Journal of Stress Physiology & Biochemistry, Vol. 8 No. 3 2012, p. S30 ISSN 1997-0838 Original Text Copyright © 2012 by Tretiakova, Voroshilova, Ivanitskya, Shuvaev, Park

THE BIOTECHNOLOGY OF EMBRYOGENIC CELL LINES OBTAINING AND PLANTLETS OF CONIFEROUS SPECIES IN SIBERIA IN CULTURE IN VITRO

Tretiakova I.*, E. Voroshilova, A. Ivanitskya, D. Shuvaev, M. Park

V.N. Sukachev Institute of Forest, Siberian Branch of Russian Academy of Science, Krasnoyarsk, Russia

*e-mail: <u>culture@ksc.krasn.ru</u>

Experiments of culturing the immature isolated embryos and megagamethophytes of Siberian coniferous species were carried out on different modified media: 1/2 LV medium for Pinus sibirica and Pinus pumila, MSG and AI media (patent № 2431651) for Larix sibirica and Larix gmelinii, DCR medium for Picea ajanensis. For induction of embryogenic callus every species needs the optimized medium supplemented with L-glutamine, casein hydrolysate, ascorbic acid and hormones with different concentrations and their different proportions. The active proliferation of embryonal masses is observed on the same medium with reduced concentration of cytokinins. The proliferation of embryonal masses was significantly improved when they were subcultured after dispersing in liquid medium. The somatic embryos from embryonal masses are matured on basal medium with ABA (60-120 mM) and PEG. In spite of species specificity the embryogenesis of morphogenic structures had the same scheme: elongation and asymmetric division of somatic cells, formation of initial cells and embryonal tubes, development of globular, torpedo and bipolar somatic embryos, embryos maturation and germination. However, not all donor-plants of coniferous species can form the embryogenic cell lines and somatic embryos. The active development of embryonal masses and formation of somatic embryos are observed from zygotic embryo of hybrid seeds of P. sibirica and L. sibirica. The obtained embryogenic lines were characterized by different proliferative activity. During 10 months cultivation the value of embryonal masses in different lines was 140-570 g. The number of somatic embryos varies from 210 to 410 per 100 mg of callus fresh weight. Decreasing proliferation activity did not observed during 24-45 months cultivation. However, development of somatic embryos in long cultivated lines decreased. Maturation of somatic embryos and development of plantlets were established in L. sibirica and P. pumila 50-60 somatic embryos were matured per 1g of callus fresh weight. Somatic embryogenesis passes over the strong genetic control. Only donor tree genotypes with high reproductive potential form embryogenic cell lines and somatic embryos. The maternal affect was very strong relative to paternal and other effects. The studying molecular mechanisms involved in the control regulation of embryo development (embryo maturation, desiccation and germination) allows to understand many aspects of molecular biology of gymnosperms.

This work was supported by Integration project № 140 and by grant from Russian government department of Science and Education to Siberian Federal University «The genetic researches of the Siberian larch».