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Theoretical and Biotechnological Approaches in the Institute of Biology Bucharest Between 1975 - 2015 based on Plant Cell and Tissue Culture Technology

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SUMMARY. In the present paper we highlight the main research stages of the laboratory of plant cell and tissue culture at the Institute of Biology, Bucharest from the moment of its beginning until present.

Keywords: electrostimulation, *in vitro* plant conservation, plant transformation, protoplasts, secondary metabolites in *in vitro* cultures.

The year 1975 marked the beginning of research on plant biotechnology at the Institute of Biology Bucharest by the study of *in vitro* culture of plant cells. The team of researchers was coordinated by Dr. Aurelia Brezeanu within the newly created Laboratory of Plant Morphogenesis and Genetic Engineering, at the initiative of AcaD.G. Zarnea.

During the first stage (1975-1983) several basic research directions were started, that contributed to a better understanding of the biology of the plant development: plant *in vitro* cytodifferentiation and morphogenesis (Badea *et al*., 1982; Brezeanu and Davis, 1979; Davis *et al.,* 1976, Davis *et al.,* 1979; Brezeanu, 1980; Brezeanu, 1983; Brezeanu *et al*., 1980; Brezeanu *et al*., 1981; Brezeanu *et al*., 1982b; Mirancea and Brezeanu, 1986; Pătraşcu, 1981); protoplasts technology, a pioneer activity in Romania (Brezeanu *et al*., 1982a; ***, 1984). The studies were focused on microbial protoplasts (yeasts, bacteria) (Anghel *et al*., 1985; Anghel *et al*., 1989a; Zarnea *et al*., 1988) and plant protoplasts, including isolation of protoplasts (Brezeanu and Roşu, 1984; Cornea *et al*., 1992; Pătraşcu and Brezeanu, 1989), their chemical or electric fusion (Anghel *et al*., 1989b; Cornea *et*

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al., 1993). Another pioneering research direction during that period was the study of androgenesis/gynogenesis as tools for plant breeding (Badea and Raicu, 1982; Badea and Raicu, 1983; Badea *et al*., 1985; Raicu and Badea, 1979).

The 2nd stage (1984-1989) was more focused on biotechnological applications using plant cell and tissue cultures. A complex network of scientific cooperation was established with research institutes, experimental stations and universities. Important results were obtained regarding: clonal multiplication (Brezeanu *et al*., 1982b; Iordan *et al*., 1981a; Iordan *et al*., 1984; Roşu and Anghel, 1985), production of virus-free plants (Brezeanu *et al*., 1994a; Pătraşcu *et al*., 1985).

The 3rd stage (1990-1998) was mostly devoted to fundamental research topics including: gene transfer in plant cells mediated by bacterial plasmid vectors using direct (electroporation and electrotransfection) or indirect methods (co-cultivation) (Brezeanu *et al*., 1993b, Brezeanu *et al*., 1994b; Brezeanu *et al*., 1999a; Călin *et al*., 1996; Fologea *et al*., 1998; Iordan-Costache *et al*., 1996; Pătraşcu-Călin *et al*., 1991); electrostimulation in *in vitro* systems of cytodifferentiation and morphogenesis by using weak electric fields (Cogălniceanu *et al*, 1998b; Cogălniceanu *et al*, 1998c; Cogălniceanu *et al*., 2000b; Radu *et al*., 1994); the effect of hipersalinity (Holobiuc *et al*., 1999) and high aluminum concentration on plant genome (Badea *et al*., 1994b); the study of chimaera gene expression (Badea *et al*., 1999c) involved in pollinic (Badea *et al*., 1994a) and somatic embryogenesis (Badea *et al*., 2000) in *in vitro* plant cultures; the role of endo- and exogenous factors in expressing the androgenetic and/or gynogenetic potential thus regenerating haploid plants used in programs of genetic improvement production of haploid plants (Badea and Răduţoiu, 1999), somaclonal variability in *in vitro* cultures (Badea *et al*., 1991; Badea *et al*., 1999a) and *in vitro* stress selection (Badea *et al*., 1999b).

The 4th stage (1999-2005) continued fundamental research directions, such as apoptosis and senescence in plants using *crown gall* tissues infected by *Agrobacterium tumefaciens* (Brezeanu *et al*., 2001; Brezeanu *et al*., 2002; Maximilian *et al*., 2003; Maximilian and Brezeanu, 2004). In the international context focused on the phytohormonal control of plant development, our laboratory initiated studies on several factors that have a potential role as signals: aliphatic polyamines (Antofie and Brezeanu, 2000b; Brezeanu *et al*., 2000; Carasan and Brezeanu, 2002; Dumitrescu and Carasan, 2002), salicylic acid (Antofie *et al*., 2000; Antofie *et al*., 2003), external electric current (Cogălniceanu, 2006, Cogălniceanu *et al*., 2001b) and on biochemical markers (Carasan and Antofie, 2007; Carasan *et al.*, 2004a, Cogălniceanu *et al*., 1998a; Voichiţă *et al*., 2013; Voichiţă *et al*., 2014) involved in the morphogenetic processes in experimental *in vitro* systems, both in test and recalcitrant plant species (Badea and Săndulescu, 2001). The mechanisms

that induce stress tolerance (Antofie et al., 1999; Brezeanu, 2009; Carasan, 2009; Carasan *et al*., 2003; Cogălniceanu and Brezeanu, 2000) were studied by simulating in *in vitro* conditions using stress inductors like PEG (Dumitrescu, 2005), mannitol (Mitoi *et al*., 2009), sorbitol (Dumitrescu and Holobiuc, 2005), and specific mediators (ABA) (Carasan *et al*., 2004b; Carasan et al., 2005; Dumitrescu and Carasan, 2006), thus isolating cell line and/or tolerant individuals (Brezeanu, 2009, Brezeanu *et al*., 2002). Another major research direction was the potential utility of *in vitro* cultures to proliferate and to biosynthesize secondary metabolites of biotechnological interest. Thus, the study of *Vitis vinifera* callus resulted in the isolation of a *long-term* cell line highly proliferative and with a significant production of compounds valued by the pharmaceutical industry: anthocyanins, pycnogenol and resveratrol (Brezeanu *et al*., 1999b; Lupşea and Brezeanu, 1999; Cogălniceanu *et al*., 2000a; Matienco *et al*., 2004).

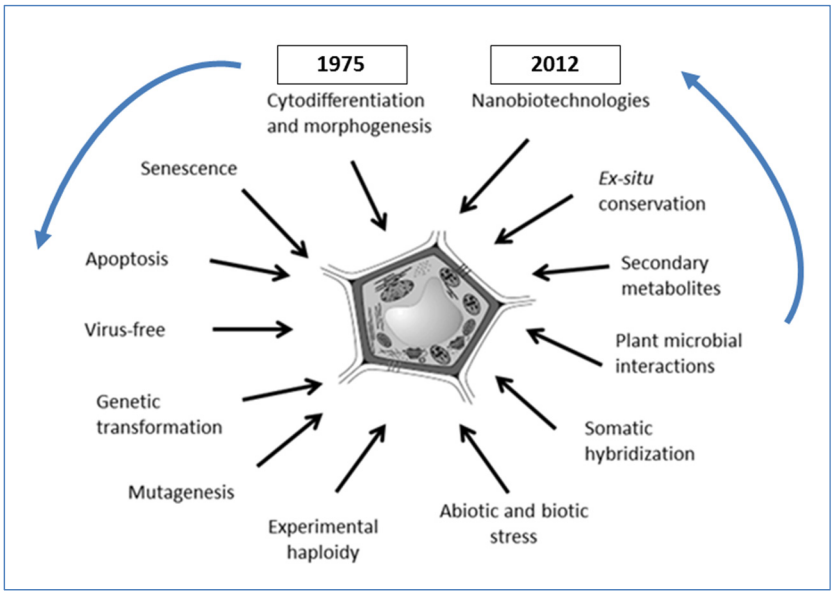

Figure 1. Schematic diagram of the main theoretical and biotechnological research topics at the Institute of Biology Bucharest, during the period 1975-2015 based on plant tissue and cell culture technologies.

The present stage includes several directions of research. One is focused on biodiversity conservation, by characterizing the *in vitro* development of protected plant species of Romania for their *ex situ* preservation (Blîndu *et al*., 2010; Cogălniceanu and Cogălniceanu, 2010; Holobiuc, 2007; Manole-Păunescu, 2014; Păunescu, 2008; Păunescu, 2009). Our studies have established micromultiplication procedures and

medium-term (Catană *et al*., 2010b; Holobiuc and Blîndu, 2006; Holobiuc *et al*., 2009c; Holobiuc *et al*., 2010) or long-term (Banciu and Cristian, 2015; Holobiuc and Catană, 2012; Holobiuc *et al.*, 2009a) storage conditions. A large variety of plant taxa have been introduced in vitrocultures: bryophyte (Brezeanu *et al.,* 2008; Brezeanu *et al.,* 2009; Cogălniceanu and Stoiculescu, 2007; Cogalniceanu, 2014), lichens (Banciu and Cristian, 2015; Cristian *et al*., 2013; Voicu and Brezeanu, 2008a; Voicu and Brezeanu, 2008b), ferns (Aldea *et al*., 2013; Banciu *et al.,* 2009a; Brezeanu and Banciu, 2009; ***, 2011) and a large number of vascular plants, from many families. Plant-microbe interactions (Cogălniceanu *et al*., 2010; Helepciuc *et al*., 2008; Helepciuc *et al*., 2014, Maximilian and Carasan, 1999; Maximilian *et al*., 1998; Maximilian *et al*., 2001, Maximilian *et al*., 2002), plant *in vitro* systems for secondary metabolites biosynthesis (Mihai *et al*., 2009; Mihai *et al*., 2010; Mihai *et al*., 2013) and plant *in vitro* systems involved in bio-nano-technologies (Matei *et al*., 2015; Mitoi *et al*., 2013) are our ongoing research topics.

Numerous plant species from different taxa have been studied over time in our lab from which we mention the most representative (species listed in alphabetical order): *Alyssum borzaeanum* Nyar. (Păunescu, 2007), *Andryala levitomentosa* (E. Nyar.) P.D. Sell. (Păunescu and Vântu, 2002), *Armeria maritima ssp. alpina* Willd (Blîndu and Holobiuc, 2006), *Arnica montana* L. (Panciu *et al*., 2014), *Artemisia tschernieviana* Besser (Holobiuc and Blîndu, 2006-2007), *Artemisia alba* Turra (Holobiuc and Blîndu, 2006-2007), *Astragalus pseudopurpureus* Guşuleac (Holobiuc *et al.*, 2005), *Bucegia romanica* Radian (Brezeanu *et al*., 2008; Cogălniceanu, 2014), *Campanula carpatica* Jacq. (Holobiuc and Blîndu, 2006-2007), *Campanula polymorpha* Witasek (Păunescu, 2010), *Campanula romanica* Săvul. (Manole and Banciu, 2014), *Centaurea pontica* Prodan & Nyar (Voichiţă *et al*., 2014), *Cerastium transsilvanicum* Schur. (Păunescu, 2005), *Cetraria islandica* (L.) Ach. (Voicu and Brezeanu, 2008b, Cristian *et al*., 2013), *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Roşu *et al*., 1987), *Cladophora vagabunda* (L.) Hoek (Bavaru *et al.,* 2000), *Convolvulus persicus* L. (Holobiuc *et al*., 2015; Voichiţă *et al*., 2013) , *Datura inoxia* Mill. (Badea and Răduţoiu, 1999), *Dianthus callizonus* Schott et Kotschy (Păunescu and Holobiuc, 2003), *Dianthus glacialis* Haencke *subsp gelidus* (Schott, Nyman et Kotschy) Tutin (Holobiuc *et al*. 2009a; Holobiuc *et al*., 2010), *Dianthus nardiformis* Janka. (Holobiuc *et al*., 2009b), *Dianthus spiculifolius* Schur. (Holobiuc *et al*. 2009; Holobiuc *et al*., 2010), *Dianthus tenuifolius* Schur. (Păunescu and Holobiuc, 2005), *Dianthus trifasciculatus* Kit. (Holobiuc *et al*., 2014), *Doronicum carpaticum* (Griseb. et Schenk) Nyman (Holobiuc and Blîndu, 2006-2007), *Doronicum orientale* Hoffm. (Holobiuc and Blîndu, 2006-2007), *Draba dorneri* Heuff. (Catană *et al*., 2013), *Ecballium elaterium* (L.) A. Rich. (Voichiţă and Brezeanu, 2005), *Erigeron nanus* Schur. (Catană *et al.,* 2010a), *Fragaria X Ananassa* Duch. (Cogălniceanu *et al*., 2010), *Gardenia jasminoides* J. Ellis (Antofie and Brezeanu, 2000b; Antofie *et al*., 2000; Antofie *et al*., 2003), *Gentiana lutea* L.

(Holobiuc *et al*., 2008a; *Holobiuc* and Catană, 2012; Holobiuc, 2015), *Helianthus annuus* L. (Brezeanu, 1980; Brezeanu, 1983), *Hieracium pojoritense* Woloszczak (Holobiuc *et al*., 2004), *Iris halophila* Pallas (Holobiuc and Blîndu, 2006-2007), *Marsilea quadrifolia* L. (Banciu *et al*., 2009a; Brezeanu and Banciu, 2009), *Medicago sativa* L. (Badea *et al*., 1994b; Holobiuc *et al*., 1999), *Nicotiana tabacum* L. (Brezeanu *et al.,* 1982a; Cogălniceanu and Brezeanu, 1995; Cogălniceanu *et al*., 1998a; Mirancea and Brezeanu, 1986), *Ocimum basilicum* L. (Brezeanu and Cogălniceanu, 2005), *Oryza sativa* L. (Brezeanu and Davis, 1979), *Papaver alpinum* L. ssp. *corona-sancti-stefani* (Zapal.) Borza (Catană and Holobiuc, 2015), *Petunia x hybrida* Vilm. (Antofie *et al*., 2004; Carasan and Antofie, 2007), *Populus* (Iordan *et al*., 1984; Iordan *et al*., 1996), *Primula halleri* J.F. Gmelin (Holobiuc and Blîndu, 2006, Holobiuc and Blîndu, 2007), *Prosopis juliflora* (Sw.) DC. (Cogălniceanu *et al*., 2001a), *Pseudevernia furfuracea* L. (Banciu and Cristian, 2015), *Quercus robur* L. (Iordan *et al*., 1981b), *Ruscus aculeatus* L. (Banciu *et al*., 2009b; Manole and Banciu, 2015), *Salix* (Iordan and Brezeanu, 1985), *Scilla autumnalis* L. (Banciu *et al*., 2010), *Sequoia sempervirens* (Lamb. ex D. Don) Endl. (Stoiculescu *et al*., 2009), *Serratula bulgarica* Acht. et Stoj. (Manole-Aiftimie *et al.,* 2013), *Solanum melongena* L. (Roşu and Anghel, 1985), *Solanum tuberosum* L. (Blîndu and Holobiuc, 2005; Călin *et al*., 1996; Pătraşcu *et al*., 1985), *Spathiphyllum patinii* (R.Hogg) N.E.Br. (Antofie and Brezeanu, 2000a; Antofie and Brezeanu, 2003), *Stevia rebaudiana* (Bert.) Bertoni (Călin and Brezeanu, 1997), *Syngonium podophyllum* Schott (Antofie and Brezeanu, 2004; Antofie *et al*., 1999), *Triticale* (Verzea *et al*., 1994), *Triticum aestivum* L. (Angheluţă *et al*., 1997; Badea *et al*., 1991), *Triticum monococcum* L. (Davis *et al*., 1979), *Ulva rigida* C. Agardh (Bavaru *et al*., 2000), *Usnea barbata* L. (Voicu and Brezeanu, 2008a; Brezeanu and Voicu, 2008), *Veronica multifida ssp. capsellicarpa* (Dubovik) A. Jelen (Holobiuc *et al*., 2006; Holobiuc *et al*., 2008b), *Vitis vinifera* L. (Brezeanu *et al*., 1980; Brezeanu *et al*., 1993a), *Xanthoria parietina* (L.) Th. Fr. (Voicu and Brezeanu, 2008b), *Zea mays* L. (Maximilian and Carasan, 1999; Maximilian *et al.,* 2000).

Our laboratory has published during this period 15 books and book chapters and about 1000 scientific papers, both at national and international level. Under the scientific supervision of Dr. Aurelia Brezeanu over 42 doctoral theses in the field of cell biology and plant biotechnologies were finalized.

The scientific recognition and importance of our results was highlighted by the medals and prizes received:

International awards:

- **The Prize of the Academy of Sciences of the Moldova Republic**, 2004, for the book: *Carpoculture in vitro. Nonmorphogenetic pathway* (Matienco *et al*., 2004)

- **The Silver Medal at the Geneva Inventions Contest**, 2006, for the patent: *Carbonic material and obtaining procedure for anthocyanin pigment biosynthesis* (Hristea *et al*., 2005)

- **The highest Prize, *Fritzphil,* at the Scientific International Film Festival in Argentina**, 1986, for *Buds*, a scientific documentary film produced by Sahia Film Studios in 1985, with the director Mircea Popescu and scientific consultant Dr. Aurelia Brezeanu

National awards:

- ***Emil Racoviţă*** Prize *(Romanian Academy),* 1982, for the atlas: *Ultrastructure of the Plant Cell* (Anghel *et al*., 1981)

- ***Ion Ionescu de la Brad*** Prize *(Romanian Academy)*, 2012, for the book: *Conservarea Geo- şi Biodiversităţii şi dezvoltarea Durabilă în Ţara Haţegului-Retezat* (Mitoi and Blîndu, 2010)

- ***Ion Haşeganu*** Prize *(Romanian Horticulture Society),* 2013, for the book: *Utilizarea experimentală a elicitorilor fungici pentru imunizarea plantelor contra putregaiului cenuşiu* (Matei *et al*., 2011)

In the 40 years of scientific activity the following researchers worked in the field of plant biotechnology at the Institute of Biology Bucharest: ALDEA Florentina, ANGHELUŢĂ Rodica, ANTOFIE Maria Mihaela, AVRAM Dorina, BADEA Elena Marcela, BANCIU Crisitan, BREZEANU Aurelia, CATANĂ (BLÎNDU) Rodica, CĂLIN (PĂTRAŞCU) Alexandrina, CÂRCIUMĂRESCU Doina, COGĂLNICEANU Gina, COMAN Ion, CORNEA Petruţa Călina, CUCU (CIUCU) Natalia, DUMITRESCU Rodica, GREGORIAN Liliana, HELEPCIUC Florenţa Elena, HOLOBIUC (LUPUŞANSCHI) Irina Mihaela, IORDAN (COSTACHE) Margareta, LUPŞEA Simona, MANOLE (PĂUNESCU) Anca, MAXIMILIAN (VOICHIŢĂ) Carmen, MIHAI (STOICULESCU) Raluca, MIRANCEA Dorina, MITOI (CARASAN) Monica Elena, ROŞU Ana, SAVU Lorand, SCRIPCARU Atena, VASSU - DIMOC Tatiana, VĂTAFU Ion, VOICU (CRISTIAN) Diana, ZAMFIR Medana.

The results briefly presented in this paper highlight the main aspects of the research topics in the Institute of Biology Bucharest in the fascinating field of plant cell and tissue culture over the 40 years of existence.

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