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Medicinal and ornamental plant tissue culture in Brazil and worldwide: Researches and patents

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Abstract

Different plant tissue culture techniques are used to produce medicinal and ornamental plants. Here, we quote such techniques, depending on the final product, and evaluate the trends of plant culture techniques used in Brazil and worldwide based on our research of patents and published papers from 2000 to 2018. We also present companies had work with plant tissue culture in Brazil. Although information is scarce, results show that patents for *in vitro* production of plants under controlled environmental conditions are expanding worldwide. While most Brazilian plant production is restricted to the domestic market, signs indicate that the international market could be expanding. Otherwise, the Chinese are the chief exporters in the production process of plantlets, which may be marketed as seedlings or used as biofactories for the pharmaceutical industry, for example. The main techniques based on plant tissue culture patents, are related to direct organogenesis, both for ornamental and medicinal tissue culture protocols. It is noteworthy that the number of patents related to tissue culture protocols for medicinal plants is higher than that for ornamental plants and that the number of published articles too, particularly those reporting on cultivation ofornamental and medicinal plants, far exceeds the number of patents related to plant tissue culture in general.

Index terms: *In vitro* culture; plant market; plant biotechnology; intellectual property.

INTRODUCTION

Plant tissue cultures incorporate techniques for *in vitro* culture of cells, tissues or organs. The process begins with the removal of any parts, i.e., explants, which, when introduced to the culture medium, can give rise to plantlets directly from the selected material, or indirectly through callus or embryo produced from the explant (Grout, 2017). Since these techniques are based on multiplication and cellular totipotentiality, each cell in theory can give rise to new cells and therefore differentiates into tissues, organs and whole organisms. These techniques allow to maintain the same genotypic characteristics of the original plant, however due to stresses of *in vitro* culture conditions, culture media and various factors as genotype and explant source, genetic characteristics of regenerants may change (Dias, 2016). Therefore, *in vitro* techniques might be a useful tool for generating genetic variability, i.e., somaclonal variations as a result of genetic and epigenetic changes at different levels - morphological, cytological, cytochemical, biochemical, and molecular (Krishna et al., 2016).

Generally, the use of plant tissue culture techniques aims to obtain plants, or plant products, such as secondary metabolites, in a uniform manner, in large-scale production and with high phytosanitary quality (Victório et al., 2008; Cardoso, 2014). The health of the seedlings produced by plant tissue culture is a requirement and an advantage over those produced conventionally; such plants are grown under sterile conditions and when they go to the acclimatization stage they present a load of microbial contamination much lower than those produced in the conventional way. Economically, contamination of plant material by pathogens, such as bacteria, virus and fungi, can cause high financial losses in medicinal and ornamental crops (Corredoira et al., 2017) and plant tissue culture can contribute to produce plants of high sanity quality mainly from meristem cultures and using thermontherapy (Balogun et al., 2017). The clonal production of plants, also termed micropropagation, is among the most used plant tissue culture techniques and, by using such techniques, it is also possible to select the genotypes aseptically and in large-scale in a short time, independent of genetic crosses/recombination (Carvalho; Rodrigues; Santos, 2016).

Many sectors are benefited by plant tissue culture techniques, such as forestry, production of ornamental and medicinal plants, production of essential oils and other secondary metabolites used by the pharmaceutical and cosmetic industries, production of synthetic seeds or synseed (Sharma et al., 2013); the production of transgenic

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plants (Dias, 2016); the evaluation of somaclonal variation, as a plant breeding strategy and conservation of genetic resources (germplasm) of diverse species through synthetic seeds or synseed, especially those with difficult sexual or asexual propagation, the seeds of which are not viable, and plants threatened with extinction (Dey; Saha; Ghosh, 2015).

In vitro production of medicinal and ornamental plants is widespread, using different techniques of plant tissue culture. Here we performed a survey of companies in Brazil that use plant tissue culture to obtain medicinal and ornamental plants, as well as a comparison between plant tissue culture patents and articles worldwide, as currently applied, to discuss the current status of techniques on plant tissue culture in the production of ornamental and medicinal plants. This research aimed to identify the main countries involved with this type of technology, as well as to evaluate which are the main techniques used in plant tissue culture.

METHODOLOGY

Research on Brazilian companies that work with plant tissue culture

In order to locate Brazilian companies that use plant tissue propagation, searches were performed using the internet as a tool and on RENASEM website - *Registro Nacional de Sementes e Mudas* (National Register of Seeds and Seedlings), an entity of the Brazilian Ministry of Agriculture, Livestock and Supply.

Patents related to plant tissue culture and main techniques

In order to compile patents related to plant tissue culture, research was carried outwith key expressions in Portuguese and English, and those with the most hits were as follows: "Plant tissue culture", "Ornamental plant tissue culture" and "Medicinal plant tissue culture". The following patent databases were consulted: Espacenet Patent Search, World Intellectual Property Organization-Pantentscope (WIPO) and, in Brazil, the National Institute of Industrial Property (Instituto Nacional da Propriedade Industrial - INPI).

Comparison between number of research articles and review articles

To survey the number of articles, the same Keywords/ Key expression were used to access platform of scientific journals searched on the database ScienceDirect, dividing our research between number of research articles and number of review articles.

RESULTS AND DISCUSSION

The Brazilian national patent database, INPI, presented only seven patents related to the expression "Plant tissue culture" (PTC), and none data for "Ornamental plant tissue culture" and "Medicinal plant tissue culture" expressions. A relevant number of patents with the more general key expression "Plant Tissue Culture" were obtained from data in the Patentscope and Espacenet (Figure 1). In order to obtain patents related to the plant tissue culture of ornamental and medicinal plants from January, 2000 to October, 2018 we chose Espacenet, an international database with world data patents, using the three terms quoted in English.

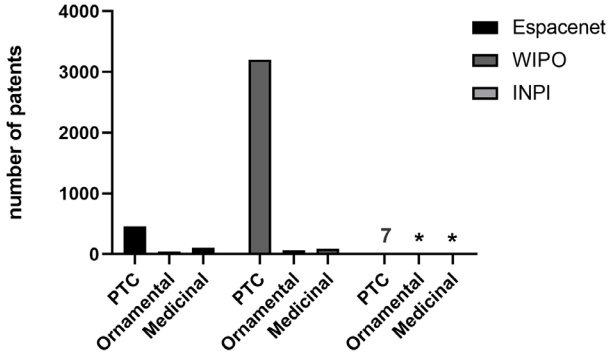

Figure 1 – Number of registered patents found on databases of National Institute of Industrial Property (Instituto Nacional da Propriedade Industrial-INPI, Brazil), World Intellectual Property Organization- Patentscope (WIPO international) and Espacenet (international) in the period of 2000-2018. Key expressions (PTC- plant tissue culture, Ornamental - Ornamental plant tissue culture and Medicinal - Medicinal plant tissue culture) searched in the databases. [7]Seven patents was found in INPI database. *Nonexistent.

Brazilian companies, according to internet search, and their respective *in vitro* produced cultures and shown in Table 1. Most companies seek to obtain seedlings in mass using micropropagation and the most propagated are from Orchidaceae family. Only 14 companies were found in an Internet search compared to the number found by Carvalho, Rodrigues and Santos (2016) who reported

139 entities registered for *in vitro* plant propagation in the RENASEM. However, according to our search, in Brazil, despite the number of companies mined by Carvalho, Rodrigues and Santos (2016), it is difficult to find companies, even though the RENASEM website. This site lists the names of individuals, instead of companies, and does not distinguish between techniques to produce seedlings grown in nurseries and those using plant tissue cultures. Although not listed, it should be noted that some "companies" are actually laboratories in universities.

Table 1 – Brazilian companies that use plant tissue culture techniques for ornamental and medicinal plants according to Internet search.

National Company/State	*In vitro* culture		Technique used
	Ornamental	Medicinal	
Biocell-Clonagem – MG	Bromeliads (Bromeliaceae)	Arrowleaf Elephant's Ear (*Xanthosoma sagittifolium* - Araceae)	Micropropagation
BioClone - CE	Orchids (Orchidaceae), Anthuriums (Araceae), Palm and Cactus (Cactaceae)	Ginger (*Zingiber oficinale* - Zingiberaceae), Fig (*Ficus carica* –Moraceae), Black sage (*Varronia curassavica* – Boraginaceae), Bamboo (*Dendrocalamus asper* – Poaceae), Yam (*Colocasia esculenta* – Dioscoreaceae), Vanilla (*Vanilla planifólia* – Orchidaceae), Aloe vera (*Aloe vera* – Asphodelaceae), Turmeric (*Curcuma longa* – Zingiberaceae), Patchouli (*Pogostemon cablin* – Lamiaceae) Guaco (*Mikania glomerata* – Asteraceae), Passion fruit (*Passiflora edulis* and *P. alata* – Passifloraceae), Pariri (*Arrabidaea chica* - Bignoniaceae) and Blackberrry (*Morus nigra* - Moraceae)	Micropropagation Temporary Immersion Bioreactors
Biofábrica da Unopar – PR	Orchids (Orchidaceae)	Lemongrass (*Cymbopogon citratus* – Poaceae) and Gingseng (*Panax ginseng* - Araliaceae)	Micropropagation
Biofábrica da Vale/Centro de Tecnologia de Ferrosos – MG	Orchids (Orchidaceae), Bromelids (Bromeliaceae) and Cactus (Cactaceae)	n.p.	Micropropagation
Biofábrica de Cacau - BA	White or purple denphal orchid (*Dendrobium*; *Phalaenopsis* – Orchidaceae)	n.p.	Micropropagation
Clona-Gen – SC	Orchids	n.p.	Micropropagation
Clonagri – SP	Avencão (*Arachniodes adiantiformis* - Dryopteridaceae), Anthurium (*Anthurium andraeanum*), *Philodendrom melinonii* and *Philodendrum* spp. and Alocasia (*Alocasia macrorrhizos* - Araceae)	n.p.	Micropropagation
Flora Biotecnologia – SC	Orchids (Orchidaceae) and another ornamental plants	Varied - unspecified.	Micropropagation
Itamudas (ES Gonçalves Empresa Agrícola Ltda) – RJ	Ornamental plants	n.p.	Micropropagation

Continue...

Table 1 – Continuation...

National Company/State	*In vitro* culture		Technique used
	Ornamental	Medicinal	
Plantech (Orquidário Takebayashi) – SP	Various orchids: *Cattleya*, *Dendrobium, Encyclia, Laelia,Schumburgkia, Stanhopea*	n.p.	Micropropagation
ProClone – SP	Orchid (Orchidaceae), Ferns (Nephrolepidaceae), Caladium (*Caladium bicolor* - Araceae), Calla (*Zantedeschia aethiopica* - Araceae)	n.p.	Micropropagation
Renato da Cunha Pereira – RJ	Ornamental plants	n.p.	Micropropagation
SBW do Brasil – SP	Gerbera (*Gerbera jamesonii*- Araceae); Tumeric (*Curcuma longa* - Zingiberaceae); *Phalaenopsis* (Orchidaceae); Statice (*Limonium sinuatum*- Plumbaginaceae); Hemerocalis (Asphodelaceae); Lilly (*Lillium* - Liliaceae); Alstroemeria (*Alstroemeria* hybrida- Alstroemeriaceae); Anthurium; (*Anthurium* - Araceae) Violet (*Viola* - Violaceae)	n.p.	Micropropagation

n.p. – no produce.

According to Espacenet, the direct organogenesis technique was the most used to obtain ornamental and medicinal plantlets, representing around 48% and 51% of the techniques used, respectively. For the cultivation of medicinal plants, indirect organogenesis (27%), callus culture (25%) and germination (16%) were the most used after direct organogenesis (Table 2). For ornamental plants, meristem culture was the second most used technique (21%), followed by strictly callus culture (20%) and indirect organogenesis (13%). Although trying to differentiate callus culture from indirect organogenesis seems redundant, it is important to highlight the context in which they are being used in the searched patents. In the case of callus culture, it is usually aiming at mass production for cultivation for subsequent extraction of metabolites, while in the case of indirect organogenesis it is more cited as a way to obtain plant regeneration from calli. Overall, China leads in the number of patents for both ornamental and medicinal plant breeding techniques, supporting the data obtained by Liu and Liu (2010) who reported that China had between 2,500 and 3,000 companies working with plant tissue culture in 2010 and generating about 100 million plants annually. According to our research, Republic of Korea and The United States of America are the second and third countries, respectively, in plant tissue culture number of patents. Brazil did not appear in our research.

Worldwide, the main medicinal plants produce by tissue cultures are from families Zingiberaceae, Lamiaceae, Poaceae and Passifloraceae, while the ornamental plants are mainly of the Orchidaceae, Bromeliaceae, Araceae and Liliaceae (Table 2). Medicinal plant cultures have more patents than ornamental plants, but without the predominance of a specific species for micropropagation.

Table 2 – Patent deposits in the period between 2000 and 2018 in the area of plant tissue culture of medicinal and ornamental plants, using the Espacenet Patent Search databases.

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
China - Jiangxi Academy of Forestry	*Citrus aurtantium* (Rutaceae)	Direct organogenesis	Seedlings	
United States - University of Massachusetts Medical Center	Lamiaceae	Meristems culture	Phenolic secondary metabolites; Essential oils	2001
China - South China Botanical Garden, CAS	*Vanda* (Orquidaceae)	Direct organogenesis	Seedlings	2005
China - Tianjin University	*Glycyrrhiza glabra* (Fabaceae)	Germination; Direct organogenesis; Callus culture; Indirect somatic embryogenesis	Seedlings	2005
China – Tianjin University	*Astragalus* (Fabaceae)	Germination; Direct organogenesis; Callus culture; Indirect somatic embryogenesis	Seedlings	2005
Taiwan - Agricultural Research Institute	*Coridalys* (Fumariaceae)	Callus culture; Direct somatic embryogenesis	Propagation of tubers; Production of alkaloids	2005
China - Nanjing Forestry University	*Polygonum multiflorum* (Polygonacea)	Direct organogenesis; Root culture	Seedlings	2007
China - Shanghai Jiao Tong University	Medicinal plants-unspecified	Bioreactor	Cultivate types of different tissues, cells and organs	2008
China - Shanghai Jiao Tong University	*Radix pseudostell*	Callus culture; Indirect organogenesis, Root culture	Roots with medicinal metabolites	2008
China - Zhejiang Research Institute Of Traditional Chinese Medicine; Zhejiang Research Institute of Traditional Chinese Medicine Co Ltd	*Anoectochilus roxburghii* (Orchidaceae)	Direct organogenesis	Seedlings	2008
China - Southwest University	*Datura stramonium* (Solanaceae)	Root culture; Direct organogenesis	Production of scopolamine and Histocyamine	2008
China - Xinjiang Uighur Autonomous Region Chinese Medicine National Drug Institute; Institute of Chinese Materia Medica and Minorities Materia Medica of Xinjiang Uygur Autonomous Region	*Ferula* (Apiaceae)	Callus culture; Indirect organogenesis	Seedlings	2008

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
Republic of Korea - Inje University Industry-Academic Cooperation Foundation	Medicinal plants-unspecified	Callus culture	Medicinal and industrial proteins	2008
Republic of Korea - World Intellectual Property Organization; Inje University Industry; Academic Cooperation Foundation; Huh, Gyung Hye; Kim, Young Wha; Kim, Bo Mi	Medicinal plants-unspecified	Callus culture; Culture of cells in suspension; Root culture	Medicinal and industrial proteins	2009
China - Kunming Institute Of Botany, Chinese Academy of Sciences	*Ypsilandra thibetica* (Melanthiaceae)	Direct organogenesis	Seedlings; Production of substances of pharmaceutical interest	2009
Republic of Korea - Supercritical Laboratory Co Ltd	Medicinal plants-unspecified	Callus culture	Callus phytohormone residues-free	2009
China - Suzhou University	*Illicium* (Schisandraceae)	Direct organogenesis	Seedlings	2010
China -Zhejiang Province Academy of Agricultural Sciences	Ornamental plants - unspecified	Bioreactor	Seedlings	2010
The United States- Inje University Industry-Academic Cooperation Foundation; Huh Gyung Hye; Kim Young Hwa; Kim Bo Mi	Medicinal plants-unspecified	Callus culture; Culture of cells in suspension; Root culture	Medicinal and industrial proteins	2010
China - Hefei University of Technology	*Huperzia serrata* (Lycopodiaceae)	Direct organogenesis	Seedlings	2010
China - Shanghai Jiao Tong University	*Hippeastrum* (Amaryllidaceae)	Direct organogenesis	Seedlings	2011
China -Tianjin City Agricultural Bio-Tech Research Center	*Maranta arundinacea (*Marantaceae)	Direct organogenesis	Seedlings	2011
Korea Republic - Republic Korea Man Rural Dev	Ornamental plants - unspecified	Bioreactor	Seedlings	2011
China - Zhejiang Senhe Seed Industry Co Ltd	*Nandina domestica* (Berberidaceae)	Meristem cultures; Direct organogenesis; Indirect somatic embryogenesis	Seedlings	2011
China - Zhang Xiyu; Li Zongju	*Begonia* (Begoneaceae)	Direct organogenesis	Seedlings	2011
China - Inst Botany Jiangsu Province & CAS	*Jasminum sambac* (Oleaceae)	Direct organogenesis	Seedlings	2011
China - Tibet Institute of Plateau Biology	*Pedunculate herpetospermum* (Curcubitaceae)	Direct organogenesis	Seedlings	2011

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
China - Wang Weimin; Lv Xinbo; Yang Bowen; Zhong Yunxiang	Medicinal plants-unspecified	Direct somatic embryogenesis	Transgenic medicinal proteins	2011
China - Guangxi Zhuang Autonomous Region Pharmaceutical Botanical Garden	*Tinospora capillipes* (Menispermaceae)	Germination; Direct organogenesis	Seedlings	2011
China - Nanjing University of Technology	*Pinellia* (Araceae)	Direct organogenesis	Seedlings	2011
China - Institute of Medicinal Plant Development; Chinese academy of Medical Sciences	*Agropyron repens* (Poaceae)	Meristem cultures; Direct organogenesis	Seedlings	2011
Republic of Korea - Samyang Genex Corporation	Medicinal plants-unspecified	Callus culture	Betulinic acid	2011
United States - World Intellectual Property Organization - Imperial Innovations Limited	Medicinal plants-unspecified	Culture of cells in suspension	Polypeptides; Medicines; Leaf biomass	2011
China - Inst Botany Jiangsu Province & CAS	*Allium tuberosum* (Amaryllidaceae)	Direct organogenesis	Seedlings	2012
China - Guangxi Institute of Botany; The Chinese Academy of Science	*Anoectochilus roxburghii* (Orchidaceae)	Germination; Direct organogenesis	Seedlings	2012
China - Jiangxi Normal University	*Huperzia serrata* (Lycopodiaceae)	Direct organogenesis	Hupenine A	2012
China - Guangdong Institute of Traditional Chinese Medicine	*Pogostemon cablin* (Lamiaceae)	Protoplast Culture	High content of patcholenone	2012
United States - World Intellectual Property Organization - Michoux Franck; Nixon Peter; Mccarthy James Gerard; Imperial Innovations Limited	Medicinal plants-unspecified	Callus culture	Biomass hardwood, Polypeptides	2012
China - University Zhejiang A & F	*Lycoris chinensis* (Amaryllidaceae)	Callus culture Indirect somatic embryogenesis	Seedlings	2013
China - University Zhejiang A & F	*Lycoris chinensis* (Amaryllidaceae)	Direct organogenesis; somatic embryogenesis	Seedlings	2013
China - Nanjing Yimei Biotechology Co Ltd	*Cymbidium* (Orchidaceae)	Callus culture; Indirect somatic embryogenesis	Seedlings	2013
China - Zhejiang A&F University	*Lycoris chinensis* (Amaryllidaceae)	Callus culture; Indirect organogenesis	Seedlings	2013
China - Zhejiang A&F University	*Lycoris chinensis* (Amaryllidaceae)	Callus culture; Indirect organogenesis	Seedlings	2013

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
China - Kunming University	*Melasma arvense* (Orobanchaceae)	Germination; Direct organogenesis	Seedlings	2013
China - Vegetable Research Institute of Tibet Academy of Agricultural and Animal Husbandry Sciences	*Lamiophlomis rotate* (Lamiaceae)	Direct organogenesis	Seedlings	2013
China - Ianjin Binhai International Flower Science & Technology Park Co., Ltd	*Chirita wentsaii* (Gesneriaceae)	Meristems culture	Seedlings	2014
China - Institute of Botany, Jiangsu Province and Chinese Academy of Sciences	*Acanthopanax gracilistylus* (Araliaceae)	Meristems culture	Seedlings	2014
China - Chen Xiaochao	Ornamental plants-unspecified	Bioreactor	Seedlings	2014
China - Liang Caiying	*Rhodomyrtus tomentosa* (Myrtaceae)	Meristems culture	Seedlings	2014
China - Luo Shilei	Ornamental plants-unspecified	Bioreactor	Seedlings	2014
China - Dalian Nationalities University; Dalian Jinguiyuan Technology Development Co Ltd	*Schizonepeta* (Lamiaceae)	Germination; Direct organogenesis	Seedlings	2014
China - Yan Liyan	*Paeonia* (Paeoniaceae)	Callus culture; Indirect organogenesis	Seedlings	2014
China - Horticultural Research Institute; Anhui Academy of Agricultural Sciences	*Dendranthema morifolium* (Asteraceae)	Callus culture; Indirect organogenesis	Seedlings	2014
China - Cas Biobreeding Research Institute Wuxi Co Ltd	*Paris polyphylla* (Melanthiaceae)	Meristems culture	Seedlings	2014
China - Li Guoqiao; Hu Sihua	*Artemisia absinthium* (Asteraceae)	Direct organogenesis	high yield of sweet wormwood leaves and the high artemisinin content	2014
China - Li Youhui	*Lilium davidii* (Liliaceae)	Meristems culture	Seedlings	2015
China - Li Youhui	*Dianthus caryophyllus* (Caryophyllaceae)	Meristems culture	Seedlings	2015
China - Liang Shihua	*Styrax tonkinensis* (Styracaceae)	Direct organogenesis	Seedlings	2015
China - Zhu Haiyan	*Sorghum versicolor* (Poaceae)	Meristems culture	Seedlings	2015
China - Feng Wenjie	*Cercis canadensis* (Fabaceae)	-	Seedlings	2015
China - Feng Wenjie	*Paeonia ostii* (Paeoniaceae)	Meristems culture	Seedlings	2015
China - Feng Wenjie	*Clematis* (Ranunculaceae)	Meristems culture	Seedlings	2015

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
China - Research Institute of Subtropical Forestry, Chinese Academy of Forestry	*Quercus nuttallii* (Fagaceae)	Meristems culture	Seedlings	2015
China - Ingdao Huasheng Green Energy Agricultural Technology Co Ltd	*Fittonia verschaffeltii* (Acanthaceae)	Meristems culture	Seedlings	2015
China - West Anhui University	*Dendrobium huoshanense* (Orchidaceae)	Culture of pollen and protoplasts; Direct organogenesis	Seedlings	2015
China - Cheng Guangyou	*Sophora flavescens* (Fabaceae)	Direct organogenesis	Seedlings	2015
China - Guangxi Botanical Garden of Medicinal Plants	*Dysosma versipellis* (Berberidaceae)	Meristems culture	Seedlings	2015
China - Hunan Institute of Agricultural and Biological Resources Utilization	*Millettia dielsiana* (Leguminosae, Fabaceae)	Callus culture; Indirect organogenesis	Seedlings	2015
China - Chen Fengjia	*Phellodendron amurense* (Rutaceae)	Meristems culture	Seedlings	2015
China - Zhu Binggui	*Aquilaria sinensis* (Thymelaeaceae)	Callus culture; Indirect organogenesis	Seedlings	2015
China - Zhu Binggui	*Sorbus alnifolia* (Rosaceae)	Germination; Direct organogenesis	Seedlings	2015
China - Feng Wenjie	*Fritillaria unibracteata* (Lilliaceae)	Direct organogenesis	Seedlings	2015
China - Feng Wenjie	*Euphorbia lathyris* (Euphorbiaceae)	Callus culture; Indirect organogenesis	Seedlings	2015
China - Chengdu University,	*Pseudolarix* (Pinaceae)	Callus culture; Indirect organogenesis	Seedlings	2015
China - Hangzhou Zhengde Agriculture Development Co Ltd	*Dendrobium candidum* (Orchidaceae)	Germination	Seedlings	2015
India - Imperial Innovations Limited	Medicinal plants-unspecified	Callus culture; Indirect organogenesis	Polypeptides; Medicinal natural products; Capture carbon dioxide	2015
China - Linyi University	*Cymbidium bicolor* (Orchidaceae)	Germination; Direct and indirect Organogenesis	Seedlings	2015
Russia - Federal Noe G Bjudzhetnoe Obrazovatel; Noe Uchrezhdenie Vysshego Professional; Nogo Obrazovanija Altaj	*Potentilla alba* (Rosaceae)	Direct organogenesis	Seedlings	2015
China - Suzhou Shenyuan Biological Science & Technology Co Ltd	*Dendrobium officinale* (Orchidaceae)	Direct organogenesis	Seedlings	2015
China - Shenzhen Polytechnic	*Rhododendron moulmainense* (Ericaceae)	Direct organogenesis	Seedlings	2016

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
China - Xu Zhenfei	*Phalaenopsis* spp. (Orquidaceae)	Direct organogenesis	Seedlings	2016
China - SHANGHAI Institute of Technology	*Chirita liguliformis* (Gesneriaceae)	Direct organogenesis	Seedlings	2016
China - Jiangsu University	*Atractylodis lanceae* (Asteraceae)	Direct organogenesis	Seedlings	2016
China - Rice Research Institute, Guangxi Academy of Agricultural Sciences	*Tylophora kerrii* (Asclepiadaceae)	Direct organogenesis	Culture media	2016
China - Liu Zhiyang; Liu Yan	Medicinal plants-unspecified	Bioreactor	Seedlings	2016
China - University Zunyi Medical	*Bletilla striata* (Orchidaceae)	Germination; Somatic embryogenesis	Seedlings	2016
China - Wang Fanglin; Chai Chengwu; Ma Junmei; Cui Jianguo; Li Aide; Wang Yuqi; Gao Songtao; Zhang Yinghua; Wei Linyuan; Zhang Jinchun; Li Jinhui; Chang Li; Hu Jing	*Lycium ruthenicum* (Solanaceae)	Direct organogenesis	Seedlings	2016
Japan - Mp Innovations Ltd	Medicinal plants-unspecified	Callus culture; Indirect organogenesis	Production of woody biomass; Production of polypeptides, Natural Medicinal Products	2016
China - University Chengdu	*Bletillae* (Orchidaceae)	Meristems culture	Seedlings	2016
China - Guangzhou Ruishen Biological Tech Co Ltd; Dongguan Ruishen Biological Tech Co Ltd	*Dendrobium officinale* (Orchidaceae)	Bioreactor	Seedlings	2016
China - University Zunyi Medical	*Bletilllae* *(*Orchidaceae)	Bioreactor	Seedlings	2016
China - Nanjing Zelang Biological Science & Tech Co Ltd	*Dalbergia hupeana* Hance (Fabaceae)	Direct organogenesis	Seedlings	2016
China - Bengbu City Jinniuwan Agricultural Science And Tech Dev Co Ltd	*Rhododendron fortunei* (Ericaceae)	Meristem culture; Direct organogenesis	Seedlings	2017
China - University Jilin Normal	*Rosa chinensis* (Rosaceae)	Direct organogenesis	Seedlings	2017
China - Jiangsu Coastal Region Agricultural Science Res Institute; University Yancheng Teachers; Yancheng Xinyu Agricultural Dev Co Ltd	*Helianthus annuus* (Asteraceae)	Direct organogenesis	Seedlings	2017
China - South China Agricultural University	*Echinacea* (Asteracea)	Direct organogenesis, Direct somatic embryogenesis	Seedlings	2017

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Table 2 – Continuation...

Depositors (Country and applicant)	Ornamental plant specie / botanical family	Tissue culture technique	Product	Published year
Denmark - World Intellectual Property Organization; University of Copenhagen; Ipalk Sas	*Thapsia* (Apiaceae)	Direct and indirect organogenesis	*Guaianolides*	2017
China - Yulin Normal University	*Helwingia japonica* (Helwingiaceae)	Direct organogenesis	Seedlings	2017
China - Yulin Normal University	*Erythropalum scandens* (Erythropalaceae)	Direct organogenesis	Seedlings	2017
China - Yulin Normal University	*Melicope pteleifolia* (Rutaceae)	Direct organogenesis	Seedlings	2017
China - Sichuan Miaoyuan Ecological Agricultural Tech Co Ltd	*Trichosanthes kirilowii* (Cucurbitaceae)	Meristem cultures; Direct organogenesis	Seedlings	2017
China - Wuhan Botanical Garden Cas; Hubei Jingfeng Agriculture Co Ltd	*Bletilla* (Orchidaceae)	Germination, Direct organogenesis	Seedlings	2017
China – Changsha University	*Galium aparine* (Rubiaceae)	Direct organogenesis	Seedlings	2018
China – Changsha University	*Aglaonema schott* (Araceae)	Direct organogenesis	Seedlings	2018
China - Foshan Hengai Network Tech Co Ltd	*Phalaenopsis aphrodite* (Orchidaceae)	Direct organogenesis	Seedlings	2018
China - Dalian Jinguiyuan Tech Development Co Ltd	*Dendrobium officinale* (Orchidaceae)	Germination; Direct organogenesis	Seedlings	2018
China - Lincang Yunruitang Biological Science & Tech Co Ltd	*Polygonatum kingianum* (Asparagaceae)	Germination; Direct organogenesis	Seedlings	2018
China -Mianyang Shuchuang Agricultural Tech Co Ltd	*Cremastra appendiculata* (Orchidaceae)	Germination; Direct organogenesis	Seedlings	2018
China - Guangxi Institute of Botany Chinese Academy of Sciences	*Anoectochilus roxburghii* (Orchidaceae)	Germination; Direct organogenesis	Seedlings	2018
China - Li Zhengme	*Radix stellariae* (Caryophyllaceae)	Germination; Direct organogenesis	Seedlings	2018
China - Biotechnology & Germplasm Resource Institute Yunnan Agricultural Academy	*Zizania* (Poaceae)	Germination; Callus culture; Indirect organogenesis	Seedlings	2018
China - Environmental Horticulture Res Institute of Guangdong Academy of Agricultural Sciences	*Arundina chinensis* (Orchidaceae)	Direct organogenesis	Seedlings	2018
China - Chen Peidang	*Morinda officinalis* (Rubiaceae)	Germination; Callus culture; Indirect organogenesis	Seedlings	2018

Total patents with the keywords from 2000 to 2018: “Plant tissue culture”: 460; Total patents with the keywords “Ornamental plant tissue culture”: 46; Total patents with the keywords “Medicinal plant tissue culture”: 104.

The production of cells, tissues, organs and plants through tissue culture is based on the use of different *in vitro* techniques including, organogenesis (direct and indirect pathways), calogenesis, excised zygote embryo culture, somatic embryogenesis (direct and indirect pathways), pollen and anther cultures, suspension cell culture, and protoplast culture. Depending on the purpose of the culture, any one of these methods can be used alone or in combination with other techniques (Ikenganyia et al., 2017; Oseni; Pande; Nailwal, 2018). The indirect pathway is interspersed by a step of de-differentiation of mature (somatic or haploid) tissue that forms a cellular mass called callus and then initiates the re-differentiation/ organization process. The direct pathway involves the formation of organs and the production of plantlets directly from the explant, effectively bypassing disorganization/ dedifferentiation (George; Hall; Klerk, 2008). Callus is a growing mass of plant parenchyma cells. By observing callus under optical microscopy, it is possible to identify, in part, unorganized and/or organized processes and structures of tissues that show plant development (Boix et al., 2013).

Different plant tissue techniques may be used to produce a high quantity and quality plant material. Cultures are classified as organized when explants present features of organized growth and defined tissue/structure that can be maintained continuously (George; Hell; Klerk, 2008). Disorganized tissue cultures are comprised of cell mass aggregates, making it impossible to visualize defined plant structures (George; Hell; Klerk, 2008), but such mass aggregates may be used to produce calli and cell suspension cultures or plantlets indirectly. Among techniques used in plant tissue culture, micropropagation is the one most commonly used by direct organogenesis. The most used techniques to obtain medicinal and ornamental plants can be enumerated in the following order: i- production of axillary buds and induction of adventitious buds by direct or indirect organogenesis; ii- somatic embryogenesis; iii– *in vitro* gemination; iv- *in vitro* culture by thin cell layer (TCL) and v- bioreactors (Rout; Mohapatra; Mohan Jain, 2006; Carvalho; Rodrigues; Santos, 2012; Croom et al., 2016).

Espacenet revealed that the main technique of patents registered for tissue culture of ornamental plants predominantly involved direct organogenesis, somatic embryogenesis and *in vitro* germination, all aimed the large scale production of plants (Table 2). For medicinal plants, the main technique was also direct organogenesis, followed by callus culture and indirect organogenesis, also mostly aimed at mass propagation of plants for cultivation for subsequent extraction of metabolites (Table 2). However, in the 18 years between 2000 and 2018, the number of patents related to plant tissue culture (Table 2) was still far behind that of research and review articles involving the subject according to researched key expressions (Figure 2). The Figure 2 shows the high number of research articles about PTC (60,305) over 18 years. Among research articles, the quantity of manuscripts about medicinal plants was 8,914 against 1,651 for ornamental plants.

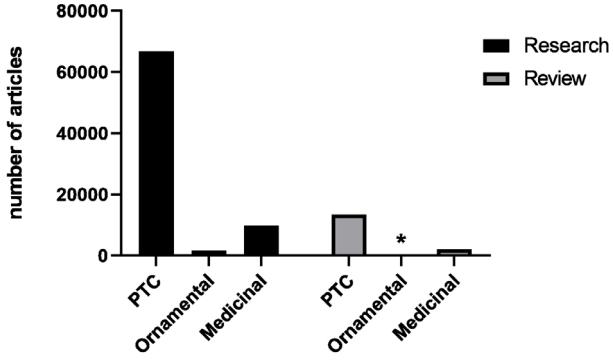

Figure 2 – Quantitative of research and review articles publishied between 2000 and 2018 related to key expressions and "Plant tissue culture" (PTC). "Ornamental plant tissue culture" (Ornamental) and "Medicinal plant tissue culture" (Medicinal). *371 review articles found.

In almost thirty years, traditional academic culture, centered on publications and recognition from peers, has not changed enough, although efforts are currently underway to change this way of thinking and integrate industry and university to encourage innovation (Sandberg et al. 2014). Although complex, the relationship between university and corporate research must be a mutually beneficial partnership: university can work and offer corporate research, while companies can rely on universities and the start-ups that to produce the technology that they need to develop themselves (Arora et al. 2017).

CONCLUSIONS

In Brazil, it is difficult to locate companies via the Internet, and the responsible institution (RENASEM) does not distinguish between *in vitro* plant production units and plantlet nurseries, making it difficult for public research on the site. However, the latest data collected in the literature from the year 2008 to 2015 (Carvalho; Rodrigues; Santos, 2016) suggest that the number of biofactories is increasing.

Companies prefer to use micropropagation to obtain standardized plantlets in mass propagation. Orchids lead the ranking of ornamental plants. Medicinal plant cultures have more patents than ornamental plants, although there is not predominance of a specific species.

Most patents related to plant tissue culture are registered by Chinese institutions according to Espacenet. Brazil haven´t relevant quantitative data on plant tissue culture patents, according to INPI, and even internationally, as claimed by Espacenet. Even so, the number of articles far exceeds the number of patents related to plant tissue culture, in general, and culture of ornamental and medicinal plants, in specific.

According to Espacenet research for tissue culture of ornamental plants, the main techniques, as determined by the number of patents, are direct organogenesis and meristem culture. For medicinal plants, the main technique used was also direct organogenesis, followed by callus culture and indirect organogenesis. The number of articles related to tissue culture of ornamental and medicinal plants is still higher than the number of patents, although more research articles on the development of protocols can be found compared to literature reviews.

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