Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2020, Vol. 4 (4), 505-523
https://doi.org/10.29329/ijiaar.2020.320.12

Review article

In Vitro Plant Tissue Culture: Means for Production of *Passiflora* Species

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Abstract

Passiflora genus includes over 600 species native to tropical and subtropical areas of America, appreciated for the production of fruit and medicinal value. Their ornamental potential is especially appreciated in North America and in Europe. With the expansion of the flower trade and the use of secondary metabolites in the pharmaceutical industry, a need for the constant monopolization of new technologies and alternative *in vitro* techniques that allow to obtain a uniform, high quality material free of pests and diseases occurs. *Passiflora's* tissue cultures began to be studied in 1966, raising more and more interest of researchers worldwide. Depending on the source and type of the explant, plant growth regulators, and the used genotype, direct and indirect organogenesis are the main regeneration pathways for *Passiflora*. The latest approaches regarding the choice of explant and its source, the plant material surface sterilization and the specific requirements of each micropropagation stage are presented within our review. To this genus, the reduced gas exchange of in vitro growing of seedlings has been shown as the main cause of lack of success. In this regard, for regeneration and obtained improvements in morphogenesis, different protocols have been developed by using inhibitors of ethylene. In recent years, studies suggest that via somatic embryogenesis, starting from mature and immature zygotic embryos, regenerated plants that have maintained their mother plant ploidy can be successfully obtained. This confirms the callus cultures as main path to obtain *in vitro* regenerated *Passiflora* plants.

Keywords: *Passiflora*, Regeneration, Organogenesis, Somatic embryogenesis.

Received: 11 September 2020 * **Accepted:** 17 December 2020 * **DOI:** https://doi.org/10.29329/ijiaar.2020.320.12

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INTRODUCTION

This review comprises the contemporary researches on advances in tissue culture of *Passiflora*. The *Passifloraceae* family includes more than 600 species distributed in 18 genera, widespread in North and South America, here being the center of biodiversity, with over 95% of species (Vanderplank, 1996). Of these, 50 species are recognized for their edible fruits, but only *Passiflora edulis* is internationally commercially exploited (Vieira, 2004). Although from an ornamental point of view, *Passiflora* is little used in Brazil, in European countries and in the United States it is highly valued (Abreu et al., 2009). About 20 species are cultivated exclusively for ornamental value. In Brazil, the most popular are *P. elegans, P. picturata, P. raddiana.* also *P. quagrangularis, P. ligularis* and *P. manicata* cultivated for ornamental purposes and for fruit production in Madeira (Silva, 2000). In Europe, *P. caerulea* is marketed for ornamental purposes. The *Passiflora* genus is known on a large area in the world, being most appreciated for the gardens and terraces decoration, but also growing in pots for bright and spacious interiors (Boboc et al., 2017). Most species are cultivated for the ornamental value of flowers due to their exotic appearance, diversity of colors and color combinations and the impressive crown of filaments, a defining characteristic of the *Passifloraceae* family. Following a survey based on a questionnaire on the degree of knowledge of *Passiflora* locally in Romania, it appears that this genus was a well-known ornamental plant, 63.5% of respondents said they know the plant, its usage and characteristics (Boboc et al., 2020).

Passiflora was first used for medicinal purposes in Peru, from where the spanish doctor, Dr. Monardes, brought it to Europe in 1569. In many European countries, in USA and Canada it has been used as a tranquilizer in neurosis for more than 200 years (Silva, 2000). Passiflora leaves contain important secondary metabolites such as: alkaloids, saponins, cyanogenic compounds and phenolic compounds (Silva & Bottoli, 2015). *Passiflora* species widely used in traditional medicine (Dhawan et al., 2004) are: *P. incarnata* (Dhawan et al., 2004; Kim et al., 2017; Singh & Pradesh, 2016; Zas & John, 2016, 2017), *P. foetida* (Bendini et al., 2006; Gaspar et al., 2019), *P. quadrangularis* (Gaspar et al., 2019; Yuldasheva et al., 2005), *P. caerulea* and *P. alata* (Ozarowski et al., 2018; Singh & Pradesh, 2016). Most studies have proved the use of the species in antimicrobial, cardiac or neurodegenerative treatments. Various types of extracts have been found to have compounds with a broad spectrum of action on certain organs, biochemical processes or physiological functions (Boboc et al., 2017). Nowadays, herbal medicines are being marketed in Romania to treat insomnia or to induce sleep, to treat mental tension, depression, irritability, or premenstrual syndrome. Also, *Passiflora* teas and tinctures are highly appreciated (Boboc & Cantor, 2017). The passion flower is a perennial, vigorous liana, with a woody or herbaceous stem that has corkscrews. The leaves are usually alternate, of different shapes depending on the species. Flowers, variously colored, may or may not have a crown of filaments (Vieira, 2004). The flowers can be bisexual or unisexual, actinomorphic, solitary or in inflorescences, usually

with bracts, five sepals, five petals and one or more series of filaments. The androecium is composed of 5 stamens and the gynoecium of 3 carpels (Vanderplank J, 2000). The flowers present decorative value from June to August (Cantor & Pop, 2008).

Considerable intra and interspecific variability, along with the exuberance of flowers and leaves led to its widespread use for decorating European greenhouses and gardens in the seventeenth century (Abreu et al., 2009).

Passiflora is an allogamous plant par excellence and its flowers are self-incompatible with most species. Thus, there is a need for pollination in order to produce fruit. Self-incompatibility has important implications for propagation methods, as commercial orchards need genetically distinct plants. The method of propagation for most fructification species is the sexual but asexual propagation (clones obtained by cuttings, grafting or tissue culture) is often used in some cases (Alexandre et al., 2004).

According to George et al., 2008, the tissue culture term is used to describe all types of *in vitro* plant cultures. *In vitro* cultures aim at two types of growth of plant material: in organized form (specialized organs and tissues) and unorganized (callus and cell suspensions) (Cătană, 2005). Cell biotechnologies, applied to passionflowers have aroused the interest of researchers around the world (Otoni et al., 2013). *Passiflora* micropropagation techniques have been studied since Nakayama, 1966 when the first *in vitro* culture of *P. caerulea* from nodal segments was initiated (Mikovski et al., 2018).

According to Fernando et al., 2007, the main pathway of *in vitro* regeneration for *Passiflora* is direct organogenesis (Becerra et al., 2004; Monteiro et al., 2000; da Gloria et al., 1999; da Silva et al., 2011; de Faria et al., 2018; Dornelas & Carneiro Vieira, 1994; Drew, 1991; Nhut et al., 2007; Otahola & Diaz, 2010; Pinto et al., 2010; Pipino et al., 2008; Prammanee et al., 2011; Vieira et al., 2014) and indirect organogenesis (Monteiro et al., 2000; Lombardi et al., 2007), depending the source of the explant and the genotype used.

PROBLEMS ASSOCIATED WITH *PASSIFLORA* MICROPROPAGATION

The production of seedlings from *Passiflora* by conventional methods has proven to be problematic due to the low germination rate of seeds but also due to plant diseases that significantly reduce the efficiency of conventional vegetative propagation (Ozarowski & Thiem, 2013). *In vitro* propagation methods are useful in breeding programs, allowing a constant and homogeneous supply of high quality phytosanitary plant materials (Ulmer & MacDougal, 2004). At the initiation of tissue cultures, the first stage consists in the disinfection of explants and seeds, a defining stage for in vitro cultures ensuring their success.

Recalcitrance

Recalcitrance is the inability of a plant tissue to respond to *in vitro* manipulations, which over time leads to cell aging and loss of morphogenetic capacity, but also the ability of cell totipotency. The reaction of tissue culture is influenced by the physiological state of the donor plant, *in vitro* manipulations and physiological stress (Benson, 2000). Tissue culture responses are influenced by donor plant physiology, *in vitro* manipulations and stress physiology. Regarding the species *P. edulis* f. *flavicarpa*, it was found that the genotype from Uganda is recalcitrant to tissue culture, without the possibility of emitting shoots or forming callus. The culture was initiated from leaf fragments, but the influence of genotype, type and age of the explant and phytohormone concentrations on the regeneration rate was not studied in detail (Tuhaise et al., 2019). Drew, 1991, states in his paper on the species *P. edulis* f. *flavicarpa* that the stage of culture stabilization is difficult to achieve by proving that adult tissue is recalcitrant to culture *in vitro*, in addition to tissue contamination. Thus, the author recommends taking explants from nodes close to growth areas.

Contamination

Contamination of biological material is one of the factors underlying the impossibility of stabilizing an *in vitro* culture. *Passiflora* secretes a sweet sap that is a nutritious environment for many microorganisms (Ozarowski & Thiem, 2013). Plants of this genus are often infected with *Xanthomonas axonopodis* pv. *passiflorae* (Boro et al., 2011) and PWV (passion fruit woodiness virus) (Arogundade et al., 2018) two pathogens that reduce its exploitation period and ornamental value.

Browning

Explants browning is caused by oxidation of phenols in tissues. Necrosis reduces cell division and thus the ability to regenerate explants leading to *in vitro* culture failure. To prevent this phenomenon, it is recommended to use absorbent substances and antioxidants. The time interval from the initiation of culture to the appearance of burnished tissue may be influenced by the presence of secondary metabolites in the tissues or by the time of sampling. Thus, it is recommended to harvest the explants in the morning before sunrise, respectively before the plant begins to do photosynthesis and immerse them in water to dissolve the secondary compounds. To avoid browning, activated charcoal and polyvinyl pyrolidine (PVP) were used (Singh, 2018). Activated charcoal is used in tissue cultures for the purpose of cell proliferation and development but especially as an absorbent of inhibitory substances, added to the culture medium before autoclaving (Thomas, 2008). Becerra et al., 2014, state that *in vitro*-derived explants from *P. edulis* did not undergo necrosis. In general, necrosis affects 50% of explants after 60 days of culture due to senescence or accumulation of toxic substances in the environment.

Sensitivity to ethylene accumulation

Ethylene is a gaseous hormone produced in most plant tissues and is involved in several stages of the plant life cycle (Dias et al., 2010). Ethylene accumulates in culture vessels, although vials allow the exchange of ethylene, often the production is higher than the loss, which leads to its accumulation in culture vessels (Pua, 1999). Analysis of the dynamics of ethylene accumulation as well as the control of its level in culture vessels is essential to establish effective *in vitro* culture protocols for these species (Otoni et al., 2013). Seedlings belonging to this genus have been shown to have difficulty growing *in vitro* due to reduced gas exchange. *P. edulis* f. *flavicarpa* has been shown to be highly sensitive to ethylene, affecting apex development, rhizogenesis, and adventitious shoot emission (Faria et al., 1997; Reis et al., 2003)

Thus, various regeneration protocols have been developed that have used ethylene inhibitors and have achieved improvements in morphogenesis. Dias et al., 2010, investigated how ethylene acts on the *in vitro* morphogenic capacity of *P. cincinata* and its effects on polyamine biosynthesis. For this purpose, two ethylene inhibitors (AVG- aminoetoxyvinyl glycine and STS - silver thiosulfate) and its promoter (ACC-1-aminocyclopro-pane-1-carboxylic-acid) were used. Treatment with the AVG ethylene inhibitor determined a higher regeneration rate and treatment with its ACC promoter decreased the regeneration rate. The data obtained suggested that the levels of biosynthesis of polyamino and ethylene are not correlated with morphogenic responses to *P. cincinnata*. However, sensitivity to ethylene appears to play an important role in the differentiation process (Dias et al., 2010). Another potent inhibitor of ethylene added to the preparation of culture media is AgNO3 (Kumar et al., 2009) which favored the differentiation of explants and seedling proliferation in *P. alata* (Pinto et al., 2010) and in *P. edulis* f. *flavicarpa* (Huh et al., 2017; Trevisan & Mendes, 2005).

MEANS FOR REGENERATING PASSIONFLOWER

Plants tissues and cells culture represents the aseptic culture in controlled nutritional and environmental conditions in order to obtain clones, identical to the genotype selected for multiplication (Oseni, 2018). Since Haberlandt's first *in vitro* culture in 1902, this segment has evolved as an indispensable tool for disease-free plant research and production, for the production of plant-derived secondary metabolites of significant economic importance (Espinosa et al., 2018).

In vitro cultures represent an alternative for the production of secondary metabolites because they ensure independence from geographical conditions (Espinosa et al., 2018).

Organogenesis

Organogenesis is a unique developmental process in the plant kingdom. Plant cells retain throughout their lives the ability to differentiate and begin a new path of development that generates completely different morphogenic structures (Cătană, 2005). Organogenesis involves the formation of

de novo organs (shoots or roots) that can evolve from differentiation of non-meristematic tissue (callus), a process known as adventitious shoot formation or pre-existing meristematic tissue, a process identified as multiple axillary shoots. Both approaches require a synergistic interaction of physical and chemical factors (Kothari et al., 2010).

In order to ensure the success of *in vitro* culture, thorough research is required, which involves choosing the right type of exponent, taken at the right age, identifying a suitable culture medium formula, with growth regulators specific to the intended objective and by adding a source of carbohydrates and a gelling agent in optimal amount. Physical factors such as light regime and temperature are important for the efficiency of the micropropagation process. Recent studies on the micropropagation of *Passiflora* species are listed in Table 1.

In most research to date on tissue cultures in *Passiflora*, the explants come from *in vitro* regenerated seedlings of the zygotic embryo (da Silva et al., 2011; de Carvalho et al., 2017; de Faria et al., 2018b; Dornelas & Vieira, 1994; Fernando et al., 2007; Guzzo et al., 2004; Isutsa, 2004; Jafari et al., 2016; Otoni et al., 2013; Pinto et al., 2010; Rocha et al., 2012; Trevisan & Mendes, 2005; Veeramohan et al., 2013; Vieira et al., 2014) to prevent crop contamination. Experiments were also performed in which the explants were taken from mature plants, grown in the greenhouse or in the field (Becerra et al., 2004; Prithviraj, et al. 2015; Shobha, 2015; Ragavendran et al., 2012; Rathod et al., 2014).

The donor plants are usually obtained by *in vitro* or *in vivo* germination of seeds, however they kept in greenhouse conditions can present the problem of contamination. Thus, it is recommended to use plant material obtained *in vitro* (Pacheco et al., 2016).

Table 1. Latest studies on *in vitro* culture of *Passiflora*

Species	Explant tissue	Culture meduim + PGR's (mg/l)	Response	References
P. alata, *P. edulis*, *P. crenata*, *P. foetida*, *P. gibertii*	ze	MS + (0.51) BA + (1.56-3.12) 2,4-D	Callus induction	(Rosa et al., 2015)
		½ MS	Germinated embryos and/or elongated plants	
P. caerulea	ls	MS + (0.5) NAA +(0.5) IAA +(0.5) 2-4-D + (0.5) KIN + (1) BAP	Shoots induction + callus formation	(Rathod et al., 2014)
		MS + (1) KIN + (2) BAP	Shoots induction	
		MS + (1) KIN + (2) BAP + (0.4) IAA	Shoots multiplication	
		MS + (2) 2,4-D + (0.8) IAA +(1) KIN +(2) BAP	Callus fully developed	
		MS + (1) NAA + (0.5) IBA + (0.5) IAA	Roots induction	
P. caerulea	ls-ad ls-ab	MS + (0.5 - 4) BAP	Shoots induction	(Prithviraj et al., 2015)
		MS + (0.5-3) BAP + (0.5 - 1) NAA	Shoots + roots induction	
		MS + (0.25 - 3) IBA + NAA (0.25 - 3)	Roots induction	
P. caerulea	sht, cn	MS + (0.5, 1, or 1.5) BAP + (0.05, 0.1 or 0.15) IBA	Shoots induction	(Jafari et al., 2016)
		MS + (0.25, 0.5 or 1) TDZ + (0.025, 0.05 or 0.1) IBA		
		MS + (1 or 2) KIN + (0.1 or 0.2) IBA		
		MS + (0.5, 1 or 2) IBA	Roots induction	
		MS + (0.5, 1 or 2) NAA		
		MS + (0.5, 1 or 2) IAA		
P. caerulea	ns	MS wihout PGR`s	Regenerated plants	(Faria et al., 2020)
		½ MS wihout PGR`s		
P. cincinnata	ze	MS + (4) 2-4-D + (1) BAP	Somatic embryos induction	(Vieira et al., 2018)
		MS + (15) activated charcoal	Somatic embryos maturation	
		MS + (0.5) GA_3 + (15) activated charcoal	Somatic embryos elongation	
P. cristalina	ls, hy, rs, co, en	MS + (0.5 - 2) BA	Shoots induction	(de Faria et al., 2018b)
		MS + (0.5 - 2) TDZ		
		MS + (0.5 - 2) KIN		
P.edulis f. *flavicarpa*	ns	MS + (1 -5) BAP + (1 -5) KIN	Shoots induction	(Shekhawat et al., 2015)
		MS + (0.5 -2.5) BAP + (0.5 -2.5) KIN	Shoot multiplication	
		MS + (0.5 - 3) IBA + (0.5 - 3) NAA	Roots induction	
P. edulis	ls	MS + (0.5 -2) BAP + (1 - 4) 2,4-D	Callus	

			formation	(Huh et al., 2017)
		MS + (0.5 - 2) BAP + (1 - 4) 2,4-D	Somatic embryo Shoot formation	
P. edulis	en	MS + (0.25 -1.01) BAP	Regenerated plants	(Antoniazzi, et al., 2018)
		MS + (0.25 – 1.3) TDZ		
		MS + (0.52 – 1.05) KIN		
P. edulis f. *edulis*	ls	MS + (1.6 - 2.2) BAP	Shoots induction	(Tuhaise et al., 2019)
		MS + (0.05) BAP	Shoots proliferation and elongation	
		MS + (0.33) GA_3		
		MS + (1 or 2) NAA	Roots induction	
P. edulis	ns	MS + (0.25-1.02) BAP + (0 or 0.33) GA_3 + (0 or 0.05) IBA	Shoots proliferation	(Khas et al., 2020)
		MS + (0.26-1.04) TDZ + (0 or 0.33) GA_3 + (0 or 0.05) IBA		
		MS + (0.12 – 1.31) IAA or (0.11-1.02) IBA or (0.12-1.23) NAA	Roots induction	
P. gibertii	c-ls	MS + (0.23 – 0.95) PIC + (0 or 0.46) KIN	Callus formation	(M. A. de F. Carvalho et al., 2015)
		MS + (0.26 – 1.04) 2.4-D + (0 or 0.46) KIN		
		MS + (0 or 0.05) IBA		
P. setacea *P. tenuifila*	sht is ss	MS wihout PGR`s	Shoots induction	(Sozo et al., 2016)
		MS + (0.14-0.57) GA_3		
		MS + (0.14-0.57) IBA	Shoots + roots induction	
		MS + (0.25) 2,4-D	Callus formation	
		MS + (0.14-0.57) NAA		
P. tenuifila	ns	MS wihout PGR`s	Regenerated plants	(Silvério Junior et al., 2020)
		½ MS wihout PGR`s		

Legend: c-ls = cotyledonary leaf segments, cn= cotyledonary node, co= cotyledons, en= endosperm, is= immature seeds, hy= hypocotyl, ls = leaf segmentsm, ls-ab/ ls-ad = leaf segments on abaxial or adaxial surface, ns = nodal segments, sht= shoot tips, ss = stem segments, rs= root segments, ze= zygotic embryo.

In the case of the *Passifora* genus, for the induction of organogenesis (Nhut et al., 2007) the main way to obtain regenerated plants *in vitro* (Otoni et al., 2013) is used mainly the culture medium MS (Murashige and Skoog, 1962) supplemented with growth phytoregulators that vary depending on the purpose for which morphogenesis is induced (Mikosvki et al., 2019). Regardless of the regeneration pathway, in addition to the complete MS medium, ½ MS (with half concentration) is also used. However, several authors have reported symptoms of mineral deficiency. Thus, in order to obtain more promising results in terms of micropropagation in *Passiflora*, Monteiro et al., 2000, proposed adjusting

the mineral composition of the MS medium based on the nutrient level of the leaves of *P. edulis* f. *flavicarpa* plants grown in the field. Modified medium (MSM) shows an increase in the concentration of P, Ca, Mg, S, Mn, Cu, Na and FeEDTA, a decrease in the concentration of Zn and does not contain Cl or I. The efficacy of the new culture medium was confirmed by analysis visual effects of plants, which were taller and formed a larger number of green leaves (Pacheco et al., 2016).

Direct organogenesis

Direct organogenesis is induced in *Passiflora* species using mainly 6-benzyladenine (BA) in concentrations between 2.2 and 8.8 μM, as such or in combination with thidiazuron (TDZ) in concentrations between 1.1 and 2.3 μM for axillary shoot stimulation (Garcia et al., 2011; Pinto et al., 2010; Trevisan & Mendes, 2005; Vieira et al., 2014) cited by (Mikosvki et al., 2019).

Among the passionflowers cultivated for ornamental purposes, *P. cincinata* proved the best regeneration performance of new seedlings, by organogenesis (da Silva et al., 2011; da Silva et al., 2009; Faleiro et al., 2019; Lombardi et al., 2007).

Jafari et al., 2017 evaluated the micropropagation potential of two types of explants from *in vitro* germination of the zygotic embryo (apexes and cotyledonary nodes). Different concentrations of BAP (0.5, 1 and 1.5 mg / l), KIN (1 and 2 mg / l) and TDZ (0.25, 0.5 and 1 mg/L) were tested in combination with AIB in a concentration of 10:1 against each phytohormone in the culture media. The results showed that the cotiledonary nodes regenerated 90%, obtaining a maximum number of 8.86 shoots in medium supplemented with 1.5 mg/l BAP and 0.15 mg/l AIB.

An efficient micromultiplication protocol by multiple axillary shoots was developed by Prithviraj, 2015 using as explants, fragments of young leaves about 1 cm. The explants were inoculated in the adaxial or abaxial position. For initiation, the MS culture medium was supplemented with different concentrations of BAP (between 1-4 mg / l) or with a hormonal balance containing combinations of 0.5-3 mg/l BAP and 0.5-1 mg/l ANA. The highest number of shoots was obtained on the MS medium containing 3 mg/l BAP. Roots were also formed on the medium containing BAP + ANA. The 8-10 cm seedlings were transferred to the rooting medium. The rooting of the shoots was performed on MS or ½ MS supplemented with AIB or ANA in concentrations between 0.25 and 3 mg/l and the best results were recorded on the variants with 0.5 mg/l (97% rhizogenic response with formation on average of 3.5 roots of 6.6 cm in length) or 1 mg / l (96% rhizogenic response with formation of on average of 3.2 roots of 6.6 cm in length). The acclimatization of the plants was done in pots with vermiculite, manure and earth (1: 1: 1).

Rathod et al., 2014, managed to stabilize an *in vitro* culture of *P. caerulea* starting from young leaf explants harvested from specimens from "Garden of Lokmangal College of Agricultural Biotechnology, Wadala, India". On variants of MS culture medium supplemented with 0.5 mg/l ANA,

0.5 mg/l AIA, 0.5 mg/l 2-4-D, 0.5 mg/l KIN and 1 mg/l BAP the explants formed callus. Direct regeneration without callus formation was obtained on the culture medium supplemented with 2 mg/l BAP and 1 mg/l KIN. In about 54 days a complete and rooted plant was obtained.

Otahola & Diaz, 2010, evaluated the regenerative potential of *P. edulis* and *P. quadrangularis* starting from explants taken from mature plants (leaf fragments and lateral buds). BAP culture medium supplemented with BAP in concentrations between 0.5 and 2 mg/l was used. In the case of variants in which foliar fragments were inoculated, no regenerants were obtained for any of the species. In the case of *P. quadrangularis* species, most shoots were formed on the medium supplemented with 1.5 and 2 mg/l BAP (6.1 and 7.4 shoots per explant, respectively).

Otoni et al., 2013, state that to date, no protocol has been shown to be effective for the regeneration of a large number of plants *in vitro*.

Callusogenesis

Callus culture can be initiated from any type of tissue, but the type of explant, its age and size and its culture are defining factors in obtaining a quality morphogenic callus (Cătană, 2005)

To date, several studies have been performed on the possibilities of inducing callus from different types of explants taken from *Passiflora*, inoculated on the basic MS medium supplemented with various combinations and concentrations of phytohormones. In most cases, the explants were taken from *in vitro* regenerated seedlings starting from seeds germinated under sterile conditions (Ozarowski, 2011). For callus induction, mainly leaf disks taken from young shoots were used (Antognoni et al., 2007; Carvalho et al., 2015; Garcia et al., 2011, Rasool et al., 2011). In the case of explants from plants regenerated from zygotic embryos, fragments of hypocotyl, cotyledonary lamina, embryonic tissue, internodal fragments or root fragments were used (Ozarowski, 2011). The explants reacted differently depending on the hormonal balance and physico-chemical factors. It has been found that callus induction occurs both in the photoperiod (16/8 h light / dark) (Antognoni et al., 2007; Machado et al., 2010; Pinto et al., 2011; Rocha et al., 2012), as well as in total darkness (da Silva et al., 2009; Carvalho et al., 2013) cited by Ozarowski, 2011. Using leaf fragments from *P. quadrangularis*, the callus was efficiently induced on the base medium MS supplemented with BA (2.0 mg/l) and GA3 (1.0 mg/l) but also in the case of the variant containing only BA (0.5 mg/l). In the case of *P. caerulea* species, organogenic callus was obtained on the MS culture medium supplemented with ANA (0.5 mg / l) and KIN (0.1 mg / l) (Ozarowski, 2011).

Despite the medicinal importance of secondary metabolites derived from *Passiflora*, a few studies have also focused on the production of bioactive compouds from biological materials obtained *in vitro* (Pacheco et al., 2016). Thus, (Antognoni et al., 2007) analyzed the production of flavonoids from the non-morphogenic callus derived from somatic explants (leaves) of *P. quadrangularis* grown on MS

medium supplemented with 2,4-D, after UV elicitation. In the case of *P. garckei* species, elicitation of cell suspension cultures with yeast extracts has led to increased production of aromatic compounds (Fraccaroli et al., 2008). Regarding the species *P. alata*, Lugato et al., 2014, analyzed the antioxidant potential and the total phenol content of derived materials from *in vivo* and *in vitro* plant extracts. In the case of leaf extracts derived *in vivo*, a higher antioxidant potential was observed, as well as an increased of phenolic content compared to the biological material *in vitro* (callus and cell suspension cultures). Machado et al., 2010, studied in *P. alata* the production of harman-type alkaloids in callus and cell suspension cultures, although the biosynthesis of these substances has not been detected.

Somatic embryogenesis

Somatic embryogenesis is a way of regenerating plants with many advantages such as: obtaining cell lines for genetic engineering, unlimited production of clones with elite characteristics, regeneration of a bipolar structure in a single cell, basic functional and molecular research, seed production synthetic and conservation of genetic resources through cryopreservation but also for large-scale plant production (Yang and Zhang, 2010; Rocha et al., 2012).

This path of regeneration differs from regeneration by meristems, having particular developmental characteristics, so the formation of the embryo begins from a single cell and passes through the globular, cordiform, torpedo and cotyledonary phase, resulting in a bipolar structure with two types of meristems (radicular and caulinar) (Cătană, 2005).

In 1995, Otoni developed the first protocol for somatic embryogenesis in P. giberti. Starting from leaf explants, the author obtained callus on MS medium supplemented with 2.4 or 4.8 mg / l PIC. Starting from this callus, the author obtained cell suspensions that developed somatic embryos (Otoni, 1995). Studies in recent years have shown the possibility of obtaining regenerated plants by somatic embryogenesis starting from mature and immature zygotic embryos on the basic MS medium supplemented with different combinations of 2,4-D (8.8-72.4 μM) and 4 ,5 μM BA (da Silva et al., 2009; Ferreira et al., 2015; Pinto et al., 2011) cited by (Mikosvki et al., 2019). Instead (Pinto et al., 2010) obtained in the case of P. cincinata species seedlings regenerated by somatic embryogenesis that maintained the ploidy of the mother plant, an aspect that confirms the possibility of using callus cultures to obtain regenerated passionflower plants in vitro.

Conclusions and future prospects

To date, significant progress has been made in improving micropropagation protocols in *Passiflora*, but many aspects remain to be studied and deepened. Important advances consist in establishing the optimal concentrations and combinations of growth phytoregulators in correlation with the purpose of the culture, the use of BAP remaining the phytohormone that provides the best results in organogenesis.

Following the application of the new protocols, a large number of plants have multiplied and taken root by micropropagation. These protocols can be used for the mass production of high-quality, free pest and disease plants, specimens that can be a source of seedlings for farmers who want to set up an industrial plantation at low cost.

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