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**Review of the PhD. thesis by Adéla Hýlová entitled  
“Study of molecular mechanism and biological activity of strigolactones”**

**The work presented in this PhD. thesis by Adéla Hýlová** is a continuation of the research carried out at the Department of Chemical Biology and Genetics of the Centre of the Region Haná for Biotechnological and Agricultural Research which is also linked to the Laboratory of Growth Regulators (LGR) of the Faculty of Science, Palacký University (PU) and the Institute of Experimental Botany, Czech Academy of Sciences (IEB CAS). The main research of this lab indeed include preparation of new low-molecular organic substances that can specifically interact with key proteins of signaling and regulatory pathways in the cell; the study of their biological activity and the development of appropriate testing methods and their introduction into routine, mostly robotic screening of existing chemical libraries, substances, plant extracts, microorganisms as well as newly prepared derivatives. Mostly the work of this lab is thus at the interface of biological, chemical and physical sciences, and it is focused on novel compounds biologically active in plants.

In this context, the main aims of this work were to implement a standard germination assay for root parasitic plants, to optimize it as a high-throughput seed germination assay in order to use it as a screening method to determine biological activity and structure-activity relationship of newly synthesized mimic strigolactones, to optimize a physiological assay (pea bud outgrowth assay) and high throughput phenotyping and to perform a routine screening of biological activity of newly synthesized mimic strigolactones.

**The manuscript** takes the form of a 181-page document composed of four main sections (although this subdivision is not identified this way): an introduction of the work followed by a description of the aims of work and a bibliographical analysis, a material and method part, a survey of results and at last, a supplement part in which all the articles published on this work are provided.



**The first part of this work** (chapters 1 to 6) gives a brief general introduction of the context in which this PhD. work takes place and from which the main aims of the work are drawn (chapter 1). Although the objectives are then clearly stated they could have been better explained through the use of more detailed paragraphs rather than a simple bullet point list (chapter 2). Then comes a bibliographical analysis of the main data already published on the topic (chapter 3 to 6). This gives a comprehensive view the different functions among which the germination of parasitic (*Striga sp*, orobanche) and autotrophic plants (*Arabidopsis thaliana*) and it describes the chemical structure of the strigolactones and especially the roles of the different cycles A, B, C and D and the different kinds of synthetic strigolactones. This study also gives an overview of the biosynthesis, perception of these molecules and the subsequent signalization. Finally, it also covers the bioassays commonly used to study the activity of strigolactones.

**The second part** (chapter 7) is a summary of the different material and methods used throughout this PhD work. This part is of main interest because some aims were specifically to adapt and set up a high throughput germination assay for parasitic plants (*Striga hermonthica*, *Orobanche minor* *Phelipanche ramosa*). The specific improvements of an already published germination assay are given in this section. It also described a high throughput branching assay for pea plants and a high throughput germination assay for *Arabidopsis thaliana*. This part is rather short but more detailed information is given in the different articles provided in the supplement section.

**The third part** is a survey of the results obtained for different kind of strigolactone mimics (butenolide D Ring linked to auxin moiety, resorcinol-type SL, triazolide) tested through the use of either the high throughput germination assay in parasitic plants. This part also explained why the pea branching assay was not finally used to study newly synthesized-strigolactone activity. The transposition of colorimetric MTT assay to assess *A. thaliana* seed germination in salt stress condition is also explained in this part. Finally, this part gives a conclusion of this work and the future prospects.

**The fourth part of the work** presented here regroups the 4 articles already published on this PhD work. The first article was published in New Biotechnology with Adéla Hýlová as first author and is entitled “New hybrid type strigolactone mimics derived from plant growth regulator auxin”.

Through the use of the high-throughput germination assay developed by Adéla Hýlová this article shows that auxin type strigolactone mimics with a normal D-ring or with an extra methyl group at C-2 were active as germination stimulants.

The second one was published in Journal of Natural Products with Adéla Hýlová as second author and is entitled “Resorcinol-Type Strigolactone Mimics as Potent Germinators of the Parasitic Plants



*Striga hermonthica* and *Phelipanche ramosa*". In this article it is shown that resorcinol-type strigolactone mimics related to debranones are highly stable and they induce seed germination of parasitic plants *Striga hermonthica* and *Phelipanche ramosa* at low concentrations. There is also a correction for this article regarding the material and methods part.

The third article was published in *Pest Management Science* with Adéla Hýlová as second author and is entitled "Triazolid strigolactone mimics as potent selective germinators of parasitic plant *Phelipanche ramosa*". Interestingly, these compounds show some specificity in inducing the germination of only *P. ramosa* and not *S. hermonthica*.

Finally, the fourth article was published in *Frontiers in Plant Science* with Adéla Hýlová as first co-author and is entitled "Characterization of Biostimulant Mode of Action Using Novel Multi-Trait High-Throughput Screening of *Arabidopsis* Germination and Rosette Growth". This part of the work is not related to parasitic plants but uses the model *A. thaliana*. It describes the use of this approach based on the semi-automated analysis of in vitro germination rate, early seedling establishment capacity, growth capacity under stress and stress response based on plant greenness.

**As a conclusion** to this report, the PhD manuscript presented here by Adéla Hýlová shows an important and very good research work. Everything is here clearly presented. She has studied the activity of molecules belonging to 3 different families of strigolactone mimics which were newly synthesized (SL mimics derived from auxins, resorcinol-type SL mimics and triazolid-type SL mimics). Adéla Hýlová's work brings new results on strigolactone mimics and especially on the most efficient chemical structures and on how these molecules can further be used in suicide germination approaches to control parasitic plants and limit their impact on crops. These results also bring new elegant methods to study of these compounds activity.

**Taking into account these elements I recommend this thesis for the defense.**

#### Questions:

- The work presented here shows that molecules harboring a D cycle on an auxin moiety can act as potent germination stimulants. Would it be of any interest, and why if so, to create SL mimics with a D cycle linked to ABA?
- The results presented in article 1 demonstrate that new auxin type SL mimics are species specific. For example, 1 and 3 are very good stimulants for *P. ramosa*, but only moderate for *S. hermonthica* and *O. minor*. How could these differences could be explained?
- How to explain triazolid mimics specificity toward *P. ramosa*?



- Would the high throughput germination assay carried out in *A. thaliana* be suitable for the analysis SL mimics and why?
- Can the complex pipe-line analysis used in *A. thaliana* be used also in parasitic plants?

A handwritten signature in black ink, appearing to be 'G. Montiel', written diagonally across the page.