Examiner’s report to Biljana Dordevic Doctoral Thesis

Title: The effect of heavy metal ions on the development of *Abies alba* Mill. and *Picea abies* (L.) H. Karst somatic embryos.
Mendel University in Brno, Faculty of Forestry and Wood Technology
Thesis supervisor: prof. RNDr. Ladislav Havel, CSc.

Heavy metal contamination of water, soil and agricultural land is a serious environmental problem with negative impact on living organism. Plants as sessile organism accumulate the toxic elements and by consuming different parts of edible plants these elements can be transferred to food chain causing serious health problems of human beings as well as animals. Owing to this fact, serious studies have been devoted to investigation of heavy metal effect on the plant organism. The majority of studies have been focused on whole plant level and relatively few studies are focused on cells or tissues isolated from plants and cultivated *in vitro*. Studies appeared in recent period and devoted to study of heavy metal effect on *in vitro* cultures have shown that these cultures are excellent objects mostly for controlled environmental conditions *in vitro*.

The submitted Thesis of Biljana Dordevič elaborated under title „The effect of heavy metal ions on the development of *Abies alba* Mill. and *Picea abies* (L.) H. Karst somatic embryos“ is in accordance with this trend. The research aimed to investigate the process of somatic embryogenesis in different developmental stages as proliferation, maturation, plantlet regeneration of two model conifer trees under the influence of heavy metal ions (Cd²⁺ and Pb²⁺ stress).

The work is well written and consists of „classical“ chapters as Introduction, Aim of the research, Literature review, Material and methods, Results, Discussion, Conclusion, Souhrn, Literature, List of tables, List of figures.

The aims are exactly and well defined, in details specified into research field as Cd²⁺ and Pb²⁺ ions effect (in concentrations 50, 250, 500 μM) on cell viability, tissue growth, somatic embryo maturation, bioenergetical parameters, detoxification potential.

The Literature review is dealing with description of tree species used in work, but mostly it is focused on the studies relevant for research field (somatic and zygotic embryogenesis, individual stages of somatic embryogenesis, bioenergetics and plant development, plant
responses to stress, heavy metal pollutants). The review is well structured and provides an excellent and complete view on present state of the art.

The chapter Material and methods – the methods in work have been chosen in accordance with defined aims. The methods comprise in vitro culture methods, standard cell viability assay, biochemical and electrochemical methods. Very important part of this chapter is induction of embryogenic cultures from megagametophyte explants containing immature zygotic embryos, because in experiments these tissues were used.

The obtained results clearly showed the harmful effect of both metal ions on cell proliferation, resulting in reduced proliferation ratio, although the negative effect was stronger in Cd\(^{2+}\) treatment and was cell line dependent in both species. The embryogenic tissues of conifer species are characterised by the presence of bipolar structures – somatic embryos. In presented experiments fluorescence microscopic observation revealed changes in structural organisation of somatic embryos under stress conditions. The cellular levels of ATP were the highest at concentration 500 μM of boths heavy metal ions and this phenomenon has not been exactly explained, only some suggestions have been done. Accumulation of heavy metal ions was proportional to the applied concentration of both elements and was lower in Picea abies than Abies alba. In chapter „Conclusions“ the author proposed testing large number of cell lines of both species to obtain better view on response based on genetic background.

The obtained results have already been published in international journal (Acta Physiologiae Plantarum, 2 publications, both in 2017) and Biljana Dordevic contributed also to publication appeared in Protoplasma (2017).

Comments or questions to the submitted work:

Material and methods:

What is/was the reason of using two different culture media for both species, although in several parts of work the two species are/were compared.

In this chapter the results of induction are given, it would be more convenient to insert small description of obtained results in chapter Results.

Results:

Fig 21 – how could be explained the heavy metal effect fluctuation on proliferation ratios during 21 days of cultivation.

Fig. 22- Please, give exact explanation.
It would be useful to follow the water uptake under the heavy metal ions influence (e.g. see PhD work of Jiří Petřek (2006, PhD Thesis with interesting results))

Conclusion: the submitted work represents valuable contribution to the knowledge of heavy metal stress response in conifer species. The experiments have been done with embryogenic tissues suggesting an evidence, these types of tissues are also convenient experimental system for such kind of study, although not very often used. The aims were clearly stated, the literature review is well elaborated, the applied methods were adequate and the interpretation of results as well as their discussion is clear. Moreover, as it was already mentioned, Biljana Dordevic has published her results in international journals. The submitted Thesis as well as publications reflect Biljana Dordevic’s activity and interest in scientific work and also suggest she fulfilled requirements of MU for Doctoral Thesis. I highly recommend accepting the submitted Doctoral Thesis.

Nitra, October 23th, 2017

RNDr. Terézia Salaj, DrSc.