

Review of the Ph.D. Thesis submitted in the Doctoral study program in Experimental Plant Biology at the Faculty of Natural Sciences of Charles University

Student name: *Mgr. Marek Šustr*

Thesis title: **KT/HAK/KUP High-affinity Transporters in Plants**

Name and affiliation of reviewer: assoc. Prof. Vít Gloser, Ph.D., Department of Experimental Biology, Masaryk University, Brno.

The thesis of Marek Šustr is focused on exploring the functional properties and physiological role of a group of high-affinity potassium transporters in plants. It presents a set of four papers (all in journals registered in WOS), three original research articles and one review. In three papers, the student was the first author and dominantly contributed to all parts of the work presented in those articles. The collection of papers is accompanied by an explanation of the scientific background, the aims of the thesis, a summary of results from papers and a general discussion that summarises the work of the PhD candidate. This format of the thesis is in agreement with the rules of the study program. The thesis is technically and formally well-prepared. It is logically divided into sections and subsections with clear relevance to the main topic of the thesis. Subsections also reflect the objectives of the thesis. Text complies with all general rules for the preparation of scientific publications. Language is intelligible and without any grammatical issues.

The aims are clearly defined in the first part of the thesis as more general and detailed in the list. The results presented later in the included publications reflect all the aims well. The candidate demonstrated a good knowledge of the scientific background connected to the thesis topic. Firstly, he summarised key information in the well-written second chapter of the thesis and another big part of the information he presented in a complex review about potassium availability on root development. A significant part of the published review wrote the student as the first author. The chapter on scientific background, as well as discussion, is accompanied by numerous references to literature that are relevant and up-to-date.

The methodology in this thesis is predominantly based on model plants *A. thaliana* modified by targeted genetic manipulations. Genomic tools are already a new standard in all fields of plant physiology for unravelling scientific questions, particularly at the cellular level. So, the use of this methodology is relevant to the aims and experimental questions addressed in the thesis. I find it a powerful combination of the use of knock-out mutant plants for exploring the role of K transporters in plant phenotype and investigating transporter localisation based on GUS or GFP constructs. Although not all planned work was successfully finished, methodological problems and uncertainties are explained and discussed. I see this as a sign of transparent and fair scientific work of the PhD candidate, which is a good asset for his future career in science.

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The results obtained by the PhD candidate are presented well in all papers as part of the thesis, but they are also summarised in chapter 4. Summary of publications. Not surprisingly, results in all published articles are presented very well, clearly and concisely. In all cases, they add essential information to the given topic.

The discussion of results is provided not only in each presented paper but also in chapter 5 of the thesis. Here, the subchapters indicate the most essential topics and findings of the thesis and somehow integrate the results into the context of the whole work of the student. This is probably the most important part of the thesis as it demonstrates the ability of the student to link individual findings from various experiments and define the role of examined transporters in the context of the whole plant and plant response to the environment. This is done well in the chapter. There are also several remarks about the limits of our knowledge and prospective research directions on K transporters or K metabolism and use in general.

I have some questions related to the thesis.

1. Plants can often cope with slightly reduced nutrient availability with no or minor growth reduction by increasing nutrient use efficiency. Did you estimate to what extent *Arabidopsis* plants can adjust their potassium use efficiency?
2. Please explain shortly the complications related to localisation of KUP9 using GFP fusion and possible ways to overcome them.
3. On page 35, you mentioned experiments testing the effect of K uptake by mutant lines in experiments conducted *in vitro* and in hydroponics. You wrote that “*atkup7-1* tested in hydroponics where this line showed no phenotype regarding K content”, which is obviously incorrect and probably a slang expression. (= not different from control?) What is more important, do you have any explanation of differences in the functional response of mutants in conditions *in vitro* and hydroponics?
4. Can you explain more in detail the possible differences between *wt* and mutants in response of K transport to light (irradiance, photoperiod) that you mentioned in the text?
5. On page 37, you claim that K transporters might be directly involved in stress responses and adaptations that do not directly involve K nutrition. Adaptation to serpentine soils in *Arabidopsis* is one case of this phenomenon. Are there more known examples of such a response?

In summary, the thesis deals with important scientific questions that directly relate to general mechanisms of plant functioning. Although the topics of some publications may seem distant (e.g. plant response to K deficiency vs. serpentine adaptation), the author convincingly shows the connecting role of high-affinity potassium transporters in these processes. It also implies that this group of transport proteins has a possible important role in plant response to environmental factors in general. The PhD candidate showed in this thesis his ability to conduct independently valuable scientific research and successfully publish the results in well-ranked international journals. I conclude that this work meets all the requirements of a good Ph.D. thesis, and I recommend its defence with pleasure.



assoc. Prof. Vít Gloser, Ph.D.

Leader of the Experimental plant biology group

In Brno 14. April 2024