

<b>Reviewer's opinion of diploma thesis</b>
<b>Reviewer of the thesis: Katarzyna Retzer</b>
Date: 27.01.2022
<b>Author of the thesis: Michal Bogdan</b>
<b>Name of the thesis: Tissue-specific knockout of starch synthesis in columella cells of Arabidopsis thaliana and gravitropic response</b>
<p><b>Objectives of the thesis</b>  The aim of the thesis was to generate tissue-specific starchless mutants to answer a crucial, but still open question of 150 years plant research, namely how gravitropic response is perceived and initiated. By applying the highly specific and modern approach to knock out genes by CRISPR, the student generated an important tool to address the aim.</p>
<p><b>Structure of the thesis</b>  Size of the thesis (number of pages): <b>93</b>  Are the English and Czech abstracts and keywords given? <b>yes</b></p>
<p><b>Formal level of the thesis</b> (visual documentation, graphics, text, list of literature)  The thesis is well written, supported by schemes and figures visualizing in a clear way the literature background and the cloning strategy. The reference list is up-to date and sufficient.</p>
<p><b>Logical structure and language quality of the thesis</b>  The thesis is clear written, well-structured and the language quality is very good.</p>
<p><b>Literature overview:</b>  Does it correspond to the topic and is it logically structured? <b>yes</b>  Is it written comprehensibly? <b>yes</b>  Are the literature sources used relevant and up-to-date? <b>yes</b>  Are the literature sources used (including pictures) correctly quoted? <b>Yes</b></p>
<p><b>Materials and Methods:</b>  The extend of methodologies used. <b>sufficient</b>  Do described methods correspond to results presented? <b>yes</b>  Are methods comprehensibly described? <b>yes</b></p>
<p><b>Experimental part:</b>  Are the aims of particular experiments explained? <b>yes</b>  Is the documentation of the results adequate? <b>yes</b>  Is the number of conducted experiments sufficient? <b>yes</b></p>

**Discussion:**

Is it really a discussion, is it not just a repetition of previously mentioned results? **yes**  
Are the results related to the literature? **yes**  
Are there any hypotheses or suggestions for further research? **yes**

**Conclusions (Summary):**

Are the main findings supported by the data? **yes**  
Are they formulated appropriately? **yes**

**Achievement of aims and overall assessment:**

Michal generated the required plasmids, transformed the plants and screened for transformed plants. Moreover, he achieved a first round of phenotyping and therefore achieved all planned aims. Finally, he prepared the selection for homozygous plants, and when these plants are confirmed, they will serve the plant research community as a mighty tool to address several open questions how directional root growth is regulated.

**Questions and comments of the reviewer (mandatory part of the report!):**

The topic of the thesis is highly important for the plant community, and it was performed and described excellent.

Q1: What are the limitations of T-DNA insertion lines, and why is the CRISPR approach such a huge improvement.

Q2: When is the CRISPR approach limited, and what must be taken into consideration when designing the plasmids.

Q3: The literature summary is well chosen, the only point I miss, although you cite Swarup et al., 2005, to mention in the overview the importance of shootward auxin transport for directional root growth depending on AUX1. It also corresponds to recent publications of your supervisor, showing how AUX1 dependent auxin uptake modulates cell expansion. Can you please describe the role of AUX1 for shootward PAT for directional root growth.

Q3: You describe that the difference at last timepoints of gravitropism test between WT and pgm mutants are non-significant, why do you think is it so.

Q4: It is known that pgm starchless mutants have less rigid cell walls as well, which impact would this have on directional root growth control, if statoliths are not the crucial player.

Q5: You apply sucrose in your growth medium, why would it be better to omit it when you look on a, starch metabolism mutants, b, compare experiments to WT and c, when you look on specifically targeted cells.

Q6: How would you manipulate endogenous sucrose levels. How is sucrose produced and transported when no exogenous sucrose is added to growth medium?

Q7: In Figure 8 you see on one side A, etiolated plants, when the whole seedling was grown in darkness. How would you guess, do sucrose/starch level and distribution differ in the individual lines along the plant. In the methods they describe they use sugar. And can you describe why the mutants deviate more from vertical. B, Why are the plants growing against the gravitropic vector when light comes from below, and why do you think is it more enhanced in the mutants.

**Reviewer's final classification proposal:**

x  **excellent (výborně)**    very good (velmi dobře)    good (dobře)    unsatisfactory  
(nevyhověl/a)

Signature of the reviewer

*Katarzyna Retzer v.r.*