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Arsenic-induced morphogenic response in roots of arsenic hyperaccumulator fern *Pteris vittata*

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HIGHLIGHTS

- We studied arsenic hyperaccumulation in *Pteris vittata* starting from the root system.
- As elicited modifications in root hair number, length and differentiation pattern.
- A modulation in nucleolar activity was detected following As treatments.
- As influenced the production of border-like cells, shown for the first time in ferns.
- As-induced morphogenic responses improved the absorbance features of root.

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ABSTRACT

On the assumption that arsenic induces stress morphogenetic responses involved in As tolerance and hyperaccumulation in the *Pteris vittata* fern, we analyzed the root system of young sporophytes grown in 250, 334, and 500 μM As for five days and for 14 days. Anatomical and histological analyses were performed in plants grown for five days to evaluate the number, position, length and differentiation pattern of root hairs. AgNOR staining, employed to study nucleolus behavior in root apices, showed that arsenic influences nucleolar activity (evaluated by nucleolus size, number and absorbance) in the root meristem. In plants treated with 250 and 334 μM As an acropetal shift of root hair development and an increase in hair length and density were observed, linked to an ectopic pattern of differentiation. The opposite trend was recorded in plants treated with 500 μM As. It is worth noting the presence of living border-like cells, not yet observed in ferns, and their increase following As treatments. Analysis and vitality of border-like cells were surveyed after 14 days of treatments. In conclusion As treatments elicited a stress-induced morphogenic response which, by modifying the differentiation pattern, number and length of root hairs, modulating nucleolar activity and interacting with the rhizosphere by inducing border-like cell production, may adjust the rate of root uptake and its metabolic activity.

1. Introduction

Developmental plasticity is essential for stress adaptation in plants, as sessile organisms have to cope with the dynamics of transiently changing environmental conditions during their lifetime [1]. A key role in this plasticity is played by a range of protective responses, many of which are morphogenic changes which have been observed in many plants exposed to suboptimal environmental conditions, following exposure to many different stressors (e.g. heavy metals) [2]. Despite the diversity of stress-induced phenotypes, the existence of a generic "stress-induced morphogenic response" (SIMR) can be recognized [3] in the roots of many seed vascular plants following exposure to different chemical, physical and biological stresses [4]. To our knowledge there is no consistent evidence of SIMR in ferns, but the ability of As-hyperaccumulation of the *Pteris vittata* fern is well known [5]. SIMR, decreasing stress exposure, limiting damage or facilitating repair of damaged systems, can be linked to the mechanism of tolerance which, in turn, is necessarily coupled to hyperaccumulation [6,7]. The complexity of hyperaccumulation is far from fully understood not only at cell level, but also at tissue level [7]: understanding how plants take up and metabolize highly toxic and ubiquitous elements such as arsenic, would be very important for developing mitigation measures to counter the problem of food-chain contamination by toxic pollutants. As-hyperaccumulation of *P. vittata* is a constitutive trait of plants

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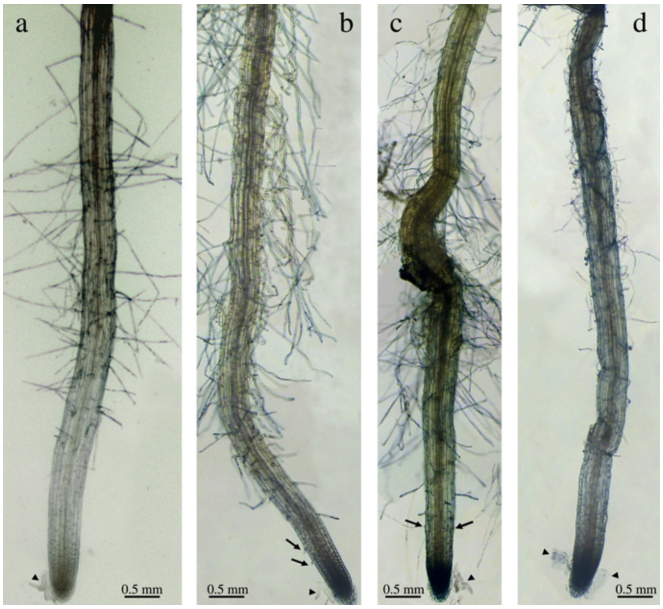

Fig. 1. Effects of different As concentrations on *P. vittata* root hair density. Hair density increases in roots from plants exposed for five days to As concentration of 250 and 334 μM (b and c respectively) if compared to control roots (a), and decreases significantly under higher As concentration (500 μM, d). Moreover ectopic hairs emerge closer to the root apex (arrows) in samples b and c. Arrowheads indicate border-like cells.

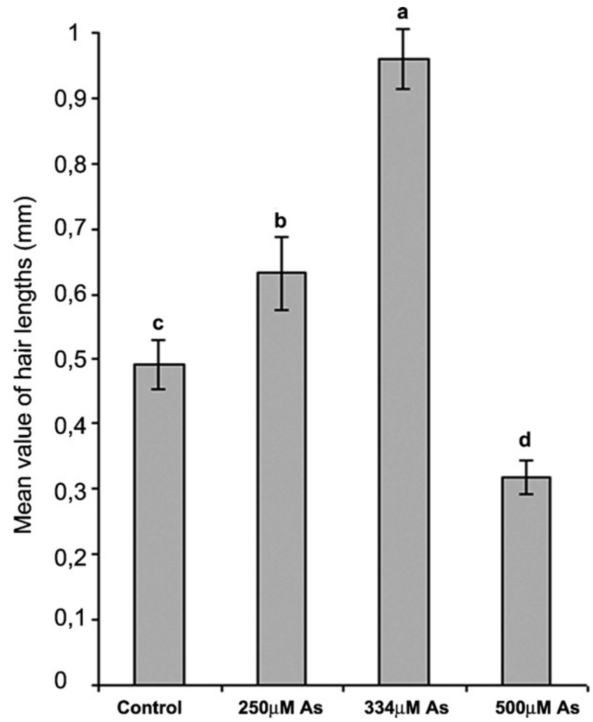

Fig. 3. Mean values of root hair length in *P. vittata* control plants and 250, 334 and 500 μM As-treated plants. Bars represent standard errors. Different letters indicate significant differences by Bonferroni's multiple comparison test ($p < 0.01$).

originating from As-contaminated and As non-contaminated environments showing broadly similar hyperaccumulation abilities [8–10].

We have hypothesized the presence of SIMR in this fern and its contribution to the ability of As hyperaccumulation. To this purpose we analyzed root SIMR at cellular and histological level in plants of *P. vittata*, exposed to different As concentrations. In particular, the aim of this study was:

1) to contribute to the knowledge of the morpho-anatomical traits underlying the hyperaccumulator phenotype, as As-hyperaccumulation is a constitutive trait of this species;
2) to supply an experimental tool to know quite speculatively the highest As concentration suitable for the utilization of *P. vittata* in phytoremediation strategies, as SIMR is an active response which is impeded under severe stress conditions [3];
3) to study nucleolus behavior in root apices in relation to different As concentrations, considering the role of the nucleolus in sensing cellular stress signals;
4) to assess the possible occurrence of border cells (BC), which have not previously been observed in ferns but which are known to be involved in protecting the root system against biotic and abiotic stresses in many angiosperms.

2. Materials and methods

2.1. Plant material

Plants of *Pteris vittata* L., kindly provided by the Dept. of Plant Biology Turin University, were grown as previously described [11] from spores collected in an industrial area of Genoa (Italy). Healthy and uniform *P. vittata* plants with three–five fronds were selected for the experiments.

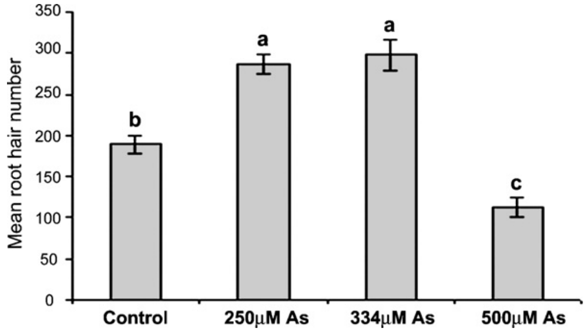

Fig. 2. Mean root hair number in *P. vittata* control plants and 250, 334 and 500 μM As-treated plants evaluated in the first cm from the apex. Bars represent standard errors. Different letters indicate significant differences by Bonferroni's multiple comparison test ($p < 0.01$).

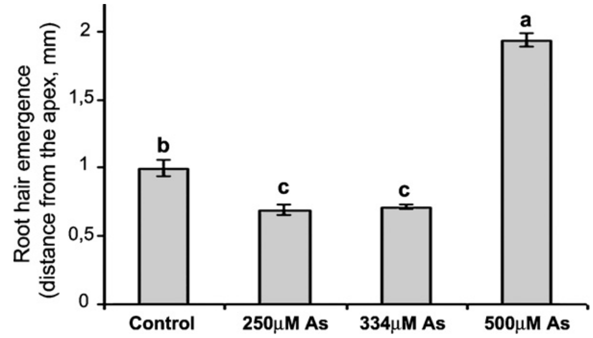

Fig. 4. Hair emergence distances from the root apices in *P. vittata* control plants and in 250, 334 and 500 μM As-treated plants. Bars represent standard errors. Different letters indicate significant differences by Bonferroni's multiple comparison test ($p < 0.01$).