



MARKERS OF SOMATIC EMBRYOGENESIS OF *PINACEAE* SPECIES IN *IN VITRO* CULTURE (CYTOHISTOLOGICAL, PHYSIOLOGICAL AND MOLECULAR GENETIC)

Tretyakova I.N., Park M.E.*

V.N. Sukachev Institute of Forest SB RAS, Federal Research Center
«Krasnoyarsk Science Center SB RAS» Siberian Branch of the Russian
Academy of Science, Krasnoyarsk 660036, Akademgorodok, 50, bld. 28,
Russian Federation

*e-mail: culture@ksc.krasn.ru

2021

SOMATIC EMBRYOGENESIS



Somatic embryogenesis is a type of asexual reproduction based on the totipotency, a unique ability of plant cells to realize their genetic information in *in vitro* culture and to give rise to a whole organism.

The study of somatic embryogenesis provides great prospects in the understanding of the cell differentiation process and the way of performing the morphogenetic programs during early ontogenesis, identification of physiological and molecular genetic events, and the use of this process in modern genetic and breeding studies in the frame work of the Multi Varietal Forestry (MVF) program.



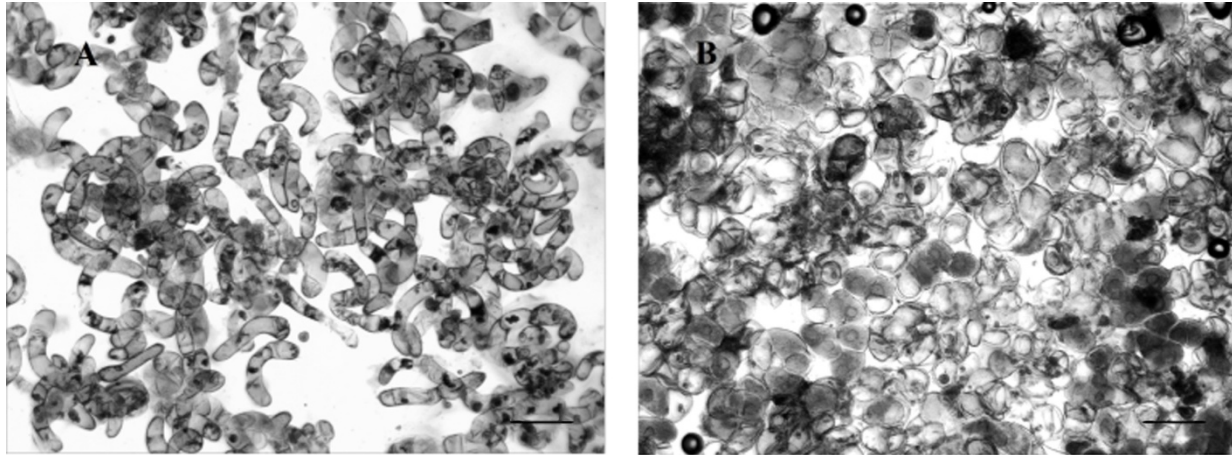
An innovative biotechnology for cloning conifers through somatic embryogenesis was developed in the laboratory of forest genetics and breeding of the Institute of Forest named after V.N. Sukachev SB RAS (Patent No. 2456344 "Method of microclonal propagation of Siberian larch in culture in vitro via somatic embryogenesis on AI medium for plantation forest growing").

A collection bank of proliferating embryogenic cultures has been created . These cultures are represented by an embryonic suspension mass, ESM (developmental stage - globular embryos). The bank consists of 30 cell lines of Siberian larch of different ages from one to 12 years old and Siberian spruce (3 cell lines), cell lines of Siberian pine, dwarf pine and Scots pine. Conditions have been created for the preservation of genetically tested planting material through cryopreservation.

INITIATION OF CALLUS CULTURES IN *PINACEAE* SPECIES

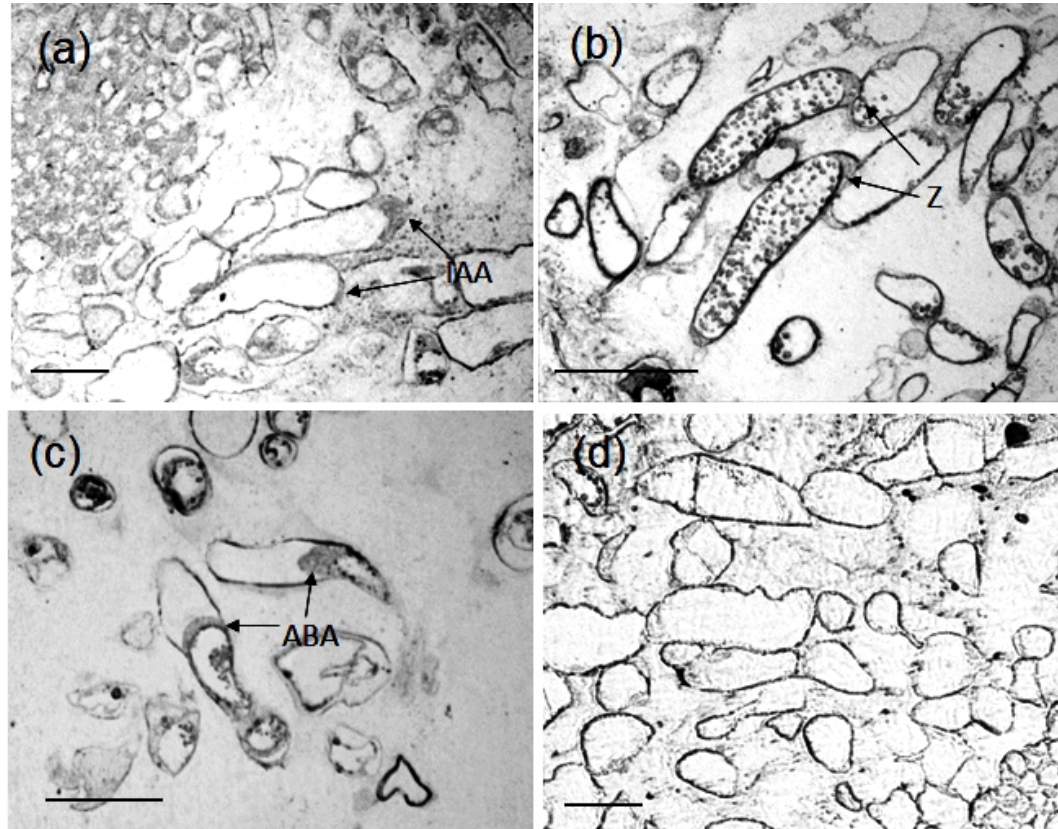


The first peculiarity of embryogenic culture (EC) in *Pinaceae* species is the elongation, polarization, and asymmetric division of somatic cells, as well as the localization of IAA at one end of the elongated cell. In *L. sibirica* the content of IAA in ESM was about 100 times higher than in non-embryogenic calli*. At the same time, a low ABA content was characteristic of embryogenic cultures.



Initiation of callus cultures in *P. sibirica*: (A) elongated cells; (B) isodiametric cells. Bars = 100 μm .

INITIATION OF CALLUS CULTURES IN *PINACEAE* SPECIES



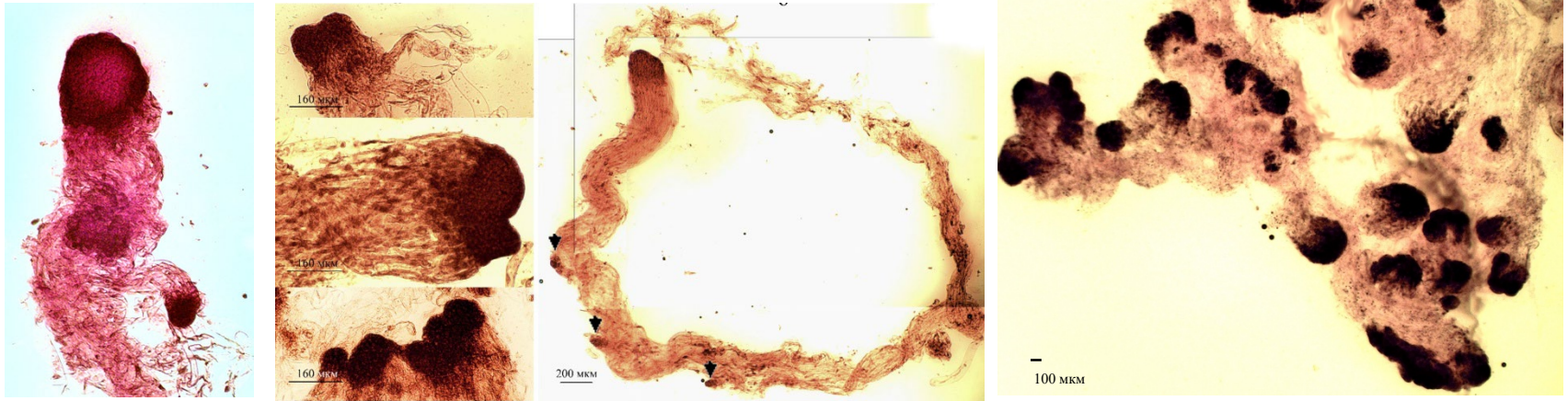
Immunohistochemical localization of IAA (a), zeatin (b) and ABA (c) during the initiation of somatic embryos in *Larix sibirica* (23 day of culturing). Non-colored cells of the initiated ESMs under the treatment of sections with non-immune serum (d). Legend: z – zeatin. Bar in all photos = 100 µm

*Tretyakova, I. N., Shuklina, A. S., Park, M. E., Yang, L., Akhiyarova, G. R., & Kudoyarova, G. R. (2021). The Role of Phytohormones in the Induction of Somatic Embryogenesis in *Pinus sibirica* and *Larix sibirica*. *Cytologia*, 86(1), 55-60.

MULTIPLICATION OF *LARIX* SOMATIC EMBRYOS



Further, a well-developed embryogenic tissue is formed, represented by the embryonal-suspensor mass (ESM), in which there is an active formation of globular somatic embryos through cleavage. In contrast to EC, non-embryogenic calli (NEC) consist of isodiametric, actively dividing cells.

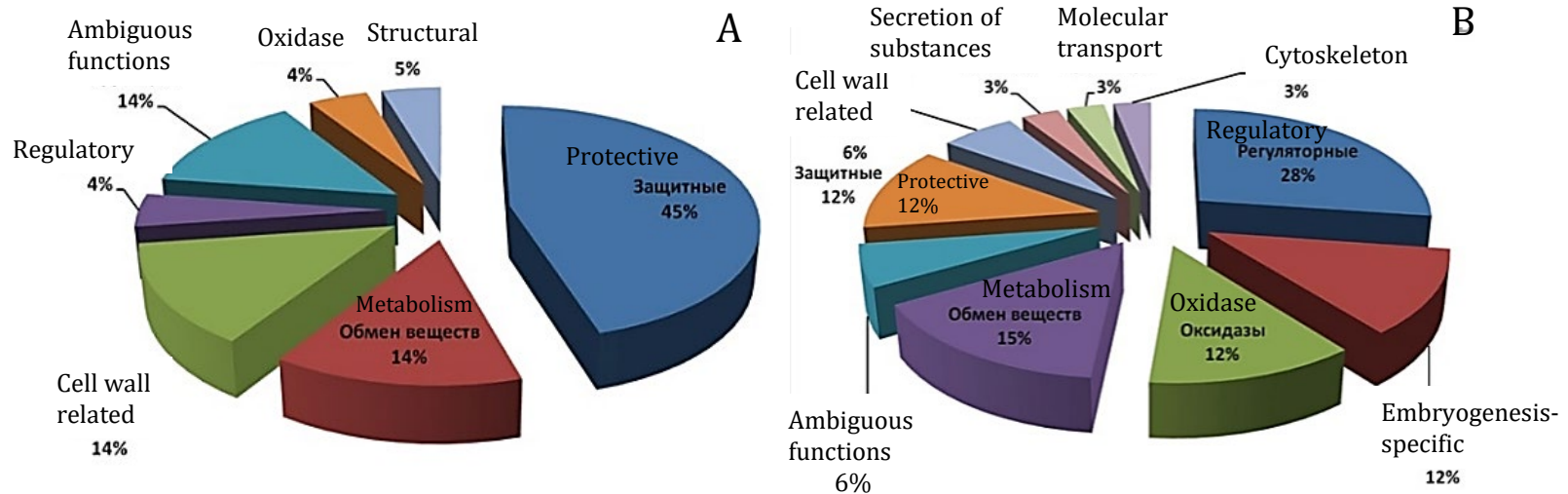


Multiplication of *Larix* somatic embryos: polyembryonal complexes and cleavage

TRANSCRIPTOMES OF EC AND NEC



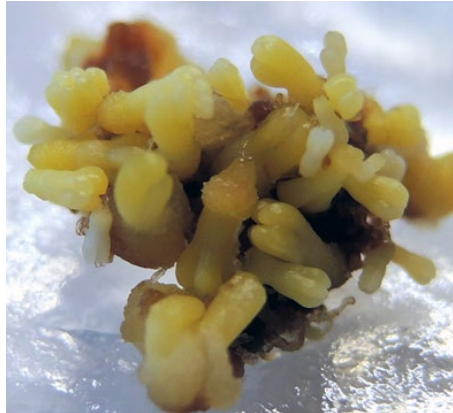
Reading of transcriptomes of EC and NEC in the culture *in vitro* of *Larix sibirica* by automated processing showed that the greatest differences in the level of expression of EC are characterized by proteins responsible for the regulation of physiological functions and associated with the development of the embryo.



Distribution of proteins associated with differentially expressed EST loci: A - in non-embryogenic cell lines, B - in embryogenic cell lines of Siberian larch.

CONCLUSION

Thus, the markers of somatic embryogenesis of *Pinaceae* species are: polarization and asymmetric division of elongated cells, formation of ESM, localization of IAA in the elongated cell, high content of IAA in the ESM, as well as the presence of proteins responsible for physiological functions and embryogenesis.



The reported study was funded by Russian Foundation for Basic Research, Government of Krasnoyarsk Territory, Krasnoyarsk Region Science and Technology Support Fund to the research projects number 19-44-240009.



THANKS FOR YOUR ATTANTION

e-mail: culture@ksc.krasn.ru