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Invited paper

Dr. Ottó Orsós, the forgotten Hungarian pioneer in plant tissue culture

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Summary: The knowledge of tissue culture deserves attention in respect of understanding the development of universal biology. This study intends to contribute to the past of the plant tissue culture by such data of the history of science which have been unprocessed so far. It seems that the life-work of the Hungarian biologist, Dr. Ottó Orsós is a missing and essential link between those early plant hormone researchers and the representatives of the pioneers of tissue culture schools who have contributed substantially to the development of the modern *in vitro* plant morphogenesis and plant cell biology. Orsós cultured kohlrabi tuber cubes on White culture medium in a sterile manner. This way, he could efficiently direct the *in vitro* morphogenesis of the kohlrabi, the regeneration of its shoot and root, and the formation and steps to subculture of pure callus tissues in 1938. He supported the correctness of its statements by means of detailed anatomical examinations. Orsós successfully rooted and aclimatized complete regenerated plants. We may as well call the above system – in remembrance of the creators of the original concept – "Haberlandt-Orsós model". Between the publishing of his main paper in 1938 and 2003, a period of 65 years has lapsed. On the occasion of this anniversary, we bow before this forgotten pioneer.

Key words: History of sciences, pioneers of plant tissue culture, Dr. Ottó Orsós, Hungary

Introduction

The very first significant pioneer of tissue culture was *Gottlieb Haberlandt* who was born in Magyar-Óvár (Hungary, Ungarische Altenburg, in German) and moved and later on to Austria and Germany where he worked. In America, it was *Ross Granwille Harrison*, researcher of the John Hopkins Hospital and Professor at the Yale University later on. *Harrison* who graduated at the medical faculty, exactly knowing the significance of aseptic intervention embedded excisions prepared out of the neural tube of frog embryo in fresh frog lymph in a sterile manner which he inspected under covered and entirely closed glass microscopic plates and he could observe, even four weeks long, the evolution of nerve fibres out of nerve cells (*Harrison*, 1907; 1925). Unlike Harrison, the botanist, Haberlandt, did not work in a sterile manner (*Haberlandt*, 1902) and the planning of the research was deficient. Both exerted an indelible impression on the future of this field of science. The most efficient continuer of Harrison's life-work became the American Nobel prize winner, *Alexis Carrel*, who was French by birth. And, Haberlandt's direct followers – as we have known so far – were mainly German scientists. At that time, in the plant physiology, the establishing the truth of *Sachs'* special organ-forming substance hypothesis (1880) and Haberlandt's totipotency theory (1902) was one of the most up-to-date research field which could only be cultivated in the hope of success by way of adopting sterile tissue culture system. The elaboration of the basic methods of plant tissue culture is owing to the discoveries of *Philip Rodney White* (1934), *Roger Gautheret* (1939) and *Pierre Nobécourt* (1939). Nevertheless, light was recently thrown on the fact that Haberlandt also had a direct impression on the life-work of *Dr. Ottó Orsós* (*Figure 1*), a pioneer who worked in Hungary and who has been forgotten since that time. We have found evidence of this during the comparative analysis of the contemporary French, German, Anglo-Saxon and Hungarian technical literature recently *(Fári*, 2002).

Professor Árpád Paál and the Budapest' School in Plant Biology

Late in 1929, in Budapest, *Árpád Paál* became the successor of *Sándor Mágocsy-Dietz* at the head of the Institute of Plant Physiology of the Pázmány Péter University (*Figure 2*) whose name had been known throughout the world for his growth (auxin) physiological works (*Paál*, 1914; 1919) as early as at that time. In the subject matter of *in vitro* organogenesis, *Ottó Orsós*, a young disciple of Árpád Paál, also published his results achieved in plant tissue culture in the issue of 1936 of the periodical entitled *Protoplasma* and in the issue of 1938 of the *Biologisches Zentralblatt*. Orsós began his research in 1934 in Budapest when *Tivadar Huzella* (*Huzella*, 1929) who had

formerly launched the tissue culture technique in Debrecen was one of the determinant international authorities in the experimental cell biology (*Figure 3*). In 1933, Huzella presided at the 3rd International Congress of Experimental Cytology who also corresponded with the Nobel Prize winner *Romain Rolland*. The title of his presidential opening speech was *"The correlations of tissue culture with the general problems of biology and with the special problems of medicine"* (*Huzella*, 1934). Huzella, after he moved from Debrecen to Budapest, also established a private biological research station in 1930 in Alsógöd which became, later on, a well-known meeting point in the circle of national and foreign biologists, thus for the tissue culturists, too. For instance, Huzella was visited by *Alexis Carrel* here, who spoke of the performance of Huzella in terms of highest praise even several years after (*Törő*, 1994). Huzella's institute in Budapest accommodated the congress of 1939 of the *Anatomische Gesellschaft* which it organised on the occasion of the centenary of the discovery of cell. Undoubtedly, the personality of Paál and that of Huzella, as well as Haberlandt's unanswered totipotency theory and plant hormone theories turned the talented Orsós, in the mid-30s of the last century toward plant tissue culture. The striving after solving the great questions of plant tissue culture, as a reception problem – was also present in Budapest's circles of biologists, simultaneously and in competition with Gautheret, Nobecourt and White *(Figure 4)*.

Reception of the Haberlandt's theories in Hungary

In Hungary, the impact of the pioneering cell biological research of Haberlandt can be demonstrated already well before Orsós, from the first half of the 20s onward. This proves that *Dániel Fehér* recapitulated the life-work of Haberlandt in connection with the plant hormone theory in his article entitled *"The role of hormones in the life of plants"* in detail as early as in 1923 (*Fehér*, 1923); presumably, that was the first paper on the plant hormones which was prepared in Hungarian language. And, *Endre Gombocz* called the attention of the Hungarian scientists in 1927 in his publication entitled *"Cell division in a new light"* to the significance of Haberlandt's work carried out in the field of the division of isolated cells (*Haberlandt, 1902*), recognising its significance in respect of history of science (*Gombocz*, 1927). Gombocz wrote in 1927 the following: *"Haberlandt was the first who wanted to give an answer to the previous question on the basis of experimentally induced examinations. Based on his research conducted with isolated cells, he thinks of a growth enzyme that would cause the division of cells, similar to chemical stimuli (Haberlandt,* 1902)" (*Gombocz*, 1927). Accordingly, Ottó Orsós had the opportunity in Budapest to learn both the original theories in detail and the Hungarian accounts made of them. In respect of the history of horticultural biotechnology, it is especially important to mention that Orsós, in May 1935, delivered a lecture entitled *"About the plant hormones and the regeneration type division of cells"* at the *Magyar Királyi Kertészeti Tanintézet* (Hungarian Royal College of Horticulture) in Budapest (*Mándy*, 1960). This event preceded by the notable book entitled *"Phytohormones"* published by *Went and Thimann* in the USA (*Went and Thimann*, 1937) and the book entitled *"The Plant Vitamins and Hormones"* published by *Raymund Rapaics* in 1942, in Budapest. About this latter work having completely been forgotten, *Sándor Sárkány* wrote in a brief review published in the Természettudományi Közlöny (Gazette of Natural Sciences) in 1942 that in this range of subjects – as far as he knew – this was the first book in Europe.

Ottó Orsós and his achievements: the Haberlandt-Orsós model

Ottó Orsós was the direct follower and continuer in Hungary mainly of the work of Haberlandt. In his research, he wanted to answer the question of wound hormone recapitulated by Haberlandt in 1922, seeking the mysterious wound hormone among the protein decomposition products. In order to achieve his goal, he further developed Haberlandt's method and elaborated a multi-function, *in vitro* callus induction type and organ regeneration model, by using tissue cubes excised out of kohlrabi tubers, raised on sterile culture medium solidified with agar-agar in Petri dishes. By adopting this system, he established that the *"wound hormone"* presumed by Haberlandt and the so-called *"lepton"* may be the tyrosine (*Orsós*, 1936). Later on, he produced *"organ-forming"* extracts from the pressed juice of kohlrabi tubers. Orsós, by adding them to sterile culture medium, could control the *in vitro* organogenesis and the rhizogenesis of the kohlrabi tuber cubes, separately which he followed up by histological analysis (*Figure 5*). He established that the *"shoot-forming factors can be separated from the substances bringing about root formation and cell division and from the additional nutrients, if any"*. By adopting modified White (1934) culture medium, he produced *"an in vitro continuously growing, pure callus culture separated from the mother tissue"* (*Orsós*, 1938a). It is still information of great significance that Orsós, subsequent to the shoot regeneration and root formation, also raised entire and fully acclimatized regenerated plants (*Mándy*, 1960; *Rapaics*, 1942).

Influence of Orsós on the contemporaries

It is presumable that the pioneer studies of Orsós on plant tissue culture influenced the research conducted by his contemporaries in Europe. Following the publishing of the paper of Orsós in 1938, the European pioneers of plant tissue culture, *Gautheret* and *Nobecourt* attempted also the whole plant regeneration from kohlrabi tuber pieces (*Gautheret*, 1939; *Nobecourt*, 1943) but, at that time, they did not refer to

the formerly published results achieved by Orsós whose other work could be read by them in French language, too. Orsós published an account, in French language, on the organ-forming and callus-forming substances of the kohlrabi, at the XXII[nd] Horticultural World Congress in Berlin, entitled "*La question des substances organogènes*" (*Orsós*, 1938b). About further details, we have no information yet, for the time being. We do not know if Orsós was in Berlin personally or not. If so, we do not know if he met the old Haberlandt or not. Nevertheless, it can be proved that the work and results of Orsós were known by the French colleagues. This was admitted by Gautheret himself, in his monograph published twenty-one years later, in 1959: "*In the similar experiments of Orsós* (1938) *and Nobecourt (1943), no moistening transformation (vitrification) could be observed, only callus- and organ-formation. It was strange that more marked phenomenon of the organogenesis was obtained with the smaller explants (Orsós)*" (*Gautheret*, 1959 p. 258); "*Orsós achieved a special result with kohlrabi extracts. The watery extracts, on the one hand, stimulated the development of callus and organogenesis on the tuber pieces. If, however, the extracts have been treated with lead acetate, it was established that the precipitated fraction stimulated the rhizogenesis but not the shoot regeneration. Orsós concluded that the two factors of the organogenesis could be separated. This relatively old work* (1938) *would, of course, deserve to be repeated.*" (*Gautheret*, 1959; p. 276). After his early death (1939), both the foreign contemporaries and the later successors entirely forgot the achievements of Orsós; his name has been omitted from among the significant pioneers of plant cell- and tissue culture till our days. The Hungarian colleagues who worked in the close vicinity of Orsós remembered his researcher's qualities and achievements for decades, e.g. *Professors Vilmos Frenyó, György Mándy, Sándor Sárkány, Géza Doby* and others. Vilmos Frenyó – immediately after the tragic death of Orsós – wrote a book entitled "*White-jacketed slaves*" which he published in private edition. In this book the friend raised a written monument to the memory of Orsós. For instance, the then Secretary of the *Természettudományi Társulat* (Society of Natural Sciences), *Raymund Rapaics* did not withdraw himself from the influence of Orsós either. *Rapaics* published a summary in the volume 73 of the year 1941 of the *Természettudományi Közlöny* (Gazette of Natural Sciences), entitled "*Culture of plant organs and tissues*". This paper may well be the first scientific publication, in Hungarian language, presenting plant tissue culture. We may well say that this publication was made, in fact, instead of being made by Orsós who died unexpectedly. In this, Rapaics presents the achievements of Orsós and the epoch-making significance of his discovery with the authenticity of a contemporary – supplemented by two original Orsós photos – in the line composed of White, Gautheret and Nobecourt. Rapaics wrote the following: "*Orsós separated a kohlrabi plantlet developed by means of an organ-forming factor from the cultured tissue, planted it in a pot where it began to grow in a regular manner...*" "*Orsós also observed in his tissue cultures that, on outer surfaces of the small kohlrabi piece, callus was formed. The callus could well be separated from the kohlrabi tissues and cultured it further, alone. However, he could not deal further with these callus cultures because of his early death. Where Orsós discontinued, White continued to address the problem*" (*Rapaics*, 1941). These rows prove that Orsós produced a pure callus culture one year earlier than Gautheret, Nobecourt and White. However, probably Orsós was the very first researcher who obtained whole rooted and acclimatized plantlets from *in vitro* regeneration system in the sense of our days, deliberately controlled by means of externally added (unspecified) plant growth regulators, which was proved by means of detailed hystological analysis and photos. Rapaics, in his book published in 1942 about plant vitamins and hormones, presented again the achievements of Orsós in detail. In honour of the twentieth anniversary of his death, György Mándy published a warm, friendly commemoration about Orsós in 1960, in the Biológiai Közlemények (Biological Gazette) (*Mándy*, 1960). Like Rapaics, Mándy also admitted that Ottó Orsós was a significant pioneer of plant tissue culture. Laterm another of his contempararies, Irma Alladiatoris also published a heartfelt, moving commemoration about Orsós mentioning his major achievements (*Allodiatoris*, 1961). Nearly two years after his death, the German botanical journal entitled *Flora* published an Orsós paper on the hystology of kohlrabi tubers (*Orsós*, 1941), not known so far or forgotten. It is an interesting fact that recently in the 66[th] Volume of the *Gartenbauwissenchaft*, a researcher of the Institute of Fruit and Vegetable, Bonn University referred to this dissertation of Orsós (*Lippert*, 2000).

Conclusion

It seems that the life-work of Dr. Orsós Ottó is a missing and essential link between those early plant hormone researchers and the representatives of the different plant tissue culture schools who have contributed to the development of the modern *in vitro* plant morphogenesis by fundamental works. Between the publishing of the paper of Orsós in 1938 and 2003, a period of 65 years has lapsed. On the occasion of this anniversary, we bow before this forgotten pioneer (*Figure 6*).

Acknowledgements

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Legend of figures

Figure 1. Posthumus portrait of *Dr. Ottó Orsós (1911–1938)* painted by *Ferenc Uzonyi* after a black-and-white photo. Courtesy of Orsós Ottó Laboratory, University of Debrecen, Centre of Life Sciences, Debrecen, Hungary.

Figure 2. *Professor Árpád Paál (1889-1943)*, a pioneer of auxin theory. Courtesy of *Prof. István Gyurján*, University of Eötvös Loránd, Department of Plant Anatomy, Budapest, Hungary.

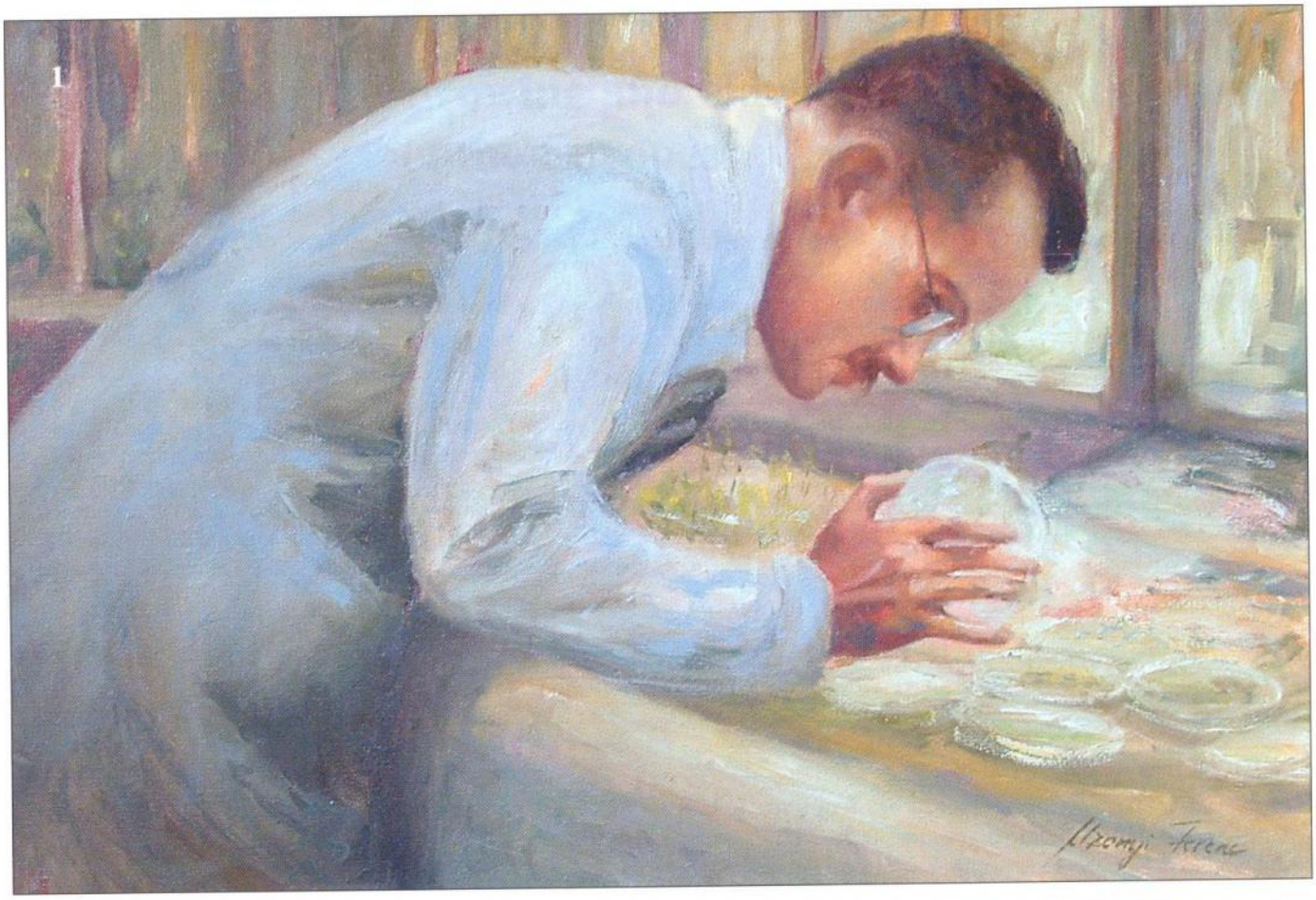
Figure 3. *Professor Tivadar Huzella (1886-1951).* In: Törő I. (1994): Dr. Huzella Tivadar (1886–1951). A Debreceni Orvosképzés Nagy Alakjai, 4. füzet. DOTE., 44p.

Figure 4. Disciples of *Árpád Paál* at the Institute of Plant Physiology, Pázmány University, Budapest. Around the mid-30'^{s} of the last century; Orsós is the second figure smoking pipe on the left.

Figure 5. Front page of Orsós's paper published in 1938. (In: Orsós O., Biol. Zentralb., 58/7–8: 366–370., 1938.)

Figure 6. Longitudinal section of *in vitro* shoot bud regeneration of kohlrabi tuber cube. (In: Orsós O., Biol. Zentralb., 58/7–8: 366–370., 1938.)

1



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(Aus dem Pflanzenphysiologischen Institut der Peter-Pázmány-Universität Budapest. Direktor: Prof. Dr. A. Paál)

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Untersuchungen über Organdifferenzierungen

(Vorläufige Mitteilung)

Von **Otto Orsós**

Mit 1 Abbildungen

Über die organbildenden Stoffe liest man in der Literatur immer mehr und mehr (Rhizocalin, Bouillenne und Went [2]; Rhizogen, Phyllogen, Blastogen, Anthogen usw., Němec [7]; Florigen, Cajlachjan [3]). Der Nachweis gelang in einzelnen Fällen sicher auf experimentellem Wege, in anderen nur mehr oder weniger wahrscheinlich. Ein Nachweis wäre überzeugend, wenn es gelänge, ein tatsächlich nichtdeterminiertes Gewebe mittels eines Organauszuges zur Differenzierung zu zwingen. Als undeterminiertes Gewebe könnte ein Meristem gelten. Doch zeigten die Versuche, isolierte Meristeme „in vitro" zu züchten, daß aus solchen sich ganz normal organisierte Organe entwickeln, auch

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