Ústav experimentální botaniky
AV ČR
Na Karlovce 1
160 00 Praha 6

Posudek disertační práce Mgr. Jany Mackové: "The role of plant cuticle in water loss protection".

Jedním ze základních předpokladů pro úspěšný přechod rostlin z vodního prostředí na souš bylo vytvoření bariery zabraňující neomezené výměně plynů a právě kutikula je touto barierou. I když obecný význam kutikuly je velmi dobře znám, teprve metodiky vyvinuté v posledních letech umožnily přesné rozlišení kutikulární a stomatární transpirace. Na vytvoření nových metod stanovení kutikulární transpirace se úspěšně podílela i laboratoř vedená Doc. Šantrůčkem. Je tedy nepochybné, že téma této disertační práce odpovídá zaměření laboratoře a je velmi aktuální.

Disertační práci Jany Mackové tvoří úvod, 4 publikace a závěry. Práce je předložena v anglickém jazyce. V úvodu autorka stručně zmiňuje složení kutikuly, její permeabilitu a možné změny vyvolané působením faktorů vnějšího prostředí. Na konci této části jsou jasně formulované cíle disertace, které jsou zároveň i cíli předložených publikací.

První publikace je metodického charakteru a autorka se podílela na vypracování nové metody umožňující rozlišení současného transportu vody kutikulou a průduchy na téže epidermis. Dříve se totiž kutikulární transpirace převážně měřila na epidermis kde průduchy nebyly přítomny nebo byly uměle zavřené. Tuto metodiku pak využila autorka pro další experimenty týkající se ovlivnění permeability kutikuly vlhkostí vzduchu u dvou druhů různého původu lišících se ve své odpovědi na sucho. Obě tyto práce byly publikovány v prestižním časopisu, „Journal of Experimental Botany", prošly náročným recenzním řízením a je tedy zbytečné mít k nim jakékoli připomínky. Chtěla bych jen zdůraznit, že se nepochybně jedná o nové a závažné výsledky. Drobným formálním nedostatkem jsou chyby tisku pravděpodobně vzniklé při kopírování publikace do této knižní podoby na str. 19 -21.

Ve třetí práci, která je předložena do časopisu „Environmental and Experimental Botany", se autorka zabývá vlivem sucha simulovaného aplikací kyseliny abscisové na chemické složení kutikulárních vosků u *Lepidium sativum*. Zde bych měla k autorce několik dotazů. Nevím do jaké míry je aplikace ABA skutečnou simulací vodního stresu. I když zvýšený obsah endogenní ABA je jedním ze signálů vodního stresu a aplikovaná ABA je obvykle dobře přijímaná rostlinou, existuje i působení vodního stresu na ABA nezávislé. Kromě toho interakce mezi ABA a půdou jsou známé a tedy koncentrace ABA v okolí kořenů se nemusí shodovat a aplikovanou koncentrací. Dále bych se chtěla zeptat, proč právě tato rostlina byla k pokusům vybrána. Na str. 78 mě překvapilo zjištění "ABA-treated plants had sighificantly higher aboveground dry biomass" když z tabulky 1 je patrné, že rostliny ošetřené ABA měly velmi sníženou celkovou biomasu.

Čtvrtá práce byla publikována v časopisu Acta Oecologica a zabývá se sledováním morfologických a fyziologických rozdílů mezi různými druhy rodu *Polylepis* rostoucími v různé nadmořské výšce na Andách. Považuji ji za dobrou ekofyziologickou práci. Pouze obrázek č. 3 mi připadá moc zmenšený ve srovnání s ostatními obrázky, ale to je věc redakce časopisu a nikoli autorů.

V závěrech autorka potvrdila splnění vytčených cílů a naznačila i perspektivy možného dalšího výzkumu v tomto oboru.

Tato disertace je založena na výsledcích získaných celými kolektivy autorů, což jistě nemálo přispělo k možnosti jejich velmi úspěšného publikování. Podíl autorky je však jasně vymezen.

Autorka ve své disertační práci nepochybně prokázala znalost oboru a schopnost být platným členem výborného vědeckého týmu a proto její disertaci doporučuji k obhajobě.

V Praze dne 8. 2. 2010

Jana Pospíšilová

RNDr. Jana Pospíšilová CSc.

universitätbonn

Rheinische Friedrich-Wilhelms-Universität Bonn
IZMB Institut für Zelluläre und Molekulare Botanik

universität bonn · IZMB · Kirschallee 1 · 53115 Bonn

Priv.-Doz. Dr. Rochus Franke
Abteilung Ökophysiologie

Kirschallee 1
53115 Bonn
Tel.: ++49 (228) 73-6525
Fax: ++49 (228) 73-6811
rochus.franke@uni-bonn.de

www.izmb.de

To the Head of the
Committee for PhD studies
Professor Jiří Šantrůček
University of South Bohemia
Branišovská 31

37005 České Budějovice

Bonn, 22. Februar 2010

Review of Ph.D. thesis

of Ms. Jana Macková with the title

„The role of plant cuticle in water loss protection"

Land-living higher plants are characterized by specific surface tissues such as periderms or the epidermis. The epidermis represents the plant/environment interface and as such a specific cell wall modification, the cuticle, build the outermost surface of the plant. The cuticle, chemically characterized by lipophilic materials such as cutin and waxes, provides a physiologically important barrier preventing uncontrolled water and nutrient loss and protection against pathogens. Despite this importance, many aspects of transport across cuticles and the factors determining or composing the barrier properties are still unknown. Understanding cuticular transport is also of agronomic interest as the application of multiple agrochemicals requires foliar uptake and cuticular transpiration is a critical factor determining the water use efficiency.

Leaf water evaporation is the sum of coordinated stomatal and cuticular transpiration. A great limitation in determining the cuticular transpiration exists for stomatal cuticles since imperfect stomatal closure could mislead leaf measurements. The use of isolated cuticles allowed only the transpiration measurement of astomatous cuticular membranes. So a major challenge in previous studies was always the differentiation of stomatal and cuticular transpiration in stomatous cuticles and this was the origin for the Ph.D. project of Jana Macková .

In a series of consecutive and complementary studies co-authored by Jana Macková - three of them already published in internationally recognized plant sciences journals and a fourth one submitted - she presented a comprehensive, sound and solid scientific thesis. Starting from method development she made the continuative steps employing this method to investigate cuticular transpiration in the relevant model plants, moved further to studies in a physiological context, and finally to investigations of ecophysiological relevance. Ms. Macková employed an array of state of the art methods including radio and stable isotope studies, scanning electron microscopy and analytical chemistry.

The solid foundation for her thesis work was established by Ms Macková developing a ground breaking method that allowed to determine water diffusion across stomata and separate it from cuticular water flux by manipulating the diffusivity of water in the gas phase. Interestingly she determined a significant difference in the permeability of stomatous and astomatous cuticular sides. In complementary control studies measuring the diffusion of radio-labelled model compounds she determined differences depending on whether the waxes originated from the stomatous or astomatous leaf side. Consequently she discusses the differences in permeability with the lateral heterogeneity in cuticular surfaces.

One of the major findings investigating the short-term and long-term effects of humidity on cuticular permeability was that astomatous cuticles are more affected by humidity than stomatous cuticles. These differences are competent discussed with possible differences in the degree of cuticular hydration advantaged by stomatal transpiration. In studies investigating the effects of drought stress induced by ABA application Ms. Macková established a set of cuticular modifications. This included changes in stomatal density and stomatal aperture and also in wax composition. In an expertly discussion, the increase in carbon chain-length in analytically determined wax monomers of *Lepidium* are compared with corresponding findings from the molecular genetic model plant *Arabidopsis*, and suggested to be a result from activation of fatty acid elongation genes. Ms. Macková further proposed that this lead to an increased hydrophobicity resulting in lower cuticular transpiration. In a very elegant way Ms. Macková verified the ABA-induced reduction in stomatal gas exchange by demonstrating a ^{13}C-enrichment in ABA-treated plants. The application of these stable isotope studies on cuticular waxes from Andes tree species indicated that ^{13}C in waxes of stomatous leaves tens to enrich with higher altitudes. From additional morphological and physiological attributes Ms. Macková concluded that so far uncovered water stress is an additional factor contributing to altitudinal distribution at the treeline.
In summary the comprehensive, multi-approach studies by Ms. Macková provided new insights and contributed significantly to the understanding of cuticular water transport and its ecophysiological importance. The thesis, written in English, is presented in a cumulative form of three publications and one manuscript. I have no doubt that the latter will be published in an internationally recognized scientific journal as the other tree. In the thesis summary the studies are appealingly presented and excitingly discussed with the adequate and up to date literature. Ms. Macková also presented a well developed, skilled outlook on the main challenges in future cuticular research and suggested appropriate future studies for which I which Ms. Macková will continue to work with similar success.

The high quality of the work presented and submitted by Ms. Jana Macková fulfils the requirements for a Ph.D. thesis and therefore I highly recommend the award of a Ph.D. scientific degree to Jana Macková.

Bonn, 22.02.2010

(Priv.-Doz. Dr. Rochus Franke)

Dr Gerhard Kerstiens
2 The Smokehouse
St George's Quay
Lancaster LA1 1RB (UK)

E-mail:
gerhard@prologos.biz

Reviewer's report on Jana Macková's thesis "The role of plant cuticle in water loss protection" for the Committee for PhD studies in Physiology and Immunology, Faculty of Science, University of South Bohemia.

This thesis contains results from three investigations relating to cuticular traits. Briefly, one is about the development of a novel technique for characterising cuticular water permeability of stomatous cuticles (Study I) and its application in a case study (Study II), one on the response of cuticular wax composition to externally applied abscisic acid (Study III), and one on the use of the carbon isotope discrimination "signature" retained in cuticular waxes to infer ecophysiological constraints of species forming the treelines in the parts of the Andes (Study IV). The Candidate is the first author of Studies II and III and contributed to Studies I and IV.

It is perhaps unavoidable that such a wide spectrum of work leaves more "loose ends" than one focussing on a single area of research in more depth (which the area of Studies I and II certainly would have deserved). I suppose there were funding constraints, and the broad range of experiments Jana was involved in certainly also has its value in terms of scientific education.

I was previously involved (at the manuscript preparation stage) with Studies I, II and IV, so most of the criticisms I might have had at some point have been dealt with. However, a few questions have remained unresolved (to me at least), and I will be putting them again below. Study III was new to me.

Questions to the Candidate:

Study II. How is the use of the same driving force for the transport of labelled water at different humidities (p. 55) justified? It seems to me that labelled water (from the donor) and unlabelled water (from the receiver reservoir at high humidity) will compete for sorption at the cuticle/receiver interface, so even though the *concentrations* of labelled water molecules in the donor and receiver reservoirs, respectively, were the *same* at both humidities, won't they be partially replaced in the steady-state by unlabelled water having entered the membrane from the receiver side under high-humidity conditions, thereby giving *different gradients* of labelled water concentration between the cuticle/donor and cuticle/receiver interfaces at different humidities? I may be wrong, but the question doesn't seem nearly trivial enough to be left un-discussed.

Study III: The suggestion that the smaller stomata found in ABA-treated plants were faster and somehow improved water use efficiency through enhanced kinetics of their response to environmental changes (p. 82, 119) seems highly speculative. I have no doubt that WUE was increased in those plants – but it always is when conductance is reduced (as will have been the case here with those high ABA concentrations), simply because the reduction affects overall resistances for water and CO2

transport differently. So I wonder if the Candidate had a particular reason to bring up the kinetics of stomatal responses in this context?

Studies III & IV: I have the impression that both studies assume that the carbon isotope signature in waxes represents a sort of time-average of isotope discrimination in newly assimilated carbon during the lifetime of a leaf up to the point when the destructive sampling occurs. However, with regard to the cotyledons used in Study III, it would be interesting to learn how the carbon content of the cotyledons compares to that of "old" carbon in the seed, which will have contributed some of its carbon to the cotyledon? And with regard to Study IV, where is the evidence in the literature of a continuous turn-over of cuticular waxes, which seems to be implied here? Yes, waxes are degraded and lost over time, but are they in fact also replaced by newly synthesised waxes? While epicuticular waxes can "grow back" after having been removed (most likely by re-equilibrating with intracuticular ones), is there any reason to assume that any carbon assimilated *after* the cuticle has been laid down by the *growing* leaf ever makes it into the waxes that were extracted here from older leaves?

Recommendation:

My questions notwithstanding, the science presented in the four Studies is solid. The Introduction and Summary parts of the Thesis could have done with a little more coherence and depth, but are satisfactory (though I'd like to mention that I strongly disagree with one point: p. 4, chapter 2.2, 4th sentence – both the "therefore", as the subsequent statement does not follow from the preceding ones, and the statement itself; see my cited paper of 2006, Fig. 2).

Subject to the Candidate dealing with the points above to the exam panel's satisfaction during the Defence of the Thesis, I recommend it be accepted for the award of a doctoral degree.

Lancaster, 15.02.2010

Dr Gerhard Kerstiens