

<b>Posudek oponenta na diplomovou práci</b>	
<b>Jméno oponenta:</b>	Dr. Said Hafidh
<b>Datum:</b>	23.05.2016
<b>Autor:</b>	Klara Aldorfova
<b>Název práce:</b>	Characterization of the exocyst complex SEC15 subunit in <i>A. thaliana</i> Charakterizace podjednotky SEC15 poutacího komplexu exocyst u <i>A. thaliana</i>
<b>Cíle práce</b>	<ol style="list-style-type: none"> <li>1) To find defects caused by <i>sec15b</i> loss of function (LOF) mutation in <i>Arabidopsis thaliana</i></li> <li>2) To analyse functional redundancy of SEC15A and SEC15B isoforms</li> <li>3) To investigate the mechanism of <i>Arabidopsis</i> SEC15B action in relation to secretory vesicles</li> </ol>
<b>Struktura (členění) práce</b>	<b>Rozsah práce (počet stran):</b> 83 pages including cover page, acknowledgements and thesis content pages.
<b>Je uveden anglický i český abstrakt a klíčová slova?</b>	Yes
<b>Formální úroveň práce</b> (obrazová dokumentace, grafika, text, seznam literatury)	The thesis has been assembled to a professional quality, graphical presentations are of good standard with clarity, readability of the text is very good and thesis includes a comprehensive coverage of literature relative to the topic presented.
<b>Logická stavba a jazyková úroveň práce</b>	The presented thesis is well structured with logical chapters breakdown
<b>Literární přehled:</b>	<p>Odpovídá tématu a je logicky členěn? Yes</p> <p>Je napsán srozumitelně? Yes</p> <p>Jsou použité literární zdroje dostatečné, relevantní a aktuální? Yes</p> <p>Jsou literární zdroje (včetně obrázků) v práci správně citovány? Yes</p>
<b>Materiál a metody:</b>	<p>Šíře použitých metodik.</p> <p>Odpovídají popsané metody prezentovaným výsledkům? Yes</p> <p>Jsou metody srozumitelně popsány? Yes</p>

**Experimentální část:**

Je vysvětlen cíl experimentů? Yes

Je dokumentace výsledků adekvátní? Yes

Je množství provedených experimentů dostačující? Yes

**Diskuze:**

Je opravdu diskuzí, nejde jen o konstatování vlastních výsledků? Yes

Jsou výsledky porovnávány s literaturou? Yes, however it could have benefited by slightly broadening the discussion and incorporate other literatures.

Jsou uvedeny nějaké hypotézy či návrhy na další řešení problematiky? Yes

**Závěry (Souhrn):**

Jsou závěry podloženy výsledky? Yes

Jsou výstižně formulovány? Yes

**Splnění cílů práce a celkové hodnocení:**

Overall, the topic of the thesis is very well presented and the objectives are fully supported by the results.

The title is succinate and clear. The aims of the thesis are clearly stated. The abstract is well structured and informative which summarised the results of the thesis in a comprehensive manner. The thesis itself is well structured, the introduction is comprehensive, focused and well researched. Materials and methods are clearly written and can be reproduced. Results are well presented. Discussion clearly discussed the results and incorporated current knowledge from the literature. The author competently generated hypothesis that originated by linking results observed with what has been documented in literature in other organisms. Collectively, this work has successfully fulfil its objectives and more, and should be finalized for publication in a very near future.

**Otázky a připomínky oponenta:**

Questions:

Q1. Are there different species of secretory vesicles, how are they distinguished? Is it based on vesicles surface proteins or is it based on vesicles cargo? If so, is SEC15B capable of binding all species of secretory vesicles?

Q2. Since vesicles tethering relies on cytoskeleton to reach the plasma membrane, how does the kinetics of exocytosis adapt in pollen tubes where cytoskeleton dynamics is extremely fast?

Q3. The role of human EXO70 and yeast EXO84 in splicing, is it a direct or indirect role?

What protein properties suggest this particular function of EXO70 and EXO84?

Q4. The loss of SEC15 C-terminal domain in rice which normally mediates SEC15 interaction with Ras GTPase, how does this loss redefine SEC15 function in rice considering its conventional/unconventional interaction?

Q5. Knockdown of *sec15a-1* results in severe defects in pollen tubes suggesting SEC15A is dominant in the male gametophyte. Can SEC15B rescue *sec15a* pollen phenotype under SEC15A promoter?

Q6. Is the subcellular localization of SEC15B1 and SEC15B2 confirmed in different tissues?

Q7. Are there planned experiments to provide independent confirmation on the interaction of SEC15B with RabA4a

Comments/suggestions:

1. The differential expression of SEC15A, SEC15B1 and SEC15B2 should not only be based on microarray dataset, instead semi-RT-PCR or qRT-PCR should be performed in different tissues to verify the differential expression observed by microarray. Promoter analysis is also informative in deciphering differential expression but it is not essential.
2. If I understood correctly, the measurements of rosette leaves diameter was done at plant maturity. I think due to rosette leaves senescence and initiation of PCD, leaves from plants at this stage of development are physiology altered and not ideal to provide accurate measurements. The measurements should be done at rosette stage prior to bolting.

**Návrh hodnocení oponenta** (známka nebude součástí zveřejněných informací)

výborně  velmi dobře  dobře  nevyhověl(a)

Podpis oponenta: