

Abstrakt

Somatic embryogenesis of Norway spruce (*Picea abies*) presents both promising means of propagation and valuable model system for investigating the structural, physiological and molecular events occurring during embryo development. Somatic embryogenesis is a process in which a bipolar structure, somatic embryo develops from non-zygotic cell through series of developmental steps similar to zygotic embryogenesis. The efficiency of every phase depends on the previous ones. Important changes in structure and physiology of somatic embryo happen in maturation phase. Embryo accumulates storage compounds (saccharides, lipids and proteins), that are necessary for post-maturation phases. Manipulation of the culture conditions during the maturation can increase both quality and number of embryos produced in culture. Between factors that influence somatic embryogenesis belong growth regulators, osmotic stress and carbohydrates. Maturation is supported considerably by abscisic acid and non-penetrating osmotica (e.g. PEG). The role of carbohydrates during maturation phase has not been fully elucidated yet. Carbohydrates serve as source of energy and carbon, osmotica, participate in protection of membrane and recently it is assumed that can be signal molecules.

Presented study was made using the embryogenic line genotyp *Picea abies* AFO 541 (AFOCEL, France). Embryogenic culture was grown on Gupta and Durzan modified liquid medium supplemented with ABA and different types of carbohydrates. To contribute to elucidation of the role of carbohydrates during somatic embryo maturation four variants of maturation of SE were conducted: 1) medium with 3% sucrose and one week subcultivation interval, 2) medium with 1,57% glucose and 1,57% fructose and one week subcultivation interval, 3) medium with 3% sucrose and subcultivation every second, fourth and seventh day in a week, which lead to limitation of sucrose hydrolysis in medium, 4) medium with 3% sucrose first two days, 2,25% sucrose next two days and 1,5% sucrose next three days in week.

Substitution of sucrose in maturation medium by glucose and fructose decrease yield of mature somatic embryos. Limitation of sucrose hydrolysis in medium lead to partial decrease of yield of mature somatic embryos. However, the significant decrease in embryo yield was also observed in cultures grown on a series of media with declining sucrose/hexoses ratio and three transfers to a new medium per week. Content and spectrum of endogenous carbohydrates in somatic embryos during maturation were similar in all variants. Analysis of maturation media confirm, that sucrose presented in media is

completely hydrolyzed to glucose and fructose after one week cultivation. Dynamics of pH of media during six weeks of maturation of SE was similar in all variants. There was a decrease of pH from the first week to the lowest value in the third week of cultivation. From the fourth week, increase of pH of media until the end of the sixth week was observed. There was not statistic difference in content of storage lipids and proteins in SE after maturation among variants.

The effect of non-penetrating osmoticum PEG in maturation media on accumulation of storage proteins and lipid in somatic embryos was studied. It was found, that the content of storage lipids was higher in somatic embryos treated by PEG during maturation.