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To Whom It May Concern:

Report about the doctoral thesis of Lucia Hlusková

Mrs Hlusková presents here a doctoral thesis that consists of three parts:

1.) Characterization of cytokinin receptors of *Brasica napus*
2.) Studies of cytokinin receptor affinities and activation using chemical biology
3.) Analysis of importance of conserved residues in the CHASE domains for AHK3 and AHK4

In the first part of her thesis she cloned and investigated three of the five putative cytokinin receptors of *Brasica napus.* When determining the cytokinin binding preferences, Mrs Hlusková discovered that although BnCHK1 and BnCHK3 are more closely related to each other BnCHK1 and BnCHK5 have more similar binding properties.

The goal of the second part was to define principles of the interaction between cytokinin receptor and the ligand. Towards this goal a combination of computer simulation and chemical biology approach was chosen. First interspecies sequence comparisons between different cytokinin receptors were combined and used with docking simulation to identify critical parts of the cytokinin binding pocket. These results were later confirmed by using different potential cytokinin analogues and testing them in various established cytokinin bioassays. In total, 19 potential agonists and 12 antagonists were identified and 10 of those further tested, confirming many of the computer predictions.

In the third part Mrs Hlusková investigated the importance of specific resides in the CHASE domain. Towards this goal, four amino acids of the CHASE domain of AHK4 were identified to be crucial for the binding properties of this particular cytokinin receptor. Then those four residues were mutated in AHK3 to test if this would be

sufficient to mimic the binding properties of AHK4 in an AHK3 background. Due to time reasons those experiments could not be finished as planned.

In all three parts Mrs. Hlusková demonstrates her abilities to conceive, design and conduction scientific experiments. Furthermore, she showed her abilities to analyses the collected data and synthesized the obtained data into new hypothesis which enabled to field of cytokinin research in general to move forward.
The quality of her work is highlighted by two articles already being published in high-ranking international journals and a third one currently being under review.

Thus, I definitely recommend this doctoral thesis for a defense.

Regards,

Garden City, the 17th of May 2019

Alexander Heyl
Assistant Professor

Questions:

1.) What could be the reason, why is there no cytokinin receptor from *Brassica napus* in the "AHK4" clade?

2.) Would you expect to see different results if you would use long-term cytokinin assays, such as chlorophyll retention or root growth assay on the AHK4 mutants, than you saw with your bacterial assay?

3.) Would it be possible to develop a high throughput cytokinin assay using plant cells, and how would it work?