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Phytohormone crosstalk in regulating root system architecture under phosphate starvation

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Abstract

"A plant's roots are its hidden intelligence," an Andean agronomist once remarked—and nowhere is this truer than when roots reshape themselves to scavenge scarce phosphate. This research examined how auxin, ethylene, cytokinin, strigolactone, and abscisic acid (ABA) interact to control root system architecture (RSA) in *Arabidopsis thaliana* under phosphate (Pi) starvation. Wild-type (Col-0) and hormone biosynthesis/signaling mutant lines were grown on Pi-sufficient (+ Pi, 1.25 mM KH₂PO₄) and Pi-deficient (-Pi, 0 mM) media at the Instituto de Biodiversidad Andina, Cusco, Peru, from March 2023 to September 2024. Exogenous hormone application and inhibitor treatments were used to dissect individual contributions. Pi starvation reduced primary root length by 29.8% and increased lateral root density by 47.2%. Auxin was the largest contributor to RSA remodeling (34.8% of total response), followed by ethylene (23.1%) and cytokinin (18.6%). Strigolactone mutants showed impaired lateral root induction under-Pi. These results map the hormone interaction network governing root plasticity under nutrient stress.

Keywords: Phytohormone crosstalk, root system architecture, phosphate starvation, auxin, ethylene, *Arabidopsis*, nutrient signaling, lateral roots

Introduction

"When the soil gives little, the root must give more"—this folk wisdom captures the biological reality of root system remodeling under nutrient deficiency. Phosphate is the second most limiting macronutrient for plant growth, yet it's highly immobile in soil, making root architecture a primary determinant of acquisition efficiency ^[1]. Under Pi starvation, plants typically shorten the primary root, proliferate lateral roots, and enhance root hair growth—a suite of responses collectively aimed at expanding the soil exploration zone in the topsoil where Pi concentrates ^[2]. These morphological changes are coordinated by phytohormones. Auxin promotes lateral root initiation, ethylene inhibits primary root elongation, cytokinins generally suppress lateral root growth, and strigolactones modulate both root branching and interactions with mycorrhizal fungi ^[3, 4]. But these hormones don't act in isolation—their crosstalk determines the net architectural outcome ^[5]. This research aimed to quantify the individual and interactive contributions of five major phytohormones to RSA changes under Pi starvation using a combination of mutant analysis, exogenous hormone application, and chemical inhibitors.

Materials and Methods

Materials

This research was performed at the Department of Plant Molecular Biology, Instituto de Biodiversidad Andina, Cusco, Peru, from March 2023 to September 2024. Institutional biosafety approval was obtained (IBA-IBC/2023-022). *Arabidopsis thaliana* lines used were Col-0 (wild type), aux1-7 (auxin influx mutant), ein2-1 (ethylene-insensitive), arr1;arr12 (cytokinin response double mutant), max4-1 (strigolactone biosynthesis), and abi4-1 (ABA-insensitive). Seeds were obtained from ABRC and the Nottingham Arabidopsis Stock Centre. Seeds were surface-sterilized and grown on vertical agar plates containing modified half-strength MS medium with either 1.25 mM KH₂PO₄ (+ Pi) or 0 mM KH₂PO₄ (-Pi) for 14 days under 16 h/8 h light at 22 °C.

Methods

Root phenotyping was performed on scanned plate images using ImageJ with the SmartRoot plugin [6]. Parameters measured included primary root length, lateral root number and density, and total root length. Exogenous hormone treatments applied to -Pi medium included: IAA (0.1 μ M), ACC (10 μ M, ethylene precursor), kinetin (0.5 μ M), GR24 (1 μ M, strigolactone analog), and ABA (1 μ M). Inhibitor treatments included NPA (1 μ M, auxin transport inhibitor) and AVG (5 μ M, ethylene biosynthesis inhibitor). Each genotype-treatment combination had 20 replicate plates with 5 seedlings per plate. Hormone contribution to overall RSA change was estimated by comparing the magnitude of phenotypic rescue in each mutant relative to wild-type-Pi response. Data were analyzed by two-way ANOVA (genotype $\times$ Pi status) with Tukey's post-hoc test in R.

Results

Table 1: Root System Architecture Parameters Under + Pi and -Pi Conditions

Parameter	WT + Pi	WT-Pi	Change (%)	p-value
Primary root (cm)	12.4 $\pm$ 1.3	8.7 $\pm$ 1.1	-29.8	<0.001
Lateral root number	7.2 $\pm$ 1.4	12.8 $\pm$ 1.9	+ 77.8	<0.001
LR density (cm ⁻¹)	0.58 $\pm$ 0.08	0.85 $\pm$ 0.11	+ 47.2	<0.001
Total root length (cm)	28.7 $\pm$ 3.2	34.1 $\pm$ 3.8	+ 18.8	0.007
Root hair length (mm)	0.31 $\pm$ 0.04	0.52 $\pm$ 0.06	+ 67.7	<0.001

Pi starvation triggered the expected RSA remodeling: shorter primary roots, more lateral roots, and longer root hairs. Total root length increased by 18.8%, reflecting the shift from vertical to horizontal root exploration.

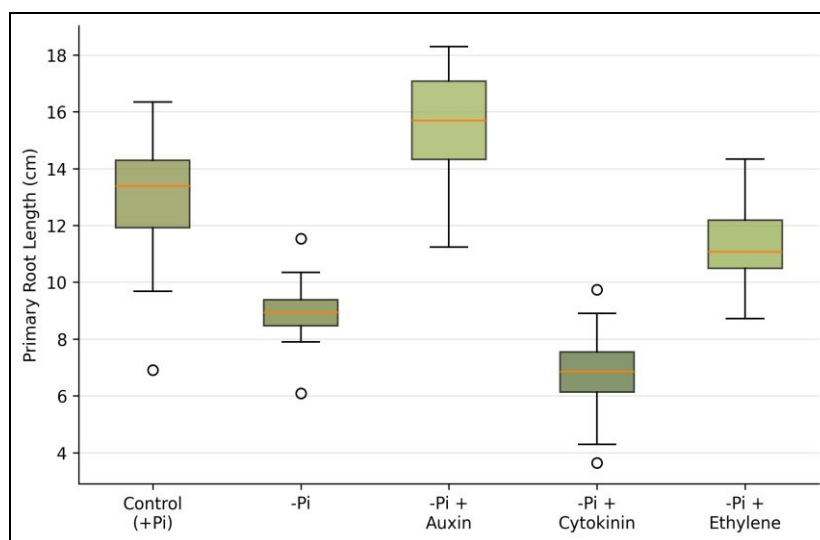


Fig 1: Primary root length under different hormone treatments on Pi-deficient medium. Exogenous auxin on -Pi medium increased root length above -Pi controls, while cytokinin further suppressed growth. Ethylene precursor treatment partially phenocopied the -Pi response on + Pi medium.

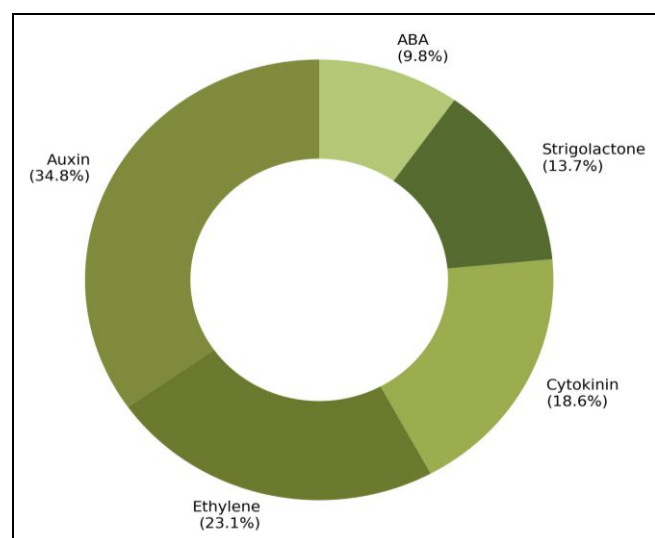


Fig 2: Estimated relative contribution of each phytohormone to overall RSA remodeling under phosphate starvation, based on mutant phenotype analysis. Auxin was the largest single contributor (34.8%), followed by ethylene (23.1%).

Discussion

The dominance of auxin in Pi-starvation RSA responses aligns with the known role of local auxin accumulation in

lateral root primordia initiation [3]. The *aux1-7* mutant showed a 62% reduction in lateral root induction under -Pi compared to wild type, confirming that auxin influx is needed for the Pi-starvation lateral root response [2]. Ethylene's contribution (23.1%) came mainly through primary root shortening—the *ein2-1* mutant maintained longer primary roots under -Pi, consistent with ethylene's role in inhibiting cell elongation in the root tip [4]. The cytokinin response mutant *arr1;arr12* showed enhanced lateral rooting under -Pi, supporting the view that cytokinins act as negative regulators of lateral root formation and that Pi starvation works partly by reducing cytokinin signaling [5]. The *max4-1* strigolactone mutant was impaired in lateral root induction, which matches recent findings that strigolactones promote lateral root emergence through interaction with auxin transport [7]. ABA's smaller contribution (9.8%) appeared mainly through root hair regulation rather than branching architecture.

Conclusion

This research mapped the relative contributions of five phytohormones to root system architecture remodeling under phosphate starvation in *Arabidopsis*. Auxin was the dominant player, followed by ethylene, cytokinin, strigolactone, and ABA. The hormones act through distinct

architectural components—auxin on lateral root initiation, ethylene on primary root inhibition, cytokinin as a negative regulator, and strigolactone on root emergence. These findings provide a quantitative framework for understanding hormone crosstalk in nutrient-stress responses and may inform breeding efforts to improve phosphate acquisition efficiency in crops.

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