

Plant Cell and Tissue Cultures – A Page in the History of Biology in Romania

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In honorem Dr. Dorina Cachiță, “*The Lady of in vitro plant culture*”

SUMMARY. Four decades have passed since a new research field started being promoted in Romania: *in vitro* cell and tissue cultures of plants. This paper highlights the outstanding contribution to its development made by a reputed specialist – Dr. Dorina Cachiță, the pioneer in this field in our country. Moreover, the paper presents the social and economic context at the time this domain was launched, the difficulties in promoting it, it describes the author’s beginnings and personal achievements together with the ones of his research group in the field of *in vitro* plant culture. On the whole, the article represents a page in the history of Romanian biology in Romania and is dedicated to the honour of PhD Prof. Dorina Cachiță.

Key words: achievements, beginnings, *in vitro* cultures, plants

Introduction

Some specialists in plant tissues and cell cultures in our country headed by Professor Elena Rakosy took the commendable initiative of organizing a jubilee session to mark the anniversary of four decades of research in this domain in Romania. A moment of assessment of contributions brought by a large number of compatriots who have devoted much of their work to this kind of research, but also an opportunity to celebrate a reputed researcher in the field, an emblematic figure of Professor Dorina Cachiță, who devoted so many decades of her professional life to *in vitro* cultures of plants. It is an area of research that this well-known specialist initiated and served with devotion.

I consider that such a moment was necessary, that the celebration of Professor Cachiță comes to recognize her undeniable merits in promoting this

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research area, because, when you say *in vitro* culture of plants in Romania, you almost automatically associate the field with a name – Dr. Dorina Cachiță. To attest this outstanding contribution, we can mention her publishing more than 270 scientific articles in various journals and books, 13 books, textbooks and book chapters devoted to the field, registering 12 patents related to *in vitro* culture, setting up (with colleagues) of the Romanian Association of Tissue and Cell Cultures (RATCC), organising (from 1981 to 2008, with collaborators) 15 editions of RATCC national symposium and the publication of papers presented at these meetings in proceedings volumes, organising and setting up specialised laboratories in several institutions (Institute of Biological Research in Cluj-Napoca, Oradea University, Biological Research Center in Jibou, "V. Goldis" University etc.), postgraduate (doctoral) supervising many young biologists from around the country, participating in numerous congresses, conferences and symposia organized in the country and abroad etc. It has been a remarkable effort and contribution to the development of science in Romania, which had to be honoured in some way, and this scientific event (19 March 2016) brings homage to the researcher, teacher and woman who was and is Dr. Dorina Cachiță. It was the reason for my decision to dedicate this article to her – a page in the history of Romanian biology – in sign of appreciation, respect and tribute to the personality of this colleague and friend.

Personal motivation to approach *in vitro* cultures of plants

It was in 1975. The first steps were taken in Romania to address a highly topical/ fashionable field in the world, but not to us –*in vitro* cell and tissue cultures of plants. I had been working for several years in an equally important area at the time – experimental mutagenesis in plants. The stake of mutagenesis research was not only knowledge of the cytogenetic, biochemical, physiological and chemical repercussions of physical and chemical mutagens in plants, but also their use in the induction of useful variations in the improvement of medicinal plants, all these representing the focal point of my research group. I knew something about the impact of *in vitro* cultures of plants, about some of their applications and we reckoned that an effective method of *in vitro* cloning of valuable genotypes isolated by us from the local populations of some species or populations mutagenized would have been extremely useful in their multiplication and obtaining seedlings necessary for the extension of these genotypes in field crops. So this is how I started.

At that time, I was conducting my research in a team in Pângărați (Neamt), at a Research Station established by “Al. I. Cuza” University, Iassy (1956); opened in 1970, for a short while it operated autonomously, before being affiliated to the Biological Research Centre (BRC) in Iassy (given that, in 1974, research centres were imposed a minimum of 60 members with higher education to preserve their status of legal entities). This centre along with a similar one in Cluj and Bucharest Institute of Biological Research constituted the Central Institute for Biological

Research (under the Ministry of Education). At the beginning of 1970, scientific research was required to function on contract, an action which was meant to streamline this sector. In retrospect, the measure had some positive effects, including empowering researchers, but the beginning was a real shock for us, researchers, because – on the one hand, we were not prepared to face this challenge, on the other hand, identifying sources of funding for research was far from being an easy task. In addition, great emphasis was placed exclusively on applied research, without taking into account the fact that, without major theoretical research, it is difficult to make progress in applicative research.

Hard times had come for research, in general, and for biological research, in particular, because, at a certain moment, it was on the brink of dissolution. Let me explain. Nicolae Ceausescu used to visit sites, enterprises, agricultural and research units, in order to witness the progress made in one area or another and feel "the devotion of the masses to the party". Such a visit took place in the mid 70s at the Institute of Biological Research in Bucharest. During that visit, some colleagues were rather uninspired and, instead of highlighting their achievements, their striving to make something out of nothing (because such were the times), they made the mistake to complain about various shortcomings faced by laboratories (some indeed ridiculously called labs), which was enough for Ceaușescu. He concluded (obviously being wrong) that biological research went the wrong way and that remedial measures had to be taken urgently. An immediate consequence of that visit was transferring some colleagues who had research interests common with agriculture to agricultural research units. Another measure imposed was that, every semester, the representatives of research teams from the Central Institute of Biology were called for meetings and had to draw up progress reports, addressing achievements and research perspectives. These meetings were usually attended by the vice president of the National Council of Science and Technology, Mihail Florescu (who had been appointed to monitor and improve the situation in biological research), and a deputy minister of education.

At Pângărați Research Station of the Centre for Biological Research in Iassy, this „earthquake" occurred somewhat later (in 1982) and it was even more painful than the episode previously reported, being caused more by subjective factors rather than by economic or professional ones. A representative centre, comprising more than 40 researchers, known and appreciated in the country (and abroad) for their achievements was broken into three smaller research groups (the one to which I belonged was about to disappear) subordinated to different ministries. After that unfortunate moment, about which I wrote on another occasion (Ghiorghiță, 2012) our mission to demonstrate our value and sustainability became more difficult, because we had to fight not only against material deprivation, but also against malevolence and prejudices of those patronising us.

Returning to *in vitro* cultures, I must say that, at that time, it was not easy to become initiated into this kind of work, because Romania faced major problems not only with the (poorly equipped) research laboratories, but with documentation and

scientific information (few subscriptions to magazines from abroad, and documentation and specialization in laboratories abroad was more a dream than reality). Fortunately, there were still Romanians who could benefit from documentation and specialized internships abroad, and those who returned to the country were also trying to capitalize experience. One such example is Professor Dorina Cachiță who worked at the Center for Biological Research in Cluj. As a young scientist, she received a grant to study in Belgium, in a laboratory of Free University of Brussels, which specialized in *in vitro* culture of plants. Once she returned home, she tried and managed to procure equipment and supplies necessary to practice these techniques and equipped a laboratory and a climate chamber to grow these cultures, which, in my opinion, turned her into a pioneer in this field in our country. Later on, a few such laboratories were established at other institutions in the country: Biology Institute in Bucharest, Bucharest University, Institute of Forest Research Ștefănești (Bucharest), ICCPT Fundulea, Agronomic Institute in Bucharest, Viticulture Research Station in Valea Călugărească and Ștefanesti (Arges), ‘Stejarul’ Research Station Pângărați / Piatra Neamt, Vegetable Research Station in Bacau etc.

The urge to promote this kind of work in my institution more than four decades ago brought me in the position of getting to know Dr. Cachiță, who was the best suited specialist for this purpose. I obtained permission over the phone to visit her lab and obtain some information to help me to approach such research at my department. The visit took place in 1975, at a time when dr. Cachiță was in full swing organizing the lab, so I saw its foundation, the necessary equipment and was provided with some information in the field, along with the promise to support such concerns at my lab. Particularly important in this context was the experience obtained by my colleague Dr. Catherine Toth, who, during the 1970s benefited from several internships in biochemistry at the Biological Research Center Szeged of the Academy of Sciences of Hungary; it was a great opportunity to visit the plant tissue culture laboratory in their centre and obtain materials and information vital to the fulfilment of our dream.

The intention of approaching this area was salutary, but its putting into practice was more difficult because we lacked in our institution what was required for such an activity. Instead, we were young and enthusiastic, and Romanians are known to be inventive when their ambition is challenged. And the challenge was too great to resist. At Pângărați, we had already set up a room for growing plants in the basement (with walls more than 1m thick), where it was easier to maintain the temperature within variation limits in summer, the lighting was ensured by fluorescent lamps, and heating with electric heaters. It was a room intended to conduct experiments in plant physiology (growth tests, hydric behaviour etc.).

The laboratory was equipped with enough air ovens and ultraviolet lamps, we could find autoclaves at our colleagues, the microbiologists, but we missed an essential piece of equipment, namely a cell culture hood (with sterile air) in which to inoculate the biological material on nutrient media sterilized under aseptic

conditions. In addition, we had to procure some specific substances: growth regulators, agar, sucrose etc., included in culture media. The action to purchase the materials necessary for *in vitro* cultures of plants took us quite some time and many of them were thanks to those whom we asked for support. Not being able to obtain a cell culture hood (the investment chapter of the ministry for Pângărați being zero), we thought of ways to manage without it in order to perform inoculations of biological material in sterile conditions. The strategy we adopted to overcome this handicap may seem now a story, or madness, but it was all true.

One day (in 1980), we decided to get to work and try our fortune. As I said, having a Laboratory of Microbiology, we could benefit from the expertise of our colleagues to work under sterile conditions. Colleague Catherine Tóth prepared some hormonal variants of Murashige-Skoog culture media (1962), in which we included sucrose (as carbon source) and agar (for hardening), the medium being distributed then in Erlenmeyer flasks 100 ml, covered with cotton plugs shrouded in gauze. To create sterile conditions, we placed a lamp on a support above the work table whose UV radiation beam was directed to it, and, on this table, we lit several lamps with alcohol. These were the conditions in which we handled the nutrient medium and performed inoculations with explants, seeking for solutions to protect our hands and face against UV radiation (but we could not do anything against air ionization). In order not to put our lives in danger, we decided to work in sessions of 10-15 minutes at that table, followed by long pauses (pauses during which only the UV lamp was functional). Despite all protective measures, my colleague (more sensitive to UV) could not avoid some burns on her face. After inoculation, the flasks were placed in a growth chamber and exposed to a photoperiod of 12 hours.

Our first experimental subjects were *Datura innoxia* and *Vinca minor* species (from which were used as explants uninodal apices and fragments of young shoots). Even if some vials got infected, the success rate was quite good, given the poor working conditions. The results that we obtained in these investigations were presented by colleague E. T. Tóth on the occasion of the first symposium of this kind, held in Cluj by Professor Cachiță, titled "*Tissue Cultures – a research tool in theoretical and practical plant biology*" and published in a proceedings volume elaborated on this occasion (Tóth and Ghiorgăiță, 1981). We were proud of our success and I think we had every reason to be, because we had done something out of nothing and the beginning was promising.

Then came the dramatic moment at the end of 1982 when our institution was practically dismantled and undeservedly dissolved. We were forced to leave the premises from Pângărați, relocate in Piatra Neamt and make a fresh start. It was a very difficult and demanding task, but all 40 researchers, technicians and workers from the former "Stejarul" Research Station adjusted to this new formula. Our main concern in the new context was to maintain our status of specialists in the field and demonstrate that the decision about disbanding Pângărați research unit had been a great error. We also resumed our work in the field of *in vitro* cultures of plants. At

first, because we did not have a plant growth chamber any longer, we arranged a closet for this purpose. Fortunately, in the building where we had moved, we had a basement which was quite spacious (but found in an advanced state of decay), which could be set up as a semi-climatic room for plant growth. With great efforts, we managed to turn this space into one that was healthy and suitable for our purpose.

After what happened to Pângărați Department, the Minister Mihail Florescu (Vice President of CNST) paid us yearly visits to check how we complied with the requirements imposed at the time, if we recovered and made progress in our projects (which, in fact, I had to report anyway every semester at any general meeting – as the head of this research group – indicating the stage at which we were and what practical achievements we had). The Minister was accompanied on his visits by the Party Secretary for economic problems in Neamt County, Eng. Mihai Roman. When the Minister repeatedly observed the conditions under which we worked and how we struggled to achieve *in vitro* cultures, he must have been impressed, so he put in the charge of the party secretary to appeal to the management staff of the Complex of Synthetic Fibres from Săvinești to assist us in arranging rooms for explants inoculation and culture incubation (including a climate control system) in our basement and to equip the lab with a more modern autoclave and a hood with sterile air, which was all achieved in a relatively short time. As an aside, despite other opinions, I believe that Minister Mihail Florescu's contribution to biological research was beneficial in those troubled times, as he was a man who proved much discernment, wisdom and tact in his actions. It was due to his intervention that we had better conditions in Piatra Neamt to assert ourselves in the field of *in vitro* cultures of plants.

It was a great achievement and satisfaction for us, but it had unintended consequences because Mihail Florescu Minister later visited Research Station for Medicinal and Aromatic Plants (SCPMA) Fundulea and reproached to the colleagues working there that there they had not developed the field of *in vitro* cultures, giving us as an example, a small group of research that was successful. Considering that some of our research contracts were financed by the Ministry of Education indirectly, through this station (to guarantee that our results in the field of medicinal plants are applied by them), the Minister's remark made the director of SCPMA Fundulea, dr. Emil Paun, take a grudge against us and, from that moment onwards, he would find every excuse to temper our enthusiasm and joy of success, always showing dissatisfied with our results.

A brief account of the objectives and accomplishments in this area

Our research group was formed in 1983 of seven researchers and four technicians and constituted the Group of Experimental Plant Biology and Genetics (which in 1990 acquired the rank of Research Laboratory). After suffering from the drama at the end of 1982, the expectations from us, the administrative and political

representatives, were very high. Every year, we made comments on dozens of lab and field experiences, planted experimental plots expanded on large areas, performed hundreds of tests etc. It was a hellish work. As there was so much field working which I was engaged together with my colleagues Florin Floria and Elvira Gille, I left *in vitro* cultures in charge of my colleague Catherine Tóth - to whom there joined in 1983 Tatiana Onisei, and in 1986 Doina Amariei.

After 1990, the external pressure which had been exerted on us (for seven years) disappeared, the field work diminished (because we had no lands in our property), which allowed me to return to *in vitro* cultures after that first attempt in the years 1980-1982. In February 1994, I became a professor at "V. Alecsandri " University of Bacau, but continued to work at my former institution in Piatra Neamt until 2007. I set up a properly equipped Laboratory of Genetics and Biotechnology in Bacau, including equipment (by means of research contracts) necessary to practice *in vitro* plant cultures, a laboratory which apprenticed many bachelor's, master's and doctoral students. I feel bound to make one more observation that may be surprising to many: in our work in the field of *in vitro* cultures of plants, we have been mostly self-taught, because we have not benefited from specializations in laboratories abroad. We told ourselves that, if others were successful in this area, so would we. And so it was. We learned from our own experience in the lab, from trial and error, and by participating in scientific meetings in the field how to overcome difficulties, how to progress.

The main objectives of the investigations in which I was involved were:

- Highlighting *in vitro* morphogenesis means for various explant types depending on explant nature, hormonal balance, growth conditions etc.;
- Regenerating plants through direct and indirect (via callus) organogenesis, analysing some morphological and physiological parameters for *in vitro* regenerants and studying their behaviour in the field;
- Analysing the biosynthesis capacity of secondary metabolites in *in vitro* and *ex vitro* cultures;
- Development of *in vitro* micropropagation technology to some of the species tested;
- Studying somaclonal variation and isolating potentially more valuable genotypes for culture;
- Haploid induction by experimental androgenesis and gynogenesis at some cultivated plants and observations on cytogenetic changes occurring in anther and ovary regenerants etc.

Some of the objectives previously mentioned were accomplished in investigating 26 medicinal and aromatic plant species: *Atropa belladonna*, *Vinca minor*, *Datura innoxia*, *Digitalis lanata*, *D. purpurea*, *Angelica archangelica*, *Anethum graveolens*, *Rosmarinus officinalis*, *Withania somnifera*, *Stevia rebaudiana*, *Stachys sieboldi*, *Catharanthus roseus*, *Trigonella foenum-graecum*, *Gentiana lutea*, *Mentha viridis*, *Mentha piperita*, *Melissa officinalis*, *Ocimum basilicum*, *Salvia*

officinalis, *Hyssopus officinalis*, *Chrysanthemum balsamita*, *Rhodiola rosea*, *Sedum hybridum*, *Sedum fabaria*, *Rosa canina*, *Hippophae rhamnoides*. For *Vinca minor*, *Stachys sieboldii*, *Datura innoxia*, *Mentha piperita*, *Mentha viridis*, *Rhodiola rosea*, there were elaborated *in vitro* micropropagation technologies.

A special achievement in this respect was obtained from *Rhodiola rosea*, which demonstrated that this technique can ensure their breeding and restocking with *in vitro* regenerated individuals in areas where the species is endangered (the Ceahlău Mountain) or disappeared (Ghiorghiță *et al.*, 2011). The investigations that followed the *in vitro* reaction of anthers and ovaries and the induction of experimental haploidy had as a subject some technical and vegetable crops: corn, sunflower, sugar beet, varieties of cabbage (for head, cauliflower, broccoli) tomatoes, potatoes, *Brassica juncea*.

I think that some technical contributions in the field of *in vitro* cultures of plants are not to be neglected either. My colleagues used as laboratory culture dishes Erlenmeyer flasks covered with cotton plug shrouded in gauze and accommodated neoplantlets regenerated *in vitro* in sterile potting soil. Both vials with nutritive medium and pots with soil (to accommodate *in vitro* regenerants) were sterilized in autoclaves. Since 1990, when I resumed my investigations in *in vitro* plant cultures, we no longer used this technique which facilitated contamination with bacteria and moulds. I decided to use two new strategies: a) I covered culture vessels (Erlenmeyer flasks) with double aluminium foil; b) to accommodate plants to septic conditions (*ex vitro*), I avoided using pots with sterile soil, finding it much easier and efficient to work in hydroponic environment. Proceeding this way, in addition to diminishing contamination hazards, the cost price decreased considerably. I shared these innovations with colleagues and many of them employed them in their research. I did not patent them as I did not consider it necessary.

The results obtained in investigations (in which I was personally involved) which aimed *in vitro* cultures of plants were the subject of 90 papers (in collaboration with some colleagues) published in various journals, but also in conference and symposia proceedings, in the country and abroad. In the *in vitro* Culture Laboratory of "Stejarul" Research Station in Piatra Neamt was conducted the doctoral research of 4 theses which included issues related to *in vitro* cultures authored by Tatiana Onisei (Onisei, 1995), Elvira Gille (Gille, 1996), Doina Amariei (Amariei, 2001), Mihaela Hârțan (Hârțan, 2009) and defended at "Al. I. Cuza" University of Iași. At the same university, as doctoral advisor, I supervised the doctoral theses elaborated by Daniela Nicuță (Nicuță, 2006), Diana-Elena Maftעי (Maftעי, 2007), Nicoleta Bădăluță (Bădăluță, 2011) whose research was conducted the Laboratory of Genetics and Biotechnology at the University "V. Alecsandri" of Bacău; Tina Oana Cristea (Cristea, 2008) – research undertaken in the laboratory for *in vitro* cultures of the Vegetable Research Station in Bacău; Daniela Ichim (Ichim, 2006) - research in the laboratories of the Faculty of Biology of "Al. I. Cuza " University); Justina-Brândușa Ciobanu (Ciobanu, 2012) – thesis elaborated in the labs of the Gene Bank in Suceava.

Based on data from specialised literature and personal results obtained in investigations conducted on *in vitro* cultures and the regenerants of various plant species, we have developed and published (in collaboration) in 2002 a monograph entitled "*Experimental Haploidy in the Context of Modern Biotechnologies*" (Prisecaru and Ghiorghiu, 2002) and a book which comprises a large chapter on *in vitro* plant cultures, entitled "*Biotechnology today*" (Ghiorghiu and Nicuța-Petrescu, 2005). Moreover, some of the results obtained in this research were valued by their presentation at numerous scientific events organized in the country and abroad, including symposia and conferences organized by us in Piatra Neamt and Bacau.

I will always have a vivid recollection of attending the International Congress of Plant Tissue and cell Culture (ICPTCC) held in Amsterdam (Netherlands) in June 1990, shortly after the events of 1989 in Romania, a congress where, among other colleagues, I could meet Professor Dorina Cachiță. It was a great joy to meet her again after many years. I lived in full satisfaction of participating in a prestigious scientific event, as we were finally free to travel anywhere in the world. Over 8 years (in June 1998), we met again in Israel (in Jerusalem, where another edition of the Congress mentioned above was held), among whom was the organizer of the current event, Professor Elena Rakosy and we were proud that Romanians were more and more numerous attending scientific conferences organized outside the country.

Unfortunately, research remained a neglected area in our country after 1989. Before 1989, I was unhappy about not being allowed to go abroad, lack of provision of equipment, scientific information, insufficient budget and other sources to support research in Romania, but now things are much worse than then. And, in my opinion, *no research means no development*. Nevertheless, this elementary truth seems to be impossible to understand for our government, regardless of political regime, experience, training and efforts made by us, researchers.

Years have passed and here we are both (Professor Cachiță and I) at the age of retirement. In recent years, we have had the pleasure to meet more frequently than in the past for doctoral defences (as referees), at some scientific meetings and, for several years, at "V. Goldis" University in Arad, where the professor ended her academic career a year ago. As dean of the Faculty of Natural Sciences, she invited me to some activities organized at the university of Arad and I can say, without reserve, that I attended there scientific meetings organized at high academic standards, I met many personalities of science in the country and abroad, I spent unforgettable moments, I appreciated the kindness, openness and generosity of the hosts.

In the end, I would like to thank Professor Dorina Cachiță for being herself and permitting me to know her, for all she has achieved in this field, for the spirit of friendship, comradeship and mutual respect manifested between us whenever it was necessary, for the times – not many, but special – spent together.

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