all these mutants. Accidentally it was observed that when seedling are germinated very closed together, undergo root hair elongation even at high PO₄ concentrations. To determine whether a compound excreted by the root is able of reverting the inhibitory effect of PO₄, we tested the effect of root exudates on seedling grown at high PO₄ concentrations. It was found that indeed root exudates contain a component able to promote root hair elongation.

Discussion

Our results show that the availability of PO_4 modulates root hair elongation but does not affect the initial steps of root hair formation. This effect does not appear to be mediated by changes in auxin or ethylene sensitivity. Root hair elongation seems to be affected not by the absolute concentration of PO_4 in the media but rather by the ratio of

this macronutrient and the concentration of micronutrients. The finding that root exudates are able to overcome the inhibition of root hair elongation not auxins or ethylene, suggest that an additional plant hormone plays an important role in this process.

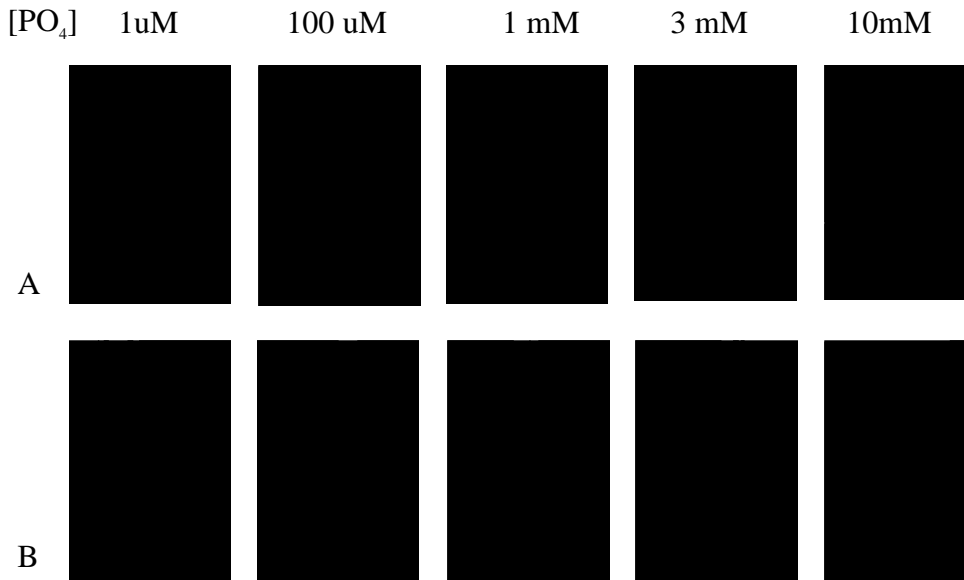


Figure 1. Effect of PO_4 on root hair development. Plants were grown in 0.1X (A) or 1X micronutrients (B)

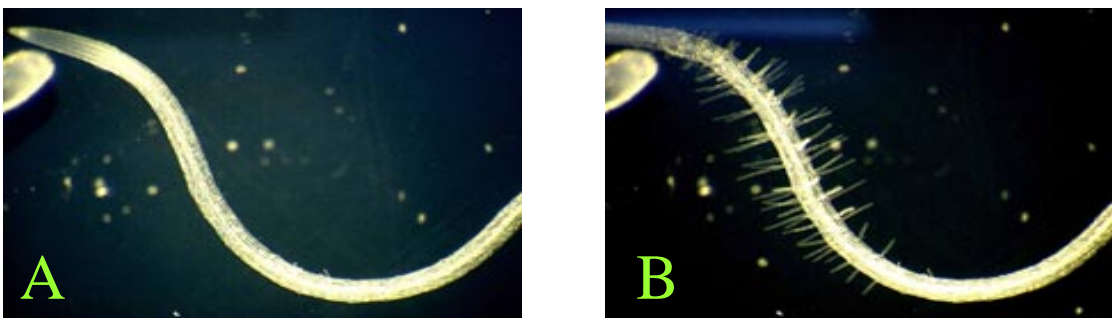


Figure 2. Effect of root exudates on root hair elongation. Roots of seedlings grown in 10 mM PO_4 were treated with a 100 μl drop of methanol (A) or concentrated root exudate dissolved in methanol (B)