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Effects of Arabidopsis thaliana Villin-4 mutations on root hair physiology, morphology and function

Maya Klem
Western Washington University

Emily Bossard
Western Washington University

Effects of *Arabidopsis thaliana* Villin-4 Mutations on Root Hair Physiology, Morphology, and Function

Maya Klem, Emily Bossard, Morgan Gingrich, Juan Garcia, Morgan Olsen, Jeff Young, Sergey Smirnov
Western Washington University, Department of Chemistry and Department of Biology

Abstract

The protein VILLIN (VLN) is found in both plants and animals across many taxa. In plants, one of the roles of VLN is to aid in root hair formation and function. They do this by assisting in maintenance of actin bundles in root hairs. It has been found that *Arabidopsis thaliana* with mutated *VLN4* have compromised actin bundles and in certain cases shorter or dysfunctional root hairs. This study examines the affect that mutations in VLN4 has on parts of plant physiology. By examining the changes in responses of *A. thaliana vln4* mutants to various external abiotic conditions, this study aims to uncover novel phenotypes. Specifically, this study looks at how VLN mutants affect the plant's ability to detect gravity in the presence or absence of salt, in addition to how *vln* mutants affect root hair growth and morphology in a sucrose environment. Although this study is ongoing, a potential phenotype has been identified. As of yet, the data suggests that sucrose and cold conditions have no apparent affect on root hair growth or morphology. However, the data is starting to suggest that the presence of 100 mM NaCl does affect *A. thaliana* gravitropism.

What are Villins and Why Study Them?

- Villins are a protein responsible for the dynamic nature of the cytoskeleton and its regulation in vertebrates and plants
- Villins are most likely an ancient protein evolved to regulate the cytoskeleton
- Research in the gelsolin/villin family began in the 1980s and has increased our understanding of actin's role in many cytoskeletal functions across many taxa
- Villins are integral in actin filament bundling and severing

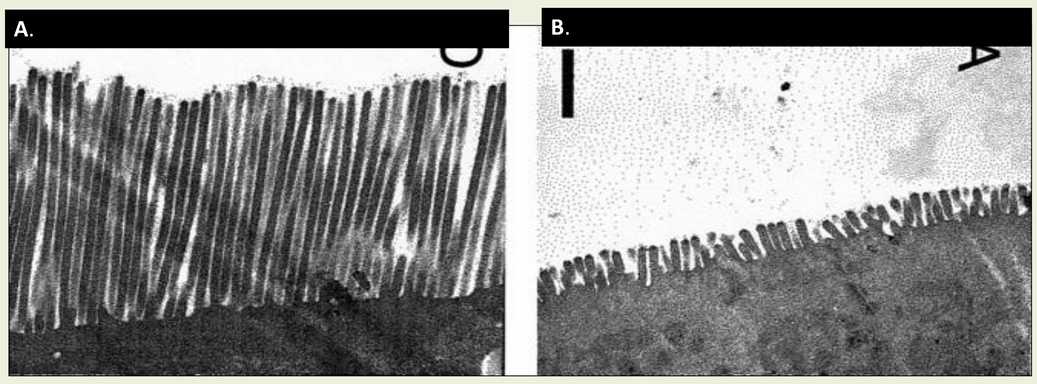

Figure 1. Python microvilli under fed versus starved states a. Microvilli are longer and show an ordered morphology under feeding conditions b. Microvilli are shortened and show a disordered morphology under fasting conditions (Secor et al., 2000.)

- While vertebrates only have one isoform of VLN, plants have 5 isoforms used in varying systems involving actin filaments
  - VLN4 plays a role in root hair morphology and F-actin bundling in plants (Du et al., 2011)
- Knowledge of the function of these proteins will aid in engineering of crops and increase our understanding of water and nutrient uptake and plant adaptation to varying environments

Effect of Sucrose on *A. thalania* VLN mutations

Methods and Results:

Seeds were vernalized, surface sterilized, and then planted on 3% sucrose, 1% Agar MS media. After four days roots were photographed and morphology was observed.

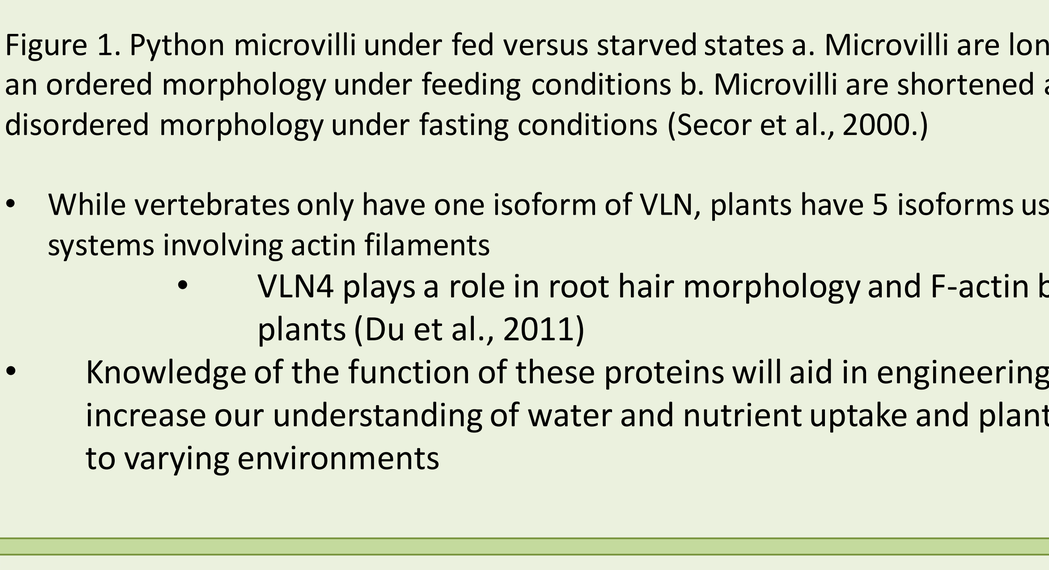

Figure 2. *vln* 4-4 and *VLN* WT root hairs after four days of growth on 3% Sucrose MS media

No differences were observed in root hair morphology between wild-type and mutants on 3% sucrose

Acknowledgements

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Effect of Salt on *A. thaliana* villin mutations

Methods and Results:

Seeds were vernalized, surface sterilized, and then planted on either 100 mM NaCl, 1% Agar MS media (Salt condition) or 1% Agar MS media (MS condition.) Plants were allowed to grow one week and then turned exactly 90°, and allowed to grow for an additional week. After the two week growth period plates were photographed and measurements 1-5 and angle of curvature were recorded using ImageJ (Fig 3,4.)

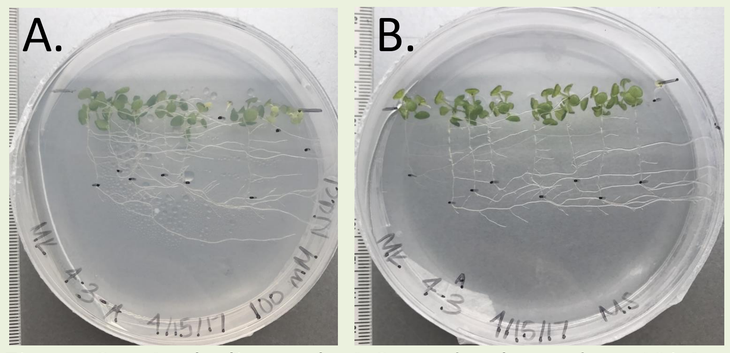

Figure 3a. *A. thaliana vln* 4-3 seeds planted on 1% Agar MS Media. 3b. *A. thaliana vln* 4-3 seeds planted on 100 mM NaCl, 1% Agar MS Media. In both images the black tics represent root growth just prior to rotation.

Measurement 1: length of root until plate was turned after one week of growth (purple length)

Measurement 2: length of root after plate was turned until root began to turn (blue length)

Measurement 3: length of root after it began to turn until it plateaued (magenta length)

Measurement 4: length of root after it plateaued until photographed (red length)

Measurement 5: Vertical length of root plate was turned until it plateaued (bright blue length)

Figure 4. Representation of measurements taken on each of the *A. thaliana* plants grown. Additionally, the angle of curvature was recorded. Black line represents root length when plate was turned.

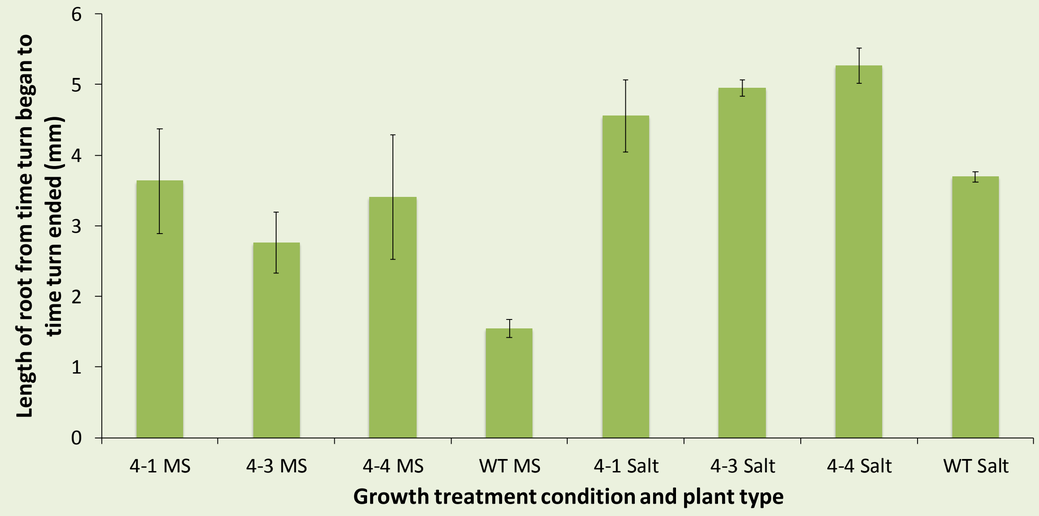

Figure 5. Measurement 3 comparison of wild-type and mutants grown MS media with or without 100 mM NaCl. Error bars represent standard error.

Table 1. Results from paired t-test comparing measurement 3 in *vln* mutants versus the wild-type for the same condition. All p-values were statistically significant.

vln and condition	4-1 MS	4-3 MS	4-4 MS	4-1 NaCl	4-3 NaCl	4-4 NaCl
P-value	0.0023	0.0119	0.0069	0.0434	0.001	0.001

No significant differences were observed between wild-type and mutants for measurements 1,2,4,5 or angle of curvature (data not shown.)

Conclusions

Previous literature indicated that salt concentrations have an effect on VLN4 function (Zhao et al., 2013.) No significant differences were found for measurements 1,2,4,5 or angle of curvature for *vln* mutants when compared to the wild-type grown on media with or without salt. However, a significant difference was found for measurement 3 between *vln* mutants compared to the wild-type when grown on media with and without salt (Fig 5, Table 1.) Although preliminary, this suggests that mutations in *VLN4* do not seem to affect overall growth (measurement 1) or ability to respond to gravity (measurement 2/ angle), but that it potentially affects how long it takes the plants to respond to gravity (measurement 3.)

Although other literature indicates potential phenotypic differences in root hairs when grown on 3% sucrose, none were observed (Fig 2.)

When combined, these data suggest that *VLN4* functions in directional growth responses in elongating root cells rather than affecting root hairs.

Literature Cited

Du F, Zhang Y, Ren H (2011) The universal bundling activity of AtVLN4 in diffusely growing cells. Plant Signal Behav 6: 1290-1293.

Secor, S. M., Whang, E. E., Lane, J. S., Ashley, S. W., & Diamond, J. (2000). Luminal and systemic signals trigger intestinal adaptation in the juvenile python. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *279*(6), G1177-G1187.

Zhao Y, Pan Z, Zhang Y, Qu X, Zhang Y, Yang Y, et al. (2013) The actin-related Protein2/3 complex regulates mitochondrial-associated calcium signaling during salt stress in Arabidopsis. Plant Cell 25: 4544-4559.