

## PLANT TISSUE AND CELL CULTURE IN VIETNAM: FORTY-FIVE YEARS OF DEVELOPMENT AND THE FUTURE

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### ABSTRACT

By 2020, plant tissue and cell culture in Vietnam had undergone 45 years of research and development. For nearly half a century, plant tissue and cell culture has been developed to its full potential, especially with the development of genetics, biochemistry and molecular biology. It has contributed significantly to basic and practical researches in our country. In addition to contributions to domestic science and technology, plant tissue and cell culture in Vietnam has also made impressive imprints in the development of plant tissue and cell culture in the world. In this review, I will summarize the process of formation, development and important achievements as well as the challenges and future prospects of this potential field in Vietnam to provide information for researchers, managers, graduate students and other interested readers.

**Keywords:** Achievement, contribution, development, plant tissue and cell culture, Vietnam.

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## INTRODUCTION

Plant tissue and cell culture (PTCC) includes techniques for culturing plant cells, tissues and organs under aseptic conditions on artificial media with known nutritional components. These techniques have been approached by Vietnamese scientists since the 70 s of the twentieth century due to their high potential for research and practical applications. Materials used in PTCC include: organs (shoot, root, anthers...), tissues and cells. Types of culture include: meristem culture, callus culture, cell culture (single cell, protoplast), embryonic culture, anther culture, pollen culture, thin cell layer culture (TCLC), etc. The basic culture methods are: culture on solid medium, culture in liquid medium (static or shaking), culture on semi-liquid medium. The main PTCC techniques include: micropropagation (or *in vitro* propagation), haploid plant production, somatic cell hybridization, embryo rescue, cell line selection and gene transfer.

PTCC is widely used in micropropagation of agricultural, forestry and flower plants as well as in the conservation of rare or endangered plant species. PTCC can be used for screening at the cellular level instead of selecting plants with beneficial traits such as disease resistance and tolerance to adverse environmental conditions. Large-scale cultivation of plant cells through liquid culture in bioreactor can generate biomass to obtain secondary substances and recombinant proteins used as biopharmaceuticals. Through PTCC, it is possible to create hybrid plants using fusion of protoplasts or rescue embryos of distant hybrid combinations. Producing haploid plants from anthers or pollen culture allows the creation of homozygous lines faster in breeding programs. Transgenic plants that express genes from animals, bacteria, viruses or other plant genes that lead to production of vaccines, recombinant proteins, plants resistant to insects, viruses and other pathogens, and crops with high nutritional quality.

In this review, the process of formation, development and important achievements as

well as the challenges and future prospects of PTCC in Vietnam will be presented. The information is based on works done in Vietnam that have been published in the conference proceedings, scientific journals and prestigious monographs in the country and abroad.

## FORMATION AND DEVELOPMENT

Since before 1975, Dr. Nguyen Van Uyen, Dr. Le Thi Muoi, Le Thi Xuan, Tran Ngoc Cat were the first officials to have an idea to set up a PTCC laboratory in Vietnam. After the complete liberation of the South and reunifying of the country (1975), Dr. Nguyen Van Uyen and a number of other researchers such as Trinh Manh Dung and Phan Xuan Thanh were assigned to the Vietnam Science Institute in Ho Chi Minh City to set up a PTCC laboratory here, later expanded to Da Lat and other localities. Especially, *in vitro* propagation of potato in “family laboratories” and the production of potato seedlings in pots were implemented by using potato seeding techniques in Da Lat. In addition, scientists in the South also studied the propagation of orchids (Huynh Van Hai, Nguyen Van Uyen, 1981; Mai Thi Phuong Hoa et al., 2011; Bui Thi Tuong, Tran Van Minh, 2011), anther culture of rice (Trinh Manh Dung et al., 1986), culture of apical buds of taro plant (Nguyen Thi Quynh & Nguyen Van Uyen, 1985; 1987), protoplast culture (Nguyen Thi Lien Chi, Nguyen Van Uyen, 1989) gene transfer (Nguyen Thi Thanh et al., 1997; Tran Thi Dung & Nguyen Huu Ho, 2003) and research on propagation of some other plants such as coffee (Nguyen Thi Quynh, Nguyen Van Uyen, 1993a; 1993b), *Artocarpus heterophyllus* Lam. (Tran Van Minh, 1997), *Polyscias fruticosa* (Nguyen Ngoc Dung & Nguyen Van Uyen, 1997), *Azadirachta indica* (Vu Ngoc Phuong et al., 2000), *Paulownia fortune* (Seem.) Hermsl (Doan Thi Ai Thuyen et al., 2001) and bamboo species (Vu Ngoc Phuong et al., 2002).

In the North, the PTCC research team included four staffs: Dr. Le Thi Muoi, Le Thi Xuan, Tran Ngoc Nguyen Hoang Uyen. In late 1975 and early 1976, Do Nang Vinh and

Nguyen Duc Thanh graduated from Azerbaijan State University (in former Soviet Union) have joined the team. At that time, working conditions were inadequate and rudimentary; the main building of the Vietnam Scientific Institute was still under construction. However, due to news and modernity on PTCC, and the attention of the leaders of the Vietnam Scientific Institute, especially Prof. Nguyen Van Hieu, two rooms located on the second floor of the main building (rooms 207 and 208 buildings A2) were urgently completed to serve as plant cell culture laboratory. The culture box was originally made of wood with two holes on both sides covered by two cloth tubes, and the inside of the box was fitted with a UV light for sterilization. The UV light must turn on at least 30 minutes before the work and the transplanting time should not exceed one hour. The culture instruments and test tubes must be brought to the yard of the 1st floor for washing. In addition, power and water outages often occur. However, even in 1976, the first success of PTCC in Vietnam was marked by the success of culturing rice pollen that was carried out by Do Nang Vinh and Nguyen Duc Thanh. In a diary written in Russian dated June 5, 1976 (Figure 1), Nguyen Duc Thanh wrote “Joy has come to us. We have received the tiny rice callus that we have been waiting for so long. We have been working for a long time with no results. And today it has arrived. When we grew tiny rice pollen in test tubes, we were certain that we would receive these calli. They are small but valuable to us. Because of them, we worked hard and waited until from the tiny anthers that inside were pollen grains that could only be seen under a magnification hundreds of times would grow into clumps of cells called “callus”. We are very joyful and happy. For me, this is the first happiness on the scientific path. Oh! How soft, precious and sacred the calli are!”. The laboratory led by Dr. Le Thi Muoi focused on the research and application of rice and tobacco anther culture techniques (Le Thi Muoi et al., 1978; Le Thi Xuan et al., 1978, 1979; Do Nang Vinh, 1979; Le Tran Binh, 1983; Nghiem Nhu Van, 1989), protoplast

culture (Le Thi Muoi & Nguyen Duc Thanh, 1978, Nguyen Duc Thanh, 1983; Le Thi Xuan, 1985), *in vitro* propagation (potato, carnation, bananas, sugarcane, agave, pineapple, medicinal plants, forestry trees, etc.), cell line selection (Nguyen Hoang Loc et al., 1990), cytoplasmic transfer (Nguyen Duc Thanh et al., 1995, 1997; Le Tran Binh, 1991) and gene transfer (Tran Thi Phuong Lien & Le Xuan Tu, 1993). Beside researching and applying PTCC techniques, the laboratory was also responsible for training researchers and technicians in this field for research institutes, universities and companies such as Agricultural University No. 1 (currently Vietnam National University of Agriculture), Institute of Medicinal Materials, Institute of Agricultural Science and Technology (currently Vietnam Academy of Agricultural Sciences), and Cao Bang Tobacco Company, etc. Later on, PTCC laboratories were also set up in the above locations. Initially, these PTCC laboratories applied mainly *in vitro* propagation techniques for multiplication of potato and lily, callus culture and biomass production of medicinal plants, and anther culture to produce double haploid lines in rice.

In the Central region, after successful defence of PhD thesis at the PTCC laboratory of the Institute of Biology (currently the Institute of Biotechnology), Dr. Nguyen Hoang Loc returned to Hue University to set-up a PTCC laboratory there. His research has focused on propagating a number of plants such as *Aquilaria crassma* Pierre (Nguyen Hoang Loc et al., 1997), *Camellia japonica* L (Nguyen Hoang Loc et al., 2001), applying PTCC to produce bioactive substance (Nguyen Hoang Loc et al., 2006) and expression of antigens in plants (Nguyen Hoang Loc et al., 2011).

The development of plant tissue and cell culture in Vietnam can be divided into three main stages:

1975–1985: This is the period of formation, approaching and mastering technologies and applying some PTCC techniques such as anther culture to produce

double haploid lines and *in vitro* propagation for rapid propagation of potato, training research staff and technicians on PTCC for

universities, research institutes and companies, establishing PTCC laboratories in some universities and research institutes.

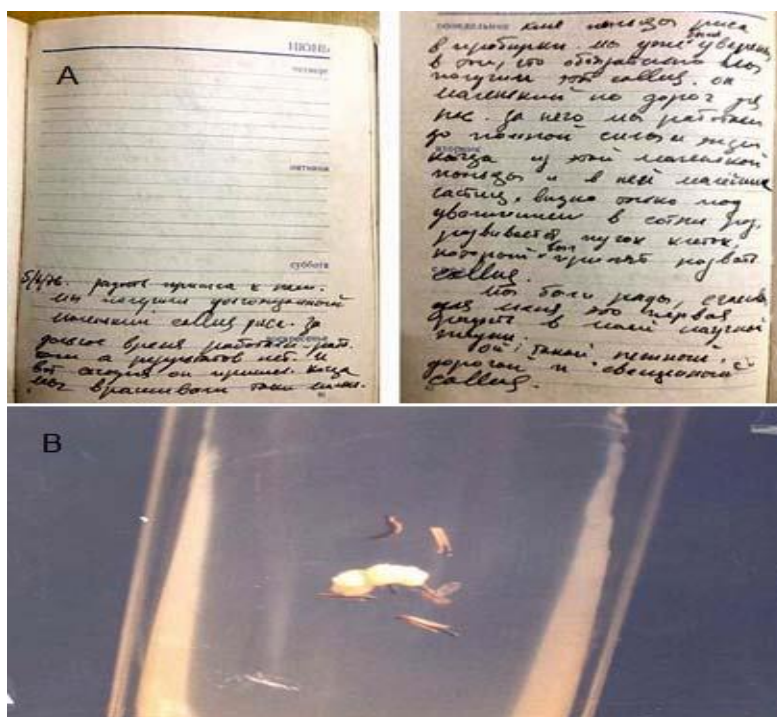


Figure 1. A. Nguyen Duc Thanh's Diary about the first success in Vietnam PTCC dated June 5, 1976; B. Callus tissue arising from rice anther culture (Original photograph taken from a 1976 experiment)

1986–1995: This is the period of application and development of PTCC methods and techniques in research and practice, and, at the same time, establishing PTCC laboratories nationwide. In addition to *in vitro* propagation and anther culture techniques, a range of other techniques such as cell line selection, callus and suspension culture for biomass collection, protoplast culture and fusion, embryo rescue, gene transfer have been studied and applied.

1996-Present: This is the period to promote the development of biotechnology; in general, genetic engineering and plant cell technology, in particular, for the industrialization and modernization of the country. Studies and applications have been focusing on creating resilient crops; fast propagating food crops, vegetables, flowers,

fruits, industrial trees and forest trees with good quality, high yield and tolerance to adverse external conditions as well as pests; conservation and development of rare and endangered genetic resources; research on expression of antigen, antibody and recombinant protein, gene transfer, thin cell layer (TCL) culture and photoautotrophic micropropagation.

## ACHIEVEMENTS

### Basic research

The basic research of PTCC in Vietnam mainly focuses on studying the factors affecting callus formation, plant regeneration, growth and development of different species under *in vitro* conditions, protoplast culture, TCL culture, cytoplasmic genetics, regeneration systems of different plants for

transgenic transformation and expression of resistance-related genes, genes involved in yield and quality, and the genes encoding antibody and recombinant proteins.

Le Xuan Tu & Nguyen Thi Xuan Giang (1981, 1982) studied the effects of gamma rays on the development of soybean callus, callus formation and plant regeneration from rice callus. The effects of gamma rays on regeneration of rice from mutated calli were also studied by Le Thi Bich Thuy et al. (2007). The conditions for culturing callus for biomass production of *Maesa balansae* Mez were investigated by Quach Thi Lien & Nguyen Duc Thanh (2004). Duong Tan Nhut et al. (2009a) studied the effect of coconut water and sucrose on the growth of callus and formation of somatic embryos in *Phalaenopsis amabilis* (L.) Blume). Influence of culture conditions such as light, CO<sub>2</sub> content (Nguyen Tri Minh et al., 2008; Duong Tan Nhut & Nguyen Ba Nam, 2009; Do Thi Gam et al., 2017), sugars (Trinh Thi Lan Anh et al., 2013), growth regulators (Phan Duy Hiep et al., 2014; Vu Thi Lan et al., 2014), amino acids (Tran Trong Tuan et al., 2015), nano silver (Duong Tan Nhut et al., 2017a) in PTCC has been studied for *in vitro* propagation.

Le Thi Muoi & Nguyen Duc Thanh (1978) for the first time in Vietnam announced the generation of a complete tobacco plants from protoplast. In 1980, Nguyen Duc Thanh & Le Thi Muoi studied the effects of the composition and concentration of plant growth regulators on the process of generating complete tobacco plants from haploid protoplasts. Potato protoplasts were also successfully cultured and the complete plants were generated (Nguyen Duc Thanh & Le Thi Muoi, 1981; Nguyen Duc Thanh, 1983, Nguyen Thi Phuong Thao et al., 2012). These results provided an important basis for further research on cytoplasmic transformation, chloroplast genetics and gene transfer. In addition, protoplasts of *Arabidopsis thaliana* (Le Thi Xuan, 1985), *Solanum laciniatum*

(Nguyen Thi Lien Chi & Nguyen Van Uyen, 1989) have also been successfully cultured.

The use of cell technology in cytoplasmic genetics research, especially the use of protoplasts has achieved impressive results in transferring cytoplasmic male sterility (CMS) into tobacco plants (Nguyen Duc Thanh & Medgyesy, 1988; Le Tran Binh, 1991), transferring whole chloroplasts or chloroplast genes (Nguyen Duc Thanh & Medgyesy, 1989; Nguyen Duc Thanh & Medgyesy, 1993; Nguyen Duc Thanh et al., 1995, 1997; Nghiem Ngoc Minh et al., 1999), creating chloroplast combinations in tobacco by fusion of protoplasts (Nguyen Duc Thanh et al., 1996), and producing cytoplasmic hybrid plants (Nghiem Ngoc Minh et al., 1997). The cytoplasmic genetic research using protoplasts is the unique research area that had only been conducted at the Institute of Biology (currently the Institute of Biotechnology, Vietnam Academy of Science and Technology) from the 80<sup>th</sup> and 90<sup>th</sup> of the twentieth century in Vietnam. Protoplasts were also used for the gene transfer into *Brassica* plants (Pham Thi Ly Thu et al., 2001; Pham Thi Ly Thu & Le Huy Ham, 2003).

The research team of the Institute of Biology in Da Lat belongs to the Institute of Tropical Biology in Ho Chi Minh City, the National Center for Natural Sciences and Technology (currently the Tay Nguyen Scientific Research Institute, Vietnam Academy of Science and Technology) has studied the method of culturing TCL in lilies (Duong Tan Nhut et al., 2006b) and cauliflower (Duong Tan Nhut & Bui Van The Vinh, 2009) and has been applied to propagate various plants such as lilies, *Jatropha curcas*, Ngoc Linh ginseng, and orchids (Duong Tan Nhut et al., 2008b; Do Dang Giap et al., 2012a; Vu Thi Hien et al., 2015; Vu Thi Hien et al., 2016; Nguyen Thi Kim Loan et al., 2016). TCL culture was also conducted at the Institute of Agricultural Biology for *in vitro* propagation of lilies and orchids (Nguyen Quang Thach & Hoang Thi Nga, 2000;

Nguyen Phuong Thao & Nguyen Quang Thach 2005; Nguyen Thanh Tung et al., 2010).

A very important basic research direction is the research on plant regeneration systems of different plants for gene transfer, as regeneration systems are very important for plant transformation. If complete plants cannot be regenerated after the transgenic process, no transgenic crops could be produced. Therefore, many plants have been studied to regenerate plants from different tissues for transgenic studies. Green bean (Mai Truong et al., 2001), rice (Cao Le Quyen et al., 2011), maize (Nguyen Van Dong et al., 2009; Vu Thi Bich Huyen et al., 2013), peanut (Bui Van Thang et al., 2004; Nguyen Thi Thu Nga & Le Tran Binh, 2012), tomato (Do Xuan Dong et al., 2007), papaya (Le Quynh Lien et al., 2003, Nguyen Minh Hung et al., 2006), *Citrus nobilis* Loureiro (Do Tien Phat et al., 2007), and *Melia azedarach* (Do Xuan Dong et al., 2008) regeneration systems were developed for gene transfer purpose.

Studies on expression of genes related to plant tolerance, yield and quality, and genes for antigen and recombinant proteins have been studied through PTCC in a number of laboratories in the Institute of Biotechnology, Institute of Tropical Biology, Institute of Agricultural Genetics, etc. *NAC2* gene from L12 peanut cultivar coding for protein related to drought tolerance was expressed in tobacco plants (Nguyen Thi Thu Nga et al., 2015). Other genes related to drought tolerance in plants such as *GmHK06*, *GmRR34*, and transcription factors like *GmNAC092*, *GmNAC083*, *GmNAC057* were successfully expressed in soybeans (Hoang Thi Lan Xuan et al., 2015; Nguyen Binh An Thu et al., 2015). Insect resistance gene *cryIA(c)* was expressed in tobacco (Huynh Thi Thu Hue et al., 2008). Many studies have been conducted to express antigens in plants such as: VP2 antigen gene that induces an immune response in chickens in duckweed (Le Huy Ham et al., 2009), HA antigen of H5N1 virus in soybean seed (Nguyen Thu Hien et al., 2013), antigen GP5 of PRRS virus in tobacco (Dao Thi Sen

et al., 2016), H7 antigen of influenza A/H7N9 virus in *Nicotiana benthamiana* (Le Thi Thuy et al., 2017). Recombinant protein interleukin-7, a major regulator of the human immune system (Nguyen Huy Hoang et al., 2017), rabies glycoprotein (Le Quynh Lien et al., 2008) and protein M of PRRS virus causing blue ear disease (Nguyen Thi Minh Hang et al., 2018) were, successfully, expressed in *Nicotiana* plants. *HIV-1-p24* gene, also, expressed in tomato-*Lycopersicon esculentum* Mill. (Phan Duc Chi et al., 2013).

### Practical application

Since the beginning of the approach to PTCC techniques, Vietnamese researchers have focused on the practice of producing pure lines, insect-resistant and disease-resistant, and tolerant to adverse environmental factors (salinity, drought) as well as high yield and high quality plant varieties. In particular, PTCC has been widely applied to the rapid propagation of agricultural, forestry, flower and medicinal plant species through stem cutting technique, apical shoot-tip culture, culture of somatic embryos, artificial seeds, etc. Researchers were also interested in the use of PTCC in biomass production to obtain bioactive substances.

### Production of double haploid lines through anther culture

The haploid plants from pollen have great practical significance, because haploid plants are ideal materials to create a pure line. Double haploid lines can be produced by colchicine treatment of haploid plants or through haploid callus tissue culture. Purebred lines are particularly significant in production of hybrids between incompatible parents and shortening the breeding time.

The production of haploid plants through anther culture has been widely used in producing double haploid lines in Vietnam. Double haploid rice (Le Thi Muoi et al., 1978) and tobacco plants (Le Thi Xuan et al., 1978) from *in vitro* anther culture were the first success of PTCC in Vietnam. These works have laid the foundation for the production of double haploid rice varieties, contributing to

shortening the time for rice breeding (Bui Ba Bong et al., 1997; Nghiem Nhu Van et al., 2002, 2004; Phan Thi Bay et al., 2004c). The two VH1 and VH2 rice lines created by the anther culture method have been varietal tested and have gone for trial production (Nghiem Nhu Van et al., 2006). Blast resistant HPMD4, HPMD6, HPMD9, HPMD13, HPMD20 and good quality HPMD4, HPMD9 rice lines have been created by culturing F1 anthers of a hybrid between quality and blast resistant rice varieties (Phan Thi Bay et al., 2004c). Anther culture was also applied to create pure lines for restoring the quality of specialty Tu Le sticky rice (Dang Thi Minh Lua et al., 2009) (Figure 2).



Figure 2. Growing Tu Le sticky rice that was improved by anthers culture in Tu Le commune, Yen Bai Province

#### **Producing disease-resistant potatoes and quality oranges through protoplast fusion**

Due to the absence of cellulose wall, protoplasts can be fused to produce somatic hybrids, including nuclear hybrid (Do Thi Thu Ha et al., 2012) and cytoplasmic hybrid (Nguyen Duc Thanh et al., 1996). The use of protoplasts in plant breeding practice has been carried out by the research team of the Institute of Agricultural Biology, University of Agriculture #1. By fusing protoplasts of the cultivated potato (*Solanum tuberosum* L. ( $2n = 4x = 48$ )) and the wild potato species (*Solanum bulbocastanum*, *Solanum tarnii*, *Solanum pinnatisectum* ( $2n = 2x = 24$ )) with

the ability resistant to late blight. Related to this, Hoang Thi Giang et al. (2013, 2014) have created a number of late blight resistant potato lines. At the Agricultural Genetics Institute, in order to improve the quality of orange varieties, Ha Thi Thuy et al. (2010) fused protoplasts between the local orange variety (*Citrus nobilis*) and sweet orange varieties (*C. sinensis*).

#### **Producing plants resistant to salinity, drought and pests through selection of cell lines**

Selection of plant cell lines is based on heterogeneity of tissues and cells in *in vitro* culture resulting in somatic variation. In Vietnam, the selection of plant cell lines was applied to select the salinity, drought and disease resistant lines. Nguyen Hoang Loc et al. (1990) selected NaCl-tolerant tobacco lines and drought tolerant sugarcane lines (Nguyen Hoang Loc et al., 2003) through callus culture. Truong Thi Bich Phuong et al. (2002) reported the selection of drought tolerant rice lines by callus culture. Vu Thi Thu Thuy et al. (2013) selected drought tolerant groundnut lines from dehydrated calli. By selecting cell lines from radiation treated calli, Nguyen Thi Huong et al. (2017) have selected a saline tolerant *Eucalyptus urophylla* callus line that can be regenerated on medium with 125 mM NaCl. Le Thi Bich Thuy et al. (1997) reported the selection of rice lines resistant to fungal pathogen (*Piricularia oryzae*) and subsequently created rice lines resistant to blast disease (Phan Thi Bay et al., 1997). Rice varieties DR1, DR2 were created by selecting dehydrated calli (Dinh Thi Phong et al., 1999), and DR2 has been recognized as a national variety.

#### **Producing transgenic plants resistant to drought, pests, diseases, and increasing productivity and quality**

In addition to the basic research orientation as described above, some laboratories have made efforts to create transgenic plant with drought tolerance (Cao Le Quyen et al., 2009), disease-resistance (Vu Thi Lan et al., 2017), increased productivity

(Nguyen Duc Thanh et al., 2015) and quality transgenic crops (Tran Thi Luong et al., 2014; Tran Thi Luong, Nguyen Duc Thanh, 2015). However, the results are still modest. *MtOsDREB2A* (Cao Le Quyen et al., 2009), *OsNLI-IF* (Nguyen Duy Phuong et al., 2015) and *OsNAC1* (Pham Thu Hang et al., 2016) control drought tolerance were transferred to rice and *NF-YB2* (Nguyen Van Dong et al., 2015) gene was transferred to maize to creating drought tolerant lines. The gene coding glycine-betaine was transferred to *Melia azedarach* L. to create salt tolerant plants (Chu Hoang Ha, Bui Van Thang, 2017). Herbicide resistant (Pham Thi Ly Thu et al., 2015) and insect resistant maize (Pham Thi Ly Thu et al., 2013), insect resistant soybean (Nguyen Van Dong, 2012) were created by transferring *GA21*, *cryAI*, *cryIb* genes into maize and soybean. Tran Thi Cuc Hoa et al. (2017) used the new vector pHOA60, pHOA100, pHOA130 to transfer *VIP3A* gene into soybean to create plants resistant to fruit flies. Transgenic tobacco plants resistant to two mosaic viruses were created by RNAi transformation (Pham Thi Van et al., 2009). Also by RNAi transfer method, Nguyen Thi Hai Yen et al. (2011) created a line of transgenic tomato PT18 resistant to viral leaf curl disease. Vu Thi Lan et al. (2018) transferred the *cry3CA1* gene to sweet potato to create sweet potato lines resistant to the *Cylas formicarius*. Vi Thi Xuan Thuy et al. (2016, 2017) reported the transformation of the *DEFENCIN (ZmDEF1)* gene from the local maize resistant to the weevil to the elite maize variety to create elite maize lines resistant to weevil.

*Shrunken 2 (Sh2)* (Tran Thi Luong et al., 2014) and *Brittle 2 (Bt2)* genes (Nguyen Thi Thu et al., 2014; Nguyen et al., 2016) encoding ADP-glucose pyrophosphorylase-an enzyme that regulates starch synthesis, were successfully transferred into maize and transgenic maize that increase starch content from 10.12 to 16.04% and yield over 5 tons/ha were obtained. As maize is a low-quality food crop (lack of lysine, tryptophan, low provitamin A including  $\alpha$ -carotene,  $\beta$ -carotene

and  $\beta$ -cryptoxanthin), with the aim of improving the quality of maize, Tran et al. (2017) had transferred the *IbOr* gene from the Hoang Long sweet potato variety into several maize lines and created transgenic maize plants with increased  $\beta$ -carotene content more than 10 fold, contributing to improving the nutritional quality of maize (Tran et al., 2017; Tran Thi Luong & Nguyen Duc Thanh, 2018). Tran Thi Xuan Mai & Tran Thi Cuc Hoa (2017) reported the production of transgenic rice plants with lysine content increased up to 38% by transferring *DHDPS-r1* gene encoding the dihydrodipicolinate synthase (DHDPS) enzyme into the Taipei 309 rice variety.

#### **Rapid propagation, conservation and development of genetic resources**

*In vitro* propagation, *in vitro* micropropagation, or simply micropropagation has the advantage of having a high multiplication coefficient, the ability to multiply large numbers of plants in a small area. Disease-free plants and no contact with disease sources should ensure seedlings are free of diseases. In addition, micropropagation makes it easy to exchange and transport the seedlings. Therefore, micropropagation has been widely applied in plant breeding, conservation and development of rare genetic resources. During the 45 years of PTCC development in Vietnam, perhaps the most important contribution is the *in vitro* propagation of agricultural, forestry, floral and medicinal plants, including some endangered plants.

#### **Agricultural plants**

Among the micropropagated agricultural plants, potato was the first and obtained impressive results. Nguyen Van Uyen had successfully implemented *in vitro* propagation of potato in Da Lat at the family laboratories and produced potato seedlings in pots by using potato seedbed method. In addition to propagating potato by cutting *in vitro* stems, propagation through producing minitubers from *in vitro* seedlings has also been conducted successfully (Nguyen Quang Thach et al., 2005).



After potato, banana (Vu Ngoc Phuong et al., 2009; Do Dang Giap et al., 2012b), pineapple (Phan Thi Bay et al., 1994a; Nguyen Duc Thanh et al., 2003), sugarcane (Ha Thi Thuy et al., 1998; Nguyen Kim Lan et al., 1998) have been propagated *in vitro* and provided millions of qualified and disease-free seedlings for breeding. The Institute of Tropical Biology, the Institute of Biotechnology, the Agricultural Genetics Institute, the Vegetable Research Institute, the Agricultural University #1 are the places that have propagated bananas, especially Cavendish species in the large scale. Vu Ngoc Phuong et al., (2009) reported about *in vitro* propagation of banana (*Cavendish* sp.) on an industrial scale.

The Cayenne pineapple propagation protocol has been developed by the Institute of Biotechnology in cooperation with the Institute of Fruit and Vegetable Research and was transferred to seedling production to supply pineapple seedlings for farms (Nguyen Duc Thanh et al., 2003) (Figure 3).



Figure 3. *In vitro* propagated Cayenne pineapple grown at Suoi Hai Farm (in 2000)

*In vitro* propagation of sugarcane has been carried out methodically at the Institute of Agricultural Genetics, especially the development and propagation of new and high yielding sugarcane at the industrial scale (Ha Thi Thuy et al., 1998, 1999., 2000a, 2000b).

#### Flower

Micropropagation has made a significant contribution to the propagation of flowering

plants, especially orchids. PTCC laboratories throughout the North, Central and South have studied and propagated these precious flowers. Tay Nguyen Institute of Biology (current Tay Nguyen Scientific Research Institute) propagates *Cymbidium* sp. (Phan Xuan Huyen et al., 2004), *Paphiopedilum callosum* (Vu Quoc Luan et al., 2012, 2013b), *Paphiopedilum graxtrixianum* (Vu Quoc Luan et al., 2013a), and *Dendrobium heterocarpum* Lindl (Dang Thi Tham et al., 2018). The Institute of Tropical Biology propagated *Dendrobium* sp., *Phalaenopsis* sp., *Cymbidium* sp. and *Rhynchostylis* sp. (Mai Thi Phuong Hoa et al., 2011; Bui Thi Tuong Thu, Tran Van Minh, 2011). The number of orchids like *Phalaenopsis* Sogo Yukidian (Nguyen Thi Son et al., 2014), *Dendrobium fimbriatum*, *Dendrobium nobile* L. (Nguyen Thi Son et al., 2012a, 2012b; Vu Ngoc Lan & Nguyen Thi Ly Anh, 2013), *Cymbidium* sp. (Nguyen Quang Thach et al., 2004), *Cymbidium iridioides* (Hoang Thi Nga et al., 2008, and *Paphiopedilum hangianum* Perner & Gruss (Hoang Thi Giang et al., 2010) were successfully propagated in the Institute of Agricultural Biology. Pham Thi Kim Hanh et al. (2009) in the Agriculture Genetics Institute reported the propagation of *Rhynchostylis gigantean* orchid. Other orchids such as *Dendrobium anosmum* (Nguyen Quynh Trang et al., 2013), *Dendrobium crepidatum* Lindl. & Paxt (Nguyen Van Ket & Nguyen Van Minh, 2010), and *Dalybium gratiosissimum* Reichenb F. (Vu Kim Dung et al., 2016), have also been propagated through micropropagation.

In addition to orchids, *in vitro* propagation of many other flowering plants have been studied e.g. *Lilium longiflorum* (Nguyen Thi Phuong Thao & Nguyen Quang Thach, 2005; Nguyen Thi Phuong Thao et al., 2006; Doan Thi Quynh Huong et al., 2013), *Lilium spp.* (Nguyen Thi Ly Anh et al., 2005; Do Minh Phu et al., 2009), *Polianthes tuberosa* (Duong Tan Nhut, 1994), carnation (Nghiem Ngoc Minh, 1992), roses (Phan Thi Bay et al., 1996; Nguyen Thi Kim Thanh, 2005; Nguyen Thi

Phuong Thao et al., 2015, Nguyen Van Viet, 2017), *Chrysanthemums* (Nguyen Thi Dieu Huong & Duong Tan Nhut, 2004), *Anthurium andraeanum* (Hoang Thi Nhu Phuong et al., 2014; Nguyen Thi Thuy Diem, 2015), and *Hydrangea macrophylla* (Thi The Luc et al., 2017) have been successfully propagated.

#### Forest trees

Among forestry trees, acacia has been the most widely and successfully used for *in vitro* propagation. Particularly, hybrid *Acacia*, *Acacia crassicarpa* A. Cunn. Ex Benth, and *Acacia auriculiformis* A. Cunn. Ex Banth (Doan Thi Mai et al., 1998, 2009b; Phi Hong Hai & Van Thu Huyen, 2016; Trieu Thi Thu Ha et al., 2014). *Eucalyptus* varieties (Le Kim Dao, 2001) have been widely propagated to provide seedling sources for reforestation. Besides, other species such as Melaleuca (Phung Thi Hang & Nguyen Bao Toan, 2011), Caribaea pine (Kieu Phuong Nam et al., 2009), resin pine-*Pinus merkusii* (Do Tien Phat et al., 2009; Pham Thi My Lan, Nguyen Xuan Cuong, 2014), *Paulownia fortunei* (Nguyen Thi Quynh et al., 2002; Doan Thi Ai Thuyen et al., 2001), *Aquilaria crassma* Pierre (Nguyen Hoang Loc et al., 1997), *Chukrasia tabularis* (Doan Thi Mai et al., 2009a), *Aquilaria crassna* Pierre (Le Van Thanh & Nguyen Thi Hien, 2010), *Azadirachta indica* A. Juss (Vu Ngoc Phuong et al., 2000) *Sinocalamus latiflorus* and *Dendrocalamus asper* (Vu Ngoc Phuong et al., 2002) were also propagated by the micropropagation method.

#### Medicinal plants

For medicinal plants, many species have been cultivated and propagated *in vitro*. However, the results are still very modest. The plant species that have been studied and propagated include *Crinum latifolium*, *Polyscias fruticosa*, *Morinda officinalis*, *Anoectochilus setaceus* Blume, *Dendrobium officinale* Kimura et Migo, and ginseng species.

Quach Thi Lien et al. (2003) studied regeneration of *Crinum latifolium* plants. *Polyscias fruticosa* was most studied for *in*

*vitro* propagation (Nguyen Ngoc Dung, Nguyen Van Uyen, 1997; Vu Hoai Sam & Pham Van Hien, 2005; Le Nhu Thao et al., 2014; Dao Duy Hung et al., 2017; Trinh Viet Nga et al., 2019). Orchid species with high pharmaceutical value such as *Anoectochilus setaceus* Blume or *Anoectochilus roxburghii* (Wall.) Lindl. (Nguyen Quang Thach & Phi Thi Cam Mien, 2012; Truong Thi Bich Phuong & Phan Ngoc Khoa, 2013; Vu Quoc Luan et al., 2015; Tran Thi Hong Thuy et al., 2015; Phan Xuan Huyen et al., 2018), *Dendrobium officinale* Kimura et Migo (Trinh Thi Thuy An & Nguyen Thi Tam, 2017; Le Thi Diem & Vo Thi Bach Mai, 2017) have been, successfully, *in vitro* propagated. Other medicinal plants like *Codonopsis javanica* Blume (Phan Xuan Huyen et al., 2014; Doan Trong Duc et al., 2015), *Milletia speciosa* Champ (Ta Nhu Thuc Anh et al., 2014), *Celastrus hindsii* (Ta Nhu Thuc Anh & Nguyen Thi Bich Thu, 2012), *Artemisia annua* (Mai Thi Phuong Hoa et al., 2012; Bui Thi Tuong Thu et al., 2012), Japanese *Angelica acutiloba* Kitagawa (Hoang Ngoc Nhung & Nguyen Thi Quynh, 2015), *Lavandula angustifolia* (Do Tien Vinh et al., 2016), *Rehmannia glutinosa* (Vu Hoai Sam et al., 2018), and *Polygonum multiflorum* (Truong Thi Bich Phuong et al., 2008; Bui Van Thang, 2017) have also been propagated *in vitro*. Ginseng, in particular, Ngoc Linh ginseng (Figure 4) have received much attention for *in vitro* propagation. Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv) has been propagated through somatic embryos from leaves, petioles or stems (Mai Truong et al., 2013; Vu Thi Hien et al., 2014), by artificial seeds (Tran Thi Huong & Duong Tan Nhut, 2011) or *in vitro* root generation (Hoang Xuan Chien et al., 2011). In addition, other ginseng species such as Lai Chau ginseng - *Panax vietnamensis* var *fuscidiscus* (Le Hung Linh & Dinh Xuan Tu, 2017), *Hibiscus sagittifolius* Kurz (Phan Duy Hiep et al., 2014) and *Milletia speciosa* Champ (Ta Nhu Thuc Anh et al., 2014) have also been studied for *in vitro* propagation.



Figure 4. Ngoc Linh Ginseng propagates *in vitro* at the National Center for Research and Development of Ngoc Linh Ginseng grown in pots on Ngoc Linh Mountain

#### Endangered plants

PTCC also contributes significantly to the conservation and development of rare and precious gene sources, especially endangered species. In Vietnam, PTCC has been applied to the conservation and development of a number of rare and endangered plants such as endangered orchid species, *Lyptostrobos pensilis* (Staunton ex D. Don) K. Koch, and *Huperzia serrata* Thunb.

Among the orchids, *Anoectochilus setaceus*, *Dendrobium chrysotoxum*, *Dendrobium heterocarpum* and *Dendrobium draconis* are the precious species that have both aesthetic and medicinal value. However, these orchids are in danger of extinction. *Anoectochilus setaveus* Blume has been studied for conservation by restricted growth culture (Nguyen Quang Thach & Phi Thi Cam Mien, 2012; Vu Hoai Sam et al., 2016) and *in vitro*

*in vitro* micropropagation through protocorm-like bodies (Tran Thi Hong Thuy et al., 2015). *Dendrobium chrysotoxum* - an endangered wild orchid species (Nguyen Van Song et al., 2011) and *Dendrobium heterocarpum* Lindl (Dang Thi Tham et al., 2018) have been studied to multiply *in vitro* for gene conservation and development.

*Lyptostrobos pensilis* (Staunton ex D. Don) K. Koch is not only an endangered gene source but also a precious medicinal plant, from which some substances can be extracted from the bark and leaves to prepare pharmaceuticals for cancer treatment. The wood is reddish-brown with beautiful wood grain, very solid, free from wood weevil, so that it is very popular and has high value. This species has been successfully propagated *in vitro* by a research team at Da Lat University (Nguyen Thanh Sum et al., 2007) and the Institute of Biotechnology, Vietnam Academy of Science and Technology (Nguyen Duc Thanh et al., 2012).

*Huperzia serrata* Thunb is a species in the RED list of the Research Program for conservation and development of precious and rare gene sources of medicinal plants. The plant contains an alkaloid called huperzine, which is effective in curing dementia, including Alzheimer's disease of the elderly. Recently *Huperzia serrata* Thunb has been successfully studied *in vitro* propagation for the purpose of conservation and development (Phan Xuan Binh Minh et al., 2019).

Application of PTCC has also been conducted for the conservation and rapid multiplication of other medicinal plants, especially rare, economically valuable, high-yield and high quality species such as: *Morinda officinalis* How., *Dendrobium nobile* Lindl., *Saussurea lappa* CB Clarke, *Fallopia multiflora* (Thunb). Haraldson), *Ligusticum wallichii* Franch, *Salvia miltiorrhiza* Bunge, and *Lilium brownii* F.E.Br. Ex Miellesz (Nguyen Minh Khoi et al., 2017).

### *Photoautotrophic micropropagation*

Photoautotrophic micropropagation, also known as photosynthesis micropropagation, inorganic micropropagation, and sugar-free medium micropropagation (Kozai et al., 2005) has been applied by Vietnam plant tissue culturists to culture different plant species. Photoautotrophic micropropagation has many advantages related to improvement of the physiology of seedlings and management during production, and it helps to reduce production costs as well as improve the quality of seedlings. Photoautotrophic micropropagation has been performed in both herbaceous and woody plants. This method has brought a breakthrough in large-scale production of disease-free, genetically homogeneous plants with the ability to outgrow and grow better than normal micropropagation and, therefore, can make a big contribution to the research and production of seedling. In Vietnam, photoautotrophic micropropagation was initiated in 1996 by Nguyen Van Uyen of the Institute of Tropical Biology and has been implemented by his colleagues Nguyen Thi Quynh, Nguyen Tri Minh and Thai Xuan Du since 1997 till now. Photoautotrophic micropropagation was developed in conjunction with the development of *in vitro* environmental control techniques such as CO<sub>2</sub> concentration, light, photosynthetic flux, relative humidity, and airflow rates in flasks. These are important environmental factors that affect the growth and development of seedlings. Pham Minh Duy et al. (2014) studied the growth and secondary compound accumulation of *Phyllanthus amarus* (Schum. & Thonn.) cultured photoautotrophically under a CO<sub>2</sub>-rich environment. The factors such as light, air permeability, humidity were also studied (Nguyen et al., 2001; Nguyen Thi Quynh et al., 2010a, 2010b; Nguyen Nhu Hien et al., 2009; Nguyen Nhu Hien & Nguyen Thi Quynh, 2010). Photoautotrophic culture for propagation and biomass production was carried out on coffee trees (Nguyen et al., 2001), yam (*Dioscorea alata*) (Nguyen Thi Quynh et al., 2002; Nguyen &

Kozai, 2007), orchids (Nguyen Thi Quynh et al., 2010a), *Phyllanthus amarus* (Schum. & Thonn.) (Pham Minh Duy et al., 2012, 2014), Ngoc Linh ginseng (Ngo Thi Ngoc Huong et al., 2015), Indian *Coleus forskohlii* (Nguyen Thuy Phuong Duyen et al., 2015) and *Hibiscus sagittifolius* Kurz (Nguyen Thuy Phuong Duyen et al., 2017).

### *Artificial seeds*

Artificial seeds are seed-like structures, created experimentally using somatic embryos derived from plant tissue culture, encapsulated by hydrogels and these encapsulated embryos have the characteristics as true seed when sown, and can be used as a substitute for natural seeds. Use of artificial seeds shortens the seeding time (no need to wait for the plants to grow, flower, set seed), free from seasonal restriction, avoiding seed sleeping, and can be done on a large scale, avoiding meiosis to stabilize the elite genetic resources. The artificial seed coat has the potential to maintain and provide nutrients, growth stimulants and insecticides. In addition, artificial seeds also help to study the role of endosperm and the formation of seed pods.

Researchers in the Tay Nguyen Institute of Biology (currently Tay Nguyen Scientific Research Institute, Vietnam Academy of Science and Technology) were the pioneers in this field. Studies on artificial seeding of *Lilium* (Duong Tan Nhut et al., 2004b), *Cymbidium* (Tran Thi Ngoc Lan et al., 2011), and garlic (Do Ngoc Thanh Mai et al., 2015) have been conducted successfully. The preservation (Duong Tan Nhut et al., 2007c) and germination ability (Trinh Thi Huong & Duong Tan Nhut, 2011; Trinh Thi Huong et al., 2013) of artificial seeds were also studied. Artificial seeds were used for propagation of lilies (Duong Tan Nhut et al., 2004b) orchids (Duong Tan Nhut et al., 2007c; Tran Thi Ngoc Lan et al., 2011), Ngoc Linh ginseng (Trinh Thi Huong & Duong Tan Nhut, 2011; Trinh Thi Huong et al., 2013) and *Codonopsis* (Tran Van Thinh et al., 2015).

### **Application PTCC for production of secondary biologically active substances**

Secondary compounds in plants are those in the plant body but have no role in the basic life process of plants (assimilation, respiration, transport, growth and development) but only play a secondary role. The primary function of secondary compounds is to protect plants against pathogens and herbivores. Many secondary biologically active substances are used as insecticides, fungicides and pharmaceuticals. In plants, the secondary compounds consist of three main groups: terpenoids, phenolic compounds and nitrogen-containing compounds. Many secondary compounds are used as valuable medicinal herbs or food additives.

The development of PTCC has opened up the ability to use this technique to produce biomass capable of synthesizing secondary substances. A large-scale biomass production in laboratories is an alternative to traditional secondary extraction methods from natural plants.

In Vietnam, research on the application of PTCC to acquire secondary substances has been started since the 80s of the last century on *Panax pseudoginseng*, *Nicotiana*, *Artemisia annua*, *Taxus wallchiana*, etc. Phan Huy Bao & Le Thi Xuan (1986) generated *Panax pseudoginseng* plants with high saponin content. The variation of nicotine content in differentiated callus tissues of tobacco was reported by Nguyen Duc Thanh et al. (1991). Tissue culture of *Taxus wallchiana* was carried out very early by Nguyen Kim Lan et al. (1996) for the purpose of acquiring paclitaxel (Taxol), a substance used to treat certain types of cancer. The acquisition of secondary substances by tissue culture methods is possible through callus culture, cell suspension culture, tuber formation, secondary rooting and hairy root formation. Phan Thi Bay et al. (1994b) cultured callus and regenerated *Artemisia annua* L. for acquisition of artemisinin, a drug that is effective against malaria. Quach Thi Lien et al. (2005) and Vu Thi Lan et al. (2008)

carried out the callus culture of *Crinum latifolium* L. to obtain saponins and some alkaloids with potential anti-cancer activity. Studies of callus culture to produce biomass to obtain secondary substances have been conducted on a number of other plant species such as: *Maesa balansae* Mez. (Quach Thi Lien & Nguyen Duc Thanh, 2004) and *Panax vietnamensis* Ha et Grushv. (Duong Tan Nhut et al., 2009b). Cultivation of cell suspension has been studied on *Artemisia annua* L. (Bui Thi Tuong Thu et al., 2010), *Taxus wallchiana* Zucc. (Le Thi Thuy Tien et al., 2006; Duong Tan Nhut et al., 2007d) and *Ehretia asperula* Zollinger et Moritzi (Tran Thi Tam Hong & Tran Van Minh, 2019). *Panax vietnamensis* Ha et Grushv has received much attention on biomass production, from *in vitro* tuber production (Hoang Xuan Chien et al., 2011), creating adventitious roots (Duong Tan Nhut et al., 2015a) and secondary roots (Nguyen Thi Nhat Linh et al., 2017, 2018) to hairy root formation (Ha Thi My Ngan et al., 2013; Mai Truong et al., 2013; Pham Bich Ngoc et al., 2013; Tran Thi Ngoc Ha et al., 2013; Trinh Thi Huong et al., 2015; Ha et al., 2016; Ha Thi Loan et al., 2014; Ha Thi Loan & Duong Hoa Xo, 2017; Ha Thi Thu Hoa et al., 2018). Hairy root culture has also been reported for biomass production to acquire artemisinin from *Artemisia annua* L. (Bui Thi Tuong Thu et al., 2012) and saponin from *Polyscias fruticosa* L. Harms (Nguyen Trung Hau et al., 2015).

### **Contribution to global PTCC**

In addition to the high impact on the development of science and practice in the country, Vietnam PTCC has made impressive contributions to the field of plant tissue and cell culture, in particular, and the world science, in general. Many works implemented in Vietnam have been published in prestigious international journals and monographs. These are studies on factors affecting plant cell tissue culture (Duong Tan Nhut, 2005; Duong Tan Nhut et al., 2005; 2006a; 2007a, 2007b; 2008a, 2015b; 2016; 2017b; Nguyen Hong Vu et al., 2006; Nguyen Ba

Nam et al., 2016; Vu et al., 2019), on *in vitro* propagation (Duong Tan Nhut, 1998, 2003; Duong Tan Nhut et al., 2004a, 2009c, 2011), on acquisition of secondary substances from plant tissues and cells (Nguyen Hoang Loc & Nguyen Thi Tam An, 2010; Nguyen Hoang Loc & Nguyen Thi Duy Nhat, 2013; Nguyen Hoang Loc et al., 2014, 2017; Nguyen Huu Thuan Anh et al., 2016; Nguyen Thanh Giang et al., 2016; Nguyen Huu Nhan & Nguyen Hoang Loc, 2017, 2018; Nguyen Thi Nhat Linh et al., 2019), on TCL culture technique (Duong Tan Nhut et al., 2007e, 2012a, 2012b, 2012c, 2013), on gene transfer in plants (Nguyen et al., 2016; Tran et al., 2017; Vi et al., 2017) and on photoautotrophic culture (Nguyen et al., 1999, 2001; Nguyen & Kozai, 2005; Nguyen et al., 2016, 2020; Hoang et al., 2017). Although these contributions are still modest, it has made a remarkable impression in the world of plant tissue and cell culture.

#### CHALLENGES AND PROSPECTS

Over 45 years of establishment and development, Vietnam's PTCC has achieved impressive results. Most PTCC techniques have been applied to basic and practical researches. It can be said that Vietnam's PTCC has developed broadly (broadly in terms of methods, technologies, and research and application facilities). However, the scale is limited. Most of the results are limited to the laboratory scale, some are small and medium pilot, not yet reaching industrial scale. The basic research has not been deeply focused. Although two National Key Laboratories for plant cell technology (one in the Institute of Agricultural Genetics and the other in the Institute of Tropical Biology) and one National Key Laboratory on Genetic Engineering (in the Institute of Biotechnology) have been set-up, but the organization and operating budget still have many problems. The cooperation between research institutions and production enterprises is very limited.

In the future, PTCC will still be an important tool in basic and practical researches. For basic research, especially for cell differentiation, gene expression, antigen

production, recombinant protein, and genetically modified plants, these are the areas that will be of great interest. *In vitro* propagation combined with hydroponic and aeroponic technologies will be a potential approach for rare and high economic value varieties. Industrial-scale cell suspension and hairy root cultures are important approaches to obtain plant-derived secondary substances for pharmaceutical and cosmetic industries.

#### CONCLUSION

PTCC was started in Vietnam in the 70s of the last century. The formation and development of Vietnam's PTCC has contributed significantly to the development of plant cell technology, in particular, and biotechnology, in general. Many impressive results have been recorded in basic and practical researches. Many effective *in vitro* propagation protocols for potato, banana, sugarcane, pineapple, eucalyptus, acacia, etc. have been developed and applied in practice to provide quality seedlings for production. Several techniques such as anther culture, apical shoot-tip culture, thin cell layer culture, and embryos culture have contributed significantly in producing and developing plant varieties (rice, potato, lilies, orchids, Ngoc Linh ginseng, etc.). However, there are some limitations in the use of PTCC to acquire secondary substances, in the studies on gene expression and recombinant protein, and in plant breeding through gene transfer. Along with the impact on domestic science and technology, Vietnam's PTCC has recorded remarkable impressions in the development of this field in the world. These are the researches carried out in Vietnam on culture conditions, TCL culture, and biomass culture for secondary substances acquisition, photoautotrophic micropropagation and gene transfer which have been published in international prestigious journals and monographs.

With high potential for basic and practical research, PTCC have been and will still be an effective tool for the studies on cell proliferation, genetics, biochemistry, improvement and development of plant

varieties. In order to bring into full potential of PTCC in Vietnam, an attention should be paid to perfecting *in vitro* propagation procedures that have been properly formulated and appropriately invested in order to expand seedling production on an industrial scale. Combining *in vitro* propagation with hydroponic and aeroponic is necessary to improve propagation efficiency and produce high quality seedlings. Building and perfecting the protocols of micropropagation, cell suspension culture, embryo culture, hairy root culture for a number of precious and rare medicinal plants of high economic value are critical to obtain secondary substances on an industrial scale. Research on expression of antigen, recombinant protein and on gene-edited crops by modern gene editing technology should be enhanced. In parallel with technical and technological issues, increasing investment in infrastructure, staff training, promoting linkages between research institute and production enterprises, and timely transfer of completed technologies to the scientific and production enterprises are important solutions for the effective use of PTCC in research and practice.

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