Subject Area: **Advanced Methods in Biotechnology and Biodiversity**
Subject: ***Plant morphogenesis in vivo and in vitro***
Level: **PhD** Year: **I-IV** Semester***:*** **1-2**
Status: **Facultative** ECTS: **3**
Department: ***Genetics***
Cooperating Department: ***Cell Biology***
Form of teaching (Number of hours; Form of assessment: Exam or Credit)

Lectures	*Seminars/Conversatoria*	*Practicals*	*Total*
4	**0**	**26**	**30**

Staff:

SUBJECT COORDINATOR: Prof. Małgorzata D. Gaj PhD
LECTURE: Prof. Małgorzata D. Gaj PhD, Prof. Ewa Kurczyńska PhD

Contents: LECTURES:

Genetic determination of plant morphogenesis in vitro

Arabidopsis as a model in studies on plant morphogenesis *in vivo* and *in vitro*. *In vitro* culture systems used to identified genes involved in plant regeneration. Somatic embryogenesis as a model for understanding genetic determination of zygotic embryogenesis. Advances in the identification of specific genes that are involved in organogenesis and somatic embryogenesis *in vitro* and selected technical tools applied.

Nuclear genome changes in morphogenesis *in vitro* and *in vivo* Modifications of the cell cycle that create endopolyploidization and their molecular mechanisms. Family-specific occurrence of endopolyploidy (polysomatic and nonpolysomatic plants). Endoreduplication in plant development. Tissue- and organ-specific endopolyploidization patterns.

Polysomatic and nonpolysomatic plant in in *vitro* culture. Somaclonal variation – chromosomal aberration in cultured cells and regenerated plant. Methods of nuclear DNA content measurement.

Symplasmic communication and plant development

Plasmodesmata – structure and function. Molecular mechanism of molecules exchange through plasmodesmata. Symplasmic fields and domains. Changes in symplasmic communication in connection with cell and tissue differentiation in *in vivo* and *in vitro* conditions. Methods used in analysis of symplasmic communication.

PRACTICALS: The practicals include the following techniques:

- RNA extraction from different stages of embryogenic *Arabidopsis* culture (control versus mutant impaired in somatic embryogenesis). Reverse transcription
- Analysis of expression of selected genes related to somatic embryogenesis process with use of Quantitative Real-Time PCR (qRT-PCR)
- Karyotype analysis of callus derived form polysomatic and nonpolysomatic plant.
- Analysis of developmental changes of polysomaty pattern using flow cytometry.
- Comparative analysis of endopolyploidy patterns in various plant organs and tissues using imaging cytometry.
- Analysis of symplasmic transport fluorochromes (low-molecular weight and with dextrans) distribution in explants during different stages of development and somatic embryos with the use of fluorescence microscopy and confocal microscopy (CLSM)
- Determination of the symplasmic domains and subdomains in relation to cell and tissue differentiation

Methods and forms of teaching: Lectures illustrated by computer presentations and video projector.
Requirements: Knowledge of genetics, cytogenetics, cell biology and molecular biology at the basic level.
Literature *(maximum 5 sources, all in English)*:

1. Shimkets RA. 2004. Gene expression profiling. Methods and protocols. Humana Press, Totowa, New Jersey, pp. 165
2. Wong ML, Medrano JF. 2005. Real-time PCR for mRNA quantitation. Biotechniques. 39 (1): 75-85.
3. Barow M, 2006, Endopolyploidy in seed plants. Bio Essays 28: 271-281
4. Doležel J, Greilhuber J, Suda J. (eds), 2007, Flow Cytometry with Plant Cells. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
5. van BEL, van Kesteren "Plasmodesmata. Structure, function, role in cell communication:, Springer, 1999

Remarks *(if necessary):*