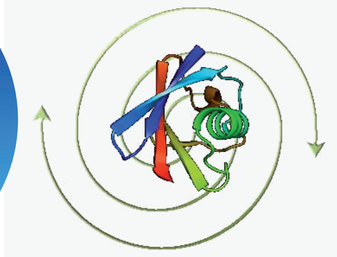
Sachsen-Anhalt
Kultusministerium

Research Cluster G

Analysis of Regulatory Networks Using Natural Variation in *Arabidopsis thaliana*

Quantitative dissection of phytohormone response pathways



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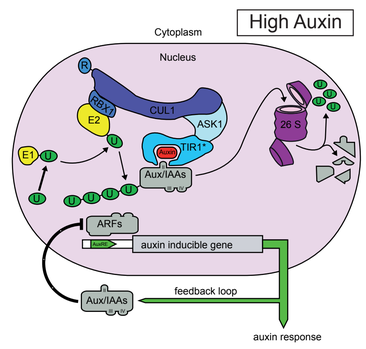
Introduction

Plant hormones are primary regulators of plant growth and development. We have previously reported naturally occuring genetic variation for auxin response phenotypes among *A. thaliana* accessions (Delker et al., 2008, *Planta* 227:929-941). Within the Research Cluster G we aim to assess and analyze natural variation for three additional phytohormones: jasmonic acid (JA), cytokinin, and ethylene. We have selected these hormones because their signaling pathways are well known for the Col-0 reference accession. While auxin and JA are both similarly regulated via proteasome-mediated degradation of transcriptional repressors, cytokinin and ethylene utilize two component histidine kinase signaling pathways. The objectives of our project are to (i) identify natural variation among 20 genetically maximally diverse accessions, (ii) examine the major signaling components of these pathways in a molecular population genetic manner, and (iii) identify quantitative trait loci (QTLs) responsible for the inheritance of genetic variation for hormone responses in populations of recombinant inbred lines (RILs).



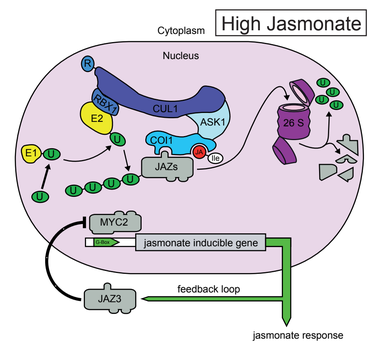


Figure 1: SCF-complex mediated signaling pathways.
Left: Auxin signaling. Auxin binding by F-box proteins (TIR1 family) enables recruitment of transcriptional repressors (Aux/IAAs) for proteasomal degradation resulting in derepression of transcription factors (ARFs). Auxin-induced gene expression of the Aux/IAAs causes a negative feedback loop.
Right: JA signaling. Similar to auxin signaling. F-box protein/receptor: COI1; transcriptional repressors: JAZs; transcription factor: MYC2.

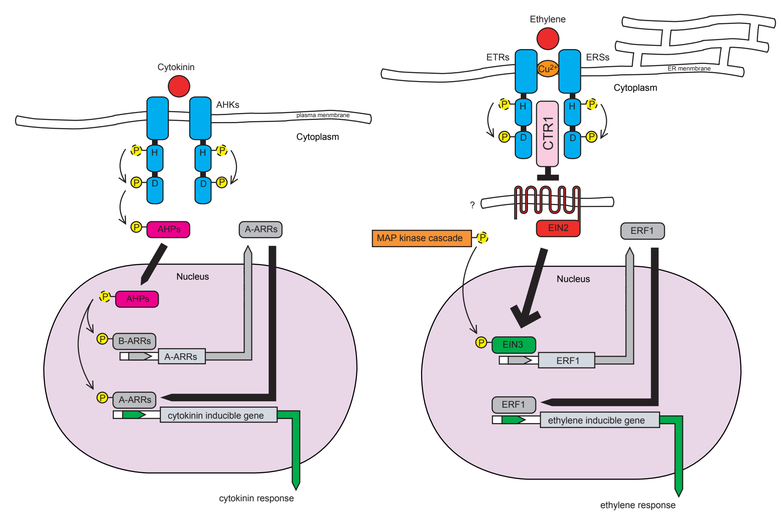
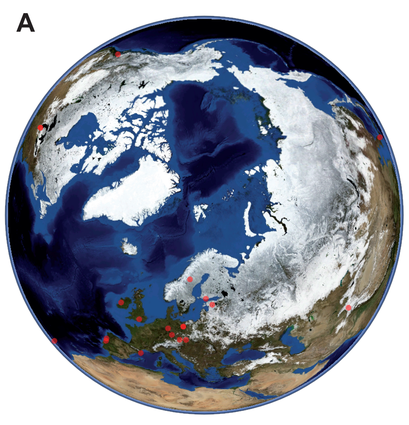
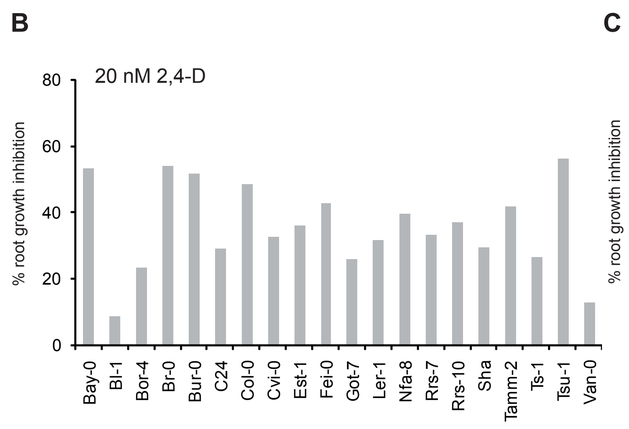


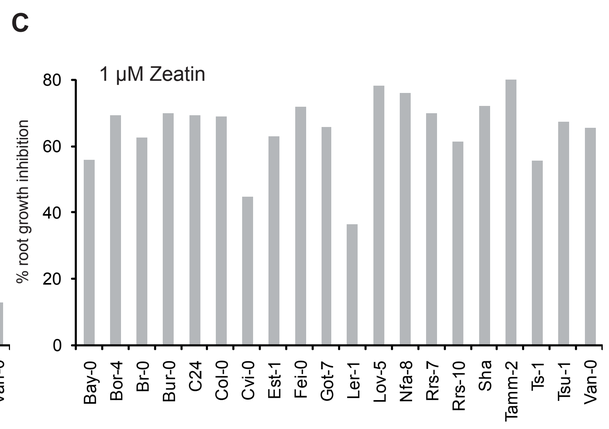
Figure 2: Two-component histidine kinase signaling pathways.
Left: Cytokinin signaling. Cytokinins trigger phosphorylation cascades in receptor proteins (AHKs) and downstream signaling components (AHPs) that result in phosphorylation of transcription factors (ARRs) in the nucleus.
Right: Ethylene signaling. Ethylene triggers inactivation of the negative regulator CTR1 via receptor proteins (ETRs/ERSs). The Activation of the signaling component EIN2 results in phosphorylation of transcription factor EIN3 in the nucleus.

(i) Natural variation in response to growth regulating phytohormones

Using a representative sample of 20 *A. thaliana* accessions from world-wide habitats we identified a tremendous degree of natural variation for hormone responses on the physiological level. Auxin and cytokinin response assays are shown here, JA and ethylene response assays reveal similar degrees of variation (data not shown).







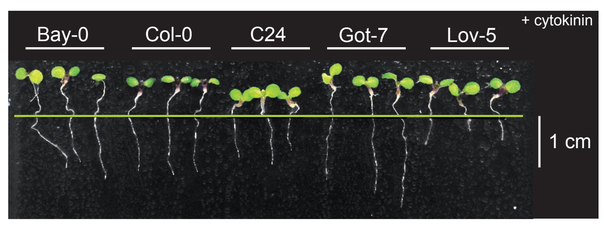


Figure 3: Natural variation in response to growth regulating phytohormones.
A: Geographical distribution of accessions used to examine genetic variation in the first stage of this project. B+C: Natural variation among accessions in response to auxin (B) and cytokinin (C). Seedlings were germinated and cultivated on hormone-supplemented medium and unsupplemented control medium for 7d; bar graphs represent root growth inhibition on hormone-supplemented plates; n=15; assays were repeated several times with similar results. D: Natural variation among 5 *A. thaliana* ecotypes in response to cytokinin.

(ii) Natural genetic variation

A possible reason for variation on the above reported physiological level may be differences in the ability of accessions to transduce the hormone signal. We therefore assessed whether hormone signaling genes are rather conserved or variable on the sequence level. While low nucleotide diversities suggest a high degree of conservation argueing against different biochemical properties of signaling components among accessions, high nucleotide diversities would indicate possible functional differences.These analyses will allow us to make assumptions on possible selective pressures acting on hormone signaling genes.
We therefore sequenced partial cds from 19 accessions for 49 (auxin), 18 (JA), 30 (cytokinin), and 22 (ethylene) signaling genes. Nucleotide diversities were computed and compared to an empirical distribution of 236 control genes. Preliminary analysis of 6 accessions indicates a rather high degree of sequence conservation for auxin signaling genes. This argues against possible functional differences among accessions on the level of signaling components.

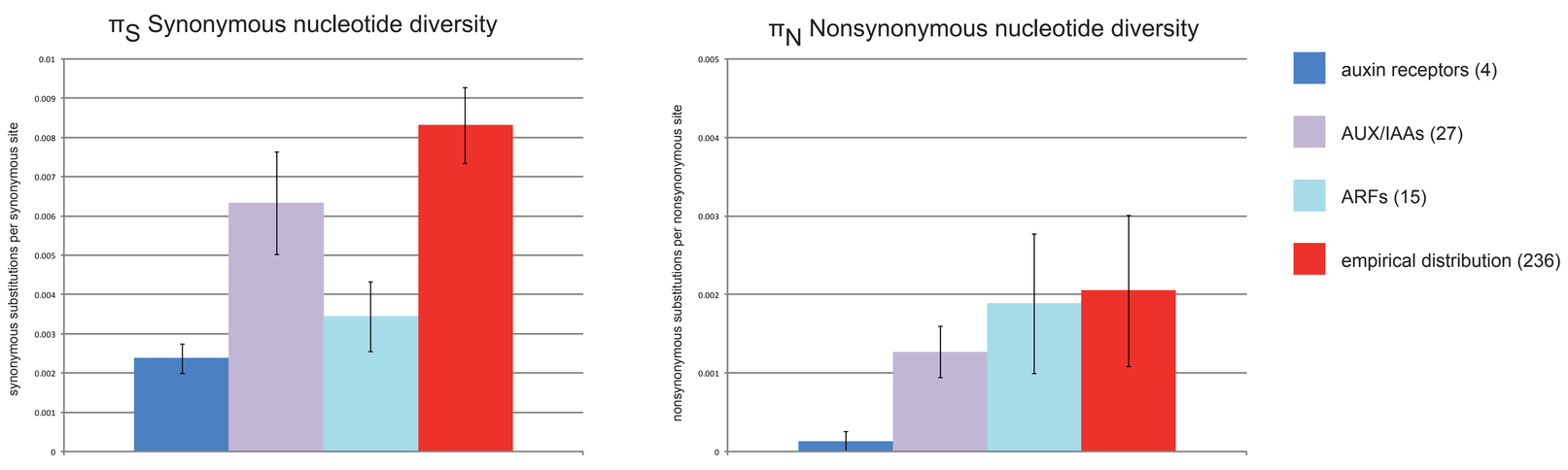


Figure 4: Nucleotide diversity of auxin signaling genes.
Synonymous (left) and non-synonymous (right) nucleotide diversities were computed for auxin signaling genes and the empirical distribution. Especially auxin receptors reveal sequence conservation. AUX/IAAs and ARFs are not significantly more variable than the empirical distribution. Error bars represent the standard error. These preliminary data are based on only 6 accessions, therefore sampling effects have to be taken into account. Similar analyses for the other hormones are in process.

Conclusions - Outlook

Our molecular population genetic analyses enable us to make assumptions on the possible role of signaling genes in the inheritance of functional variation of signaling abilities among accessions. Based on our preliminary results we find no evidence for significantly increased nucleotide diversity in auxin signaling genes. In a parallel study we identified significant transcriptional variation for auxin signaling genes indicating that different abilities to transduce hormone signals among accessions may be regulated on the transcriptional level.
Future QTL analyses will enable us to identify possible players in an untargeted approach. Furthermore, utilizing the same RIL populations as the other collaborators within Research Cluster G will enable us to identify possible pleiotropically acting QTLs. This data mergence will be especially interesting for the QTLs of the Altmann group which analyzes growth phenotypes that are likely regulated by phytohormones.
As a second quantitative genetic approach we plan to perform association mapping with a panel of 360 deeply genotyped natural accessions.

Cooperations

Thomas Altmann and Renate Schmidt (IPK Gatersleben), Thomas Lahaye (LMU München), Ivo Grosse (MLU Halle), Steffen Neumann (IPB)

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