

Transnational Access Report

1. General Information

Project Acronym (ID):	PINs and gravitropism 2
Project Title	A root gravitropic response requireskinase-mediated activation and/or retargeting of PIN auxin efflux carriers in gravity sensing columella cells
Installation used	Vertical Confocal
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2. Project summary (max. 250 words)

The auxin efflux carriers PIN3 (and PIN7) have been shown to be required for the initial differential auxin gradient. Other proposed components of the gravity-sensing machinery in the root include amyloplasts present in columella cells thought to mechanically sense changes in gravity orientation that might trigger Ca^{2+} and/or pH mediated signaling pathways. Recently, several classes of kinases have been shown to be involved in polar targeting and activation of plasma membrane (PM) associated PIN proteins. We seek to draw a possible link between lateral amyloplast sedimentation, changes in proton and Ca^{2+} currents across the PM and PIN3 polarization/activation following changes in the gravity vector. This is based on the knowledge that kinases present in the same tissue, such as D6PK, which have been shown to activate PINs at the PM, are trafficking between the PM and internal compartments with very fast kinetics. The Vertical Confocal microscope could be instrumental in studying the dynamics of D6PK trafficking in columella cells during the gravitropic response.

3. Description of work(max. 250 words)

In order to investigate whether dynamically trafficking kinases in columella cells that respond to gravity by changing their subcellular localization are directly involved in the differential polarization/activation of PIN proteins, we first needed to analyze whether these kinases are able to phosphorylate PIN proteins in the relevant tissues. For D6PK, we had previously identified targeted serines in PIN3. Using a phospho-site specific antibody for one of these targeted serines in whole-mount immunlocalizations, we sought to visualize this phosphorylation event following a gravity stimulus. The majority of the stay was used to optimize the protocol for these immunlocalizations. As analysis of this kind does not strictly require the use of a Vertical Confocal microscope, a standard confocal microscope was used instead.

4. Main achievements (max. 250 words)

Preliminary results indicate that phosphorylation of PIN3 in columella cells does indeed occur at the site previously identified to be important for PIN3 activation. We further seek to investigate whether D6PK (and related kinases) respond to a gravity stimulus and/or changes in proton and Ca^{2+} currents by changing their subcellular localization. For this analysis, the Vertical Confocal microscope will be essential. Furthermore, a correlation between changes in PIN3 phosphorylation and the sub-cellular localization of the kinase during the response to gravity would be strong evidence that these events play an important role during gravitropism of the root