

✓ CAN!

The evaluation of PhD thesis submitted by Mgr. Adéla Hýlová „Study of molecular mechanism and biological activity of strigolactones“ (PALACKÝ UNIVERSITY OLOMOUC, study program Biology, field Botany, supervisor Dr. Lukáš Spíchal, 2020)

Strigolactones released from plant roots known before as regulators of parasitic Orobanchaceae (broomrapes) seeds germinations are youngest family of recognized plant growth regulators (or phytohormones) – discovered only about two decades ago. Genetic analyses in model plant *Arabidopsis*, but also pea and rice mutants, lead to the discovery of the crucial contribution of strigolactones to the overall aboveground plant architecture – branching. Moreover it turned out that strigolactone signalling pathway is interconnected with the karrikin pathway – both using an unusual type of receptors (D14 or KAI2 proteins) belonging to the superfamily of α/β -hydrolases whereby these receptors perform an irreversible enzymatic reaction to generate its own ligand. Karrikins are especially known as a seed germination stimulants.

Mgr. Adéla Hýlová undertook efforts to contribute especially to the improvement of high throughput screening procedures to monitor biological activity of strigolactones and discover new artificial classes of molecules with strigolactone biological activity. Such molecules are good candidates for practical application in fight against Orobanchaceae infestations of crop fields – using suicidal germination approach. This PhD thesis is based on two first author and two co-author published reports attached as a part of the thesis.

Results of the thesis are summarized in chapter 8. where major contributions and advancements of appended publications are highlighted. First the improvement of previously published seed germination assay based on redox activity of germinating seeds monitored colorimetrically by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay; original Mangus et al. 1992b simple seed germination assay substantially modified and improved by Pouvreau et al. 2013) is detailed. Bunch of technical modifications improved efficacy and reliability of the assay and after the validation it was used to successfully test new synthetic SL mimics derived from natural plant hormones auxins. Along with frequent species specificity of seed germination stimulation (of selected and compared broom rates) authors found several compounds with slightly higher biological activity than standard synthetic strigolactone GR24. In case of substance 5 enhanced stability in alkaline pH was uncovered in comparison with GR24 making it promising starting point for practical applications.

Next SLs imposed pea branching inhibition phenotyping assay was considered and attempted to adopt and optimize. But due to poor description in the original article with technical details missing (Boyer et al., 2012) it was found to be hard to reproduce. Moreover this assay turned out to be laborious with poor reproducibility of data between repetitions so it was not developed and used further.

Instead MTT assay was adapted as a part of the new semi-automated analysis of Arabidopsis germination and rosette growth analyses based on four traits: in vitro germination rate, early seedling establishment capacity, growth capacity under stress and stress response based on plant greenness. This assay was validated by studying effects of known biostimulants on the moderate or severe salt stress reactions of Arabidopsis seeds.

I was surprised not to see general Discussion of the data published by the PhD student – it would be important to try to see once more the data in the bigger context with more elaborated perspective of the future challenges and questions. This is especially true also due to the mixture of Results and discussion in Hýlová et al. (2019) and two co-author reports (Dvořáková et al. 2018 and 2019) are not mentioned in the overview of Results at all.

Six aims of this PhD thesis are defined too broadly – and aim 4 and 6 largely overlap. The title of this thesis includes ...“molecular mechanism”... , but in fact whole thesis is focused on the development and implementation of bioassays to monitor biological activity of SLs and biostimulants. To tackle molecular mechanism would require to use genetic and biochemical tools to study binding of SL-mimics to receptors and signalling pathway activated. Moreover part of the aim 4 – “...structure-activity relationship of new strigolactones...” is covered only in related paper but not at all in the text of thesis. Here in all above mentioned points possibly supervisor should had intervened. The text is partly internally redundant (including extremely short Conclusions and perspectives) and not always well organized – it would also necessitate native speaker corrections.

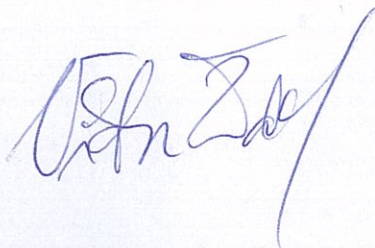
Here are some questions:

- Are you considering for the future work to adopt biosensor based or luminescence quenching based activity assays in planta? What new aspects of strigolactone-like molecules action would you be able to monitor in comparison with current approaches?
- Would it be possible to uncouple or clearly discern action of intact auxin-SL-mimics molecules effect without released free auxins interference?
- Is it excluded that some of the SL-mimics might function especially in seed germination via karrikins pathway (receptor)?
- What is known about the molecular mechanism of positive effect of SLs on salt and drought stress?
- Would you envisage the use of Arabidopsis mutants in bioassays you developed to address interesting questions about SLs, their mimics or biostimulants mode of molecular action?

Overall based on “Survey of results” it seems that major focus of this thesis is methodological advancement of assays for monitoring biological activity of low molecular weight substances under normal and stress conditions. Adéla Hýlová certainly demonstrated great technical skills, intuition and hard work in developing new biological activities assays.

She also saw how specific chemical modifications might affect biological activity of compounds she studied. Due to the fact that I am not a specialist in the field of strigolactones chemistry, chemical biology and bioassays I postpone my final advise to this thesis to the defence proper.

Viktor Žárský

A handwritten signature in blue ink, appearing to read 'Viktor Žárský', with a long, sweeping flourish extending from the end.

Prague, June 21st, 2020