



Charles University in Prague

Faculty of Science  
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## Reviewer's opinion on the Ph.D. Thesis

Author : Mgr. Daniel Vrábľ

Thesis Title: Carbon dioxide transport within the leaf mesophyll:  
physico-chemical and biological aspects

Reviewer: **prof. RNDr. Jana Albrechtová, Ph.D.**

Supervisor: doc. Ing. Jiřĩ Šantrůček, CSc.

The present thesis is aimed on investigation of nature of mesophyll conductance ( $g_m$ ) for CO<sub>2</sub> transport and its regulation per se and in respect to stomatal conductance ( $g_s$ ). It represents a valuable contribution summarized in three papers included in the thesis to the physiology of leaf photosynthetic performance with respect to CO<sub>2</sub> mesophyll conductance. Since we are in the period of ever increasing atmospheric CO<sub>2</sub> concentration, response of  $g_m$  to elevated CO<sub>2</sub> concentration and mechanisms governing  $g_m$  and the  $g_s$ - $g_m$  relationship are important to be revealed and better understood.

The first paper **Vrábľ et al. (2009)** brings an important contribution to the understanding of the  $g_s$ - $g_m$  relationship,  $g_m$  is strongly sensitive to CO<sub>2</sub> concentration in sub-stomatal cavity (Ci), and that the relationship between  $g_s$  and  $g_m$  is not conservative, differing between control and ABA-treated plants. From the methodological point of view, the paper brought an interesting comparison of two independent methods for measurement of variation of  $g_m$  in response to CO<sub>2</sub> concentration based on measurements of variable electron transport rate (J) and instantaneous carbon isotope discrimination. The second included paper **Štroch et al. (2010)** investigated  $g_s$ - $g_m$  relationship in the experimental setting of a gradual acclimation of the Norway spruce seedlings to high irradiance and to elevated temperatures. The third paper **Hřem and Vrábľ (submitted)** focused more on the effect of abscisic acid on  $g_s$ - $g_m$  relationship and revealed that  $g_m$  response to ABA is concentration dependent. A very important finding there is about varying response of  $g_s$  and  $g_m$ , to different ABA concentrations, which could lead to enhancement of water use efficiency without any depression of photosynthesis under the condition of reduced  $g_s$  and enhanced  $g_m$ . The authors there hypothesized that, under conditions of moderate water shortage, low concentrations of ABA could positively affect carbon gain and transpiration efficiency.

Let me now mention the structure of the present thesis. The thesis starts with the list of 3 publications, which are then presented as a core research part of the present thesis. The thesis comprises of an original text of the total length of 21 pages including list of references. Namely it is composed of the Introduction presented on 16 pages, Summary presented on 2 pages, References to an original text counting 6 pages. The following part of the thesis represents 3 original scientific papers. The last part included is CV of the author.

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Two of three papers presented in the second part of the thesis are already published papers – The first one, Vrábl et al. 2009, is the first-author paper published in the high-impacted prestigious Journal of Experimental Botany and I regard this paper as a core contribution of the author to the topic presented in the thesis. In the second paper Štroch et al. 2010, Daniel Vrábl is on the second position in the collective of authors. The paper was also published in high-impacted Journal of Plant Physiology . The last manuscript Hisem and Vrábl is in the thesis presented as submitted to JXB (I hope we will learn about its current state during the defence).

Contents of the thesis, does not include the item References, which should have been there, since the length of Summary is not 8 pages what implies from the Table of Contents. Thus, a reader is expecting detailed summary maybe with discussion of important aspects of contribution of the research presented in the thesis to current state of the art of the topic of mesophyll conductance.

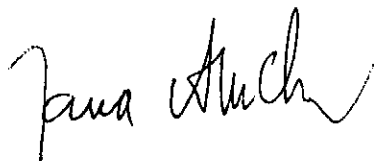
What I am missing in the theses is overall goals of the thesis with hypotheses, which have been tested in the present papers. These should have been presented before the paper part. Then the evaluation of the goals and hypotheses should have been presented in short Summary. The version of Summary, which is presented on p. 17-18, unfortunately, only little mentions contribution of the 3 papers included in the thesis – in fact only in the last sentence and only 2 papers are mentioned: "It is promising that both of this papers ((Thollen et al., 2012; Buckley and Warren, 2013), confirm our major results of variability of mesophyll conductance to CO<sub>2</sub> concentration (I–Vrábl et al., 2009) and the non-uniform response of stomatal and mesophyll conductance after low concentration of abscisic acid (III–Hisem and Vrábl, 2013). " Could author summarize clearly the major scientific conclusions of the 3 presented papers? I ask the author to prepare it for the presentation at the defence.

As conclusion of the above paragraph, I let the author to regard including ERRATA in the thesis, where overall goals and hypotheses tested in the thesis would be presented, their evaluation and summary of the major findings presented in the thesis. I understand that the author wanted to present minimalistic introductory part though some simplifications or omitting of traditional parts could be disadvantageous for fast understanding of thesis importance to a reader.

Questions:

1. What is opinion of the author about the response of  $g_m$  to increasing CO<sub>2</sub> concentration, if the real variation in  $g_s$ - $g_m$  is affecting CO<sub>2</sub> transport inside the leaf?
2. What is opinion of the author about the  $g_s$ - $g_m$  relationship affected by low concentrations of ABA in the view of economics of nitrogen and water use efficiency?

In the end I would like to express my conclusion about very good quality of the theses and the research presented there by the Ph.D. Candidate Daniel Vrábl and I recommend the thesis to the defence. If defended positively I strongly recommend awarding the title Ph.D. to Daniel Vrábl.



Jana Albrechtová

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To whom it may concern

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Review Ph.D Thesis

**Carbon dioxide transport within the leaf mesophyll:  
physico-chemical and biological aspects**

by Mgr. Daniel Vrábl

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Faculty of Science  
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The present Thesis describes short-term responses of mesophyll conductance to changes in atmospheric CO<sub>2</sub>-concentration as affected by application of exogenous ABA. It provides a valuable contribution to our understanding of CO<sub>2</sub> transport within leaves as it confirms findings, that mesophyll conductance is not only determined by leaf anatomical features, but plants seem to have the ability to actively regulate CO<sub>2</sub> transfer in response to environmental conditions. A shortcoming of the Thesis is, however, that it does not extend our mechanistic understanding of these processes.

In the introduction, the state-of-the-art on mesophyll conductance and its response to exogenous ABA is well presented and shows that the candidate has gained a thorough knowledge of this topic during his Ph.D studies. However, some more care could have been bestowed on the language. Also, what is missing is a general discussion, which synthesises the results of the first and third publication (the second one is somehow exotic with respect to the topic of the thesis) more than just the observation that the third paper is a confirmation of the findings of the first one.

Clearly the strongest part of the Thesis is the first publication with the candidate as first author. The second paper is not really in the scope of the thesis, as it does not deal with leaf internal CO<sub>2</sub> conductances. However, it shows that the candidate has acquired substantial technical expertise in gas exchange measurements. The third paper is essentially an extension of the first study, using the same scientific approach as the first study and confirming the former's result.

A finding of this work would have merit more observance (although this holds for nearly all studies that are cited in this Thesis). Data presented in I-Vrabl and III-Hisem strongly indicate that ABA treatment has no effect on Calvin-cycle enzymes; especially, catalytic properties of RubisCO seem to be completely unaffected, as indicated by the similarity of  $A_N$ ,  $C_C^*$  and  $V_{Cmax}$ . The evaluation of the data could have been extended, for example presenting  $A_N$  vs.  $C_C$  and discussing the fact, that ABA exerts an effect purely via affecting CO<sub>2</sub>-transport. As mentioned above, this is the biggest shortcoming of the Thesis that the results obtained are not used to further extend our understanding of the underlying mechanisms.

The experiments were conducted with high diligence, but some methodological aspects of the isotope approach could have been addressed in more detail. It is well known, that the gas exchange system used in these studies is prone to leakage. While the CO<sub>2</sub> exchange rates were corrected, leakage artefacts on the isotopic composition of CO<sub>2</sub> obviously were neglected. Also, possible confounding effects of (photo)respiratory fractionation are mentioned, but assumed to be negligible, while it is shown that photorespiration is a major component of the net CO<sub>2</sub> exchange especially at low  $C_a$  (Fig. 1 in I-Vrabl). The isotope approach is very sensitive to isotopic disequilibria. A difference in  $\delta^{13}C$  of the growth- and measurement-CO<sub>2</sub> causes an unintentional labelling leading to an apparent fractionation of dark respired CO<sub>2</sub>. However, values for the growth- and measurement-CO<sub>2</sub> are not presented. A sensitivity analysis would have been very helpful and would possibly explain the offset in the results obtained by the fluorescence and the isotope approach.

In conclusion, while the present Thesis is not extraordinary, it meets the demands of sound scientific work and, thus, I recommend the acceptance.

Dr. Rudi Schäufele

