
The effect of heavy metal ions on the development of *Abies alba* Mill. A *Picea abies* L. (H.) Karst somatic embryos

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The focus of the thesis is given to the study of the ability of different cell lines of *Abies alba* and *Picea abies* to undergo somatic embryogenesis under the heavy metal stress induced by ions of Cd and Pb. Heavy metals are becoming more abundant in the environment due to different anthropogenic activities and plants can be used for phytoremediation techniques. As one of the most interesting findings of the thesis I regard the use of somatic embryogenesis as the *in vitro* laboratory testing system of tolerance of different cell lines of *P. abies* and *A. alba* to heavy metal exposure. A simple laboratory tests proposed for testing tolerance to polluted soils by heavy metals are only 3-week long, what is very advantageous in case of coniferous tree species with a long lifespan. The thesis brought a complex information on physiological response of cell lines undergoing the process of somatic embryogenesis to heavy metal stress.

The text of the Thesis is presented on 112 pages. In the beginning there are abstracts in Czech and English. Introduction to the aims of the Thesis is given on 3 pages. The aims of the Thesis are then summarized on 1 page – I have one minor question to aims: I would appreciate explanation, definition, what is exactly meant by „detoxification potential“? This term I have not found to be defined throughout the thesis – it is only used in the abstract, aims and summary.

Literary review is presented on 29 pages and covers in very details topics of general introductory information on both studied tree species, basic principles of zygotic and somatic embryogenesis, developmental stages of somatic embryogenesis (SE) and practical applications of SE. The last part of the Introduction is devoted to plant responses to stress and heavy metals studied as pollutants – Cd and Pb. There I have questions: regarding the Fig. 7, p. 23 – there are described two types of cells – highly vacuolated and cytoplasmic cells but no further explanation to what these cell types give an origin to. Could you specify it more precisely? The legend of Fig. 8, p. 24 – there is mentioned histochemical staining with acetocarmine and Evan’s blue. But no explanation is given what should be stained and how by these two stains. Could it be explained?

Material and methods are presented on 12 pages in concise but very efficient manner. However, I am missing there more precise definition of early pre-cotyledonary and cotyledonary stages on p. 50, Fig.
16. In my opinion, the definition of developmental stages, which were used for studying timing of the maturation process, should be based on histological study, which I miss in the present Thesis. What does characterize early pre-cotyledonary developmental stage on a structural level? Is it compatible to cylindrical stage as defined in our previous study (Svobodova et al. 1999)?

The results are presented in very instrumental manner with very good graphics on 23 pages. Discussion presented on 11 pages is of very good quality. However, I miss there a more detailed part aimed on phytoremediation potential of both species in practice, which is shortly mentioned in subchapter 6.4., p. 81. As very useful for the thesis reader there are conclusions of the Thesis, presenting main outcomes of the Thesis in very concise but very instrumental way. One of the interesting findings, I found, is about a possibility to test both tree species as potential phytoremediators. **I would like to ask: 1) what type of phytoremediation is proposed to be tested, and 2) what is already known about a possibility of both tree species to be used as hyperaccumulators?**

As the main positive features of the Thesis I would like to emphasize an interesting and novel approach in the study of energetic status and a complex response to oxidative stress of SE in relation to the effect of heavy metal stress. Being a plant anatomist who studied processes of somatic embryogenesis in *P. abies*, I regret that histological study has not been included in the present research, that observations of morphogenetic processes during SE remained on macroscopic and biochemical levels. I expect that histological study could reveal a different, more detailed insight in the processes studied. However, it does not lower the quality of the Thesis, which I regard as high.

The Thesis brings very interesting and original findings, which are in the beginning of the thesis stated to be part of 2 impacted publications. However, it is not clear if all results published in the thesis are part of the mentioned 2 publications. Are there some other results not published yet? Is there a prospective for another publication?

In the end I would appreciate better specification about what a portion of the presented research was actually performed by the Author. I am not sure if I found clear statement in the Thesis. **Please, could you give us this review during defense?**

Regarding formal quality of the Thesis – I regard it as a very good again. Only small remarks regarding figure captions. I would appreciate more detailed Figure captions in some case. For example: Fig 16 – better description of morphological characteristics of the presented developmental stages of somatic embryos.

**Questions for discussion:**

1) What would be suggestion of following research on tolerance of both species to heavy metals?
2) What is a potential of transfer of the Thesis findings into practical applications?
To conclude my review, I would like to state that I was pleased by the quality of the Thesis presented by Biljana Dordevič. The results bring very important knowledge on possible use of different cell lines of both tree species for phytoremediation of heavy metal polluted sites.

It is possible to state, in my opinion, that the present Thesis introduces high quality research. I strongly recommend to accept the Thesis for the defense and after successful defending the Thesis to award the author of the Thesis, Ing., Biljana Dordevič the Title Ph.D.

Reviewer of the Thesis
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Signature:

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