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REVIEW ARTICLE

Tissue culture and biotechnological techniques applied to passion fruit with ornamental potential: an overview

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Abstract

The ornamental flower sector has growing over the past years worldwide with potential for further expansion. Among the ornamental plants, *Passiflora* species have been gaining ground in the market, mainly in European and North American countries. However, the market aiming the use of these species in ornamentation is still poorly explored. The inclusion of passion flower in the list of ornamental plants is related to the peculiar characteristics of the flower as it is complex structure, capacity of flowering all year long and also by the abundance and exuberance of the leaves, which in many species adds an ornamental value. Among the biotechnological tools for the production of ornamental plants, tissue culture has been outstanding in the cloning of elite genotypes, with high phytosanitary quality and large scale production. In addition, it offers possibilities of producing new varieties with characteristics peculiar to the market of ornamental plants. The diversity of wild *Passiflora* opens perspectives to the conservation, market and production of ornamental *Passiflora* cultivars.
Keywords: Biotechnology, hybrids, micropropagation, ornamental *Passiflora*, polyploids.

Resumo

Cultura de tecidos e técnicas biotecnológicas aplicadas em maracujá com potencial ornamental: uma revisão

O setor de flores ornamentais vem crescendo ao longo dos últimos anos em todo o mundo com potencial expansao. Entre as plantas ornamentais, espécies de *Passiflora* vem ganhando espaço no mercado, principalmente nos países da Europa e América do Norte. Contudo, o uso destas espécies na ornamentação ainda é pouco explorado. A inclusão da flor do maracujazeiro, na lista de plantas ornamentais está relacionada às características peculiares da flor como sua estrutura complexa, capacidade de floração durante o ano todo e também pela abundância e exuberância das folhas, que em muitas espécies agrega valor ornamental. Entre as ferramentas biotecnológicas para a produção de plantas ornamentais, cultura de tecidos tem sido proeminente na clonagem de genótipos elite, com alta qualidade fitossanitária e produção em larga escala. Além disso, oferece possibilidades de produzir novas variedades com características peculiares ao mercado de plantas ornamentais. A diversidade de espécies silvestres abre perspectivas para o mercado e produção de cultivares de *Passiflora* ornamental.
Palavras chave: Biotecnologia, híbridos, micropropagação, *Passiflora* ornamental, poliploides.

Introduction

Commercial floriculture is recognized as one of the most promising segments of the contemporary agribusiness. The expansion of the flower market is mainly a consequence of the constant incorporation of new techniques and technologies of production and logistics as well as the domestication and/or introduction of new species and cultivars into the market (Junqueira and Peetz, 2017; Rabobank, 2016). Among the ornamental plant species, those of the genus *Passiflora* have gained prominence in the ornamental sector, especially in European and in North American countries. Although the *Passiflora* species are mostly native from South America, they have not been used as ornamental plants, in those countries, especially due to the low number of breeding programs for such purposes (Santos et al., 2012).

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Passiflora species are commonly used for their fruits and derivatives, medicinal plant and as a source of oils for cosmetic industry (Pacheco et al., 2016; Cerqueira-Silva et al., 2014). The use of passion flowers as an ornamental plant is due their beauty and exuberance. These flowers are found in the most diverse forms, sizes, and colors and have a distinguished scent (Montero et al., 2013). In the view of the recognized economic importance of the wild and commercial passion fruit species several tissue culture and biotechnological techniques have been applied to the genus (Silva et al., 2009; Otoni et al., 2013; Ozarowski and Thiem 2013; Pacheco et al., 2016; Carvalho et al., 2017; Faria et al., 2018). The present review proposes to summarize the results achieved thus far in the biotechnology of wild *Passiflora* species with ornamental potential.

The ornamentation potential of passion flowers

The inclusion of passion flowers in the list of ornamental plants is related to the highly morphological complexity observed in the reproductive organs of these species. Further, some of these species flourish all year long and have a vigorous and exuberant vegetative growth, with leaves of diverse morphologies, which adds ornamental value to it (Abreu et al., 2009; Meletti et al., 2011; Faleiro et al., 2017). The use of *Passiflora* as ornamental plants started in the 15th century and continues to this day in the market of hybrid plants, especially in Europe and in the North American countries. Over 400 hybrids for ornamental purposes have been registered across the world (Vanderplank, 2000).

*Passiflora*ceae has a pantropical distribution, with approximately 630 species, and great diversification in terms of leaf, fruit, and flower characteristics (Ocampo et al., 2007; Souza and Hopkins, 2011). The genus *Passiflora* comprises approximately 530 species (Ulmer and Macdougal, 2004), 150 to 200 of which originate in Brazil and can be used for multiple purposes such as food, medicine, cosmetics, or ornamentation (Abreu et al., 2009; Pipino et al., 2010). The genus *Passiflora* is divided into four subgenera: Decaloba, Astrophea, Deidamioides, and *Passiflora*. The species of greatest importance belong to the subgenus *Passiflora* (Ulmer and Macdougal, 2004). *Passiflora* flowers are pentamerous, bisexual, and actinomorphic, with a distinct perianth presenting sepals and petals of different shapes and sizes. The petals are usually membranous and develop on the border of the calix tube. All *Passiflora* flowers have an androgynophore that elevates the five fused stamens up to the base and which are also united with a gynoecium composed of three fused carpels (Vanderplank, 2000; Ulmer and Macdougal, 2004; Abreu et al., 2009; Scorza et al., 2017) (Figure 1).

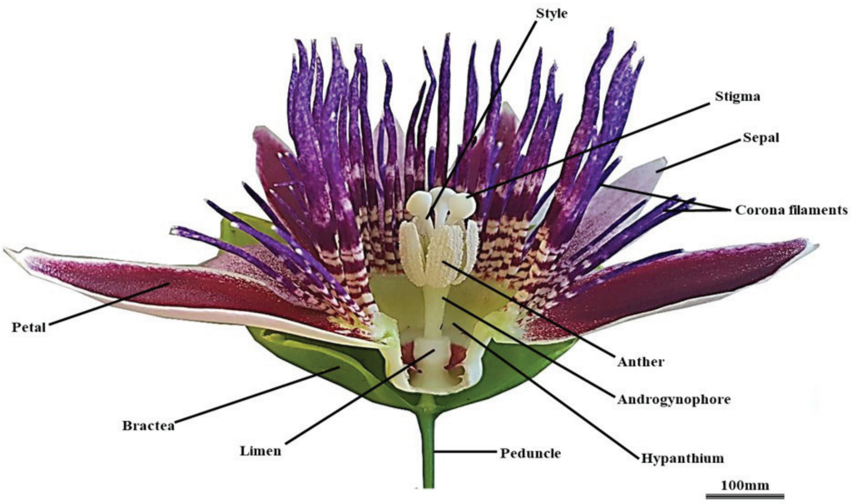

Figure 1. Floral structure of the wild passion flower of *Passiflora capparidifolia.*

Passiflora flowers are considered exotic because of the presence of a multiple series of corona filaments observed between the petals and the androgynophore column. The corona is a distinctive feature of the genus *Passiflora*, and the diversity of shapes, sizes and colors of these filaments seems to be related to the different pollinating systems observed in the genus (Aizza and Dornelas, 2011). Sporophytic self-incompatibility is another important feature of the floral biology of *Passiflora*, which determines allogamy. This characteristic contributes to the increased genetic variability, which may favor the use of those species as ornamental plants (Bruckner et al., 1995; Ferreira et al., 2010).

Several wild *Passiflora* spp. are already described as ornamental plant such as; *Passiflora alata* (Figure 2A), *Passiflora setacea* (Oliveira and Ruggiero, 2005), *Passiflora amethystina*, *Passiflora actinia*, *Passiflora triloba* Ruiz & Pav. EX DC (Peixoto, 2005), *Passiflora caerulea* (Conceição et al., 2011), *Passiflora cincinnata* (Figure 2B) (Oliveira and Ruggiero, 2005); *Passiflora mucronata* (Meletti et al., 2011), *Passiflora morifolia*, *Passiflora suberosa litoralis*, *Passiflora palmeri* var. *sublanceolata* (Pires et al., 2012), *Passiflora capsularis*, *Passiflora rubra* (Amorim et al., 2011). Other species stand out for the beauty and exuberance of their flowers showing a great ornamental potential to be explored, such as; *Passiflora nitida* (Figure 2C), *Passiflora capparidifolia* (Figure 2D), *Passiflora miniata* (Figure 2E) *Passiflora cristalina* (Figure 2F).

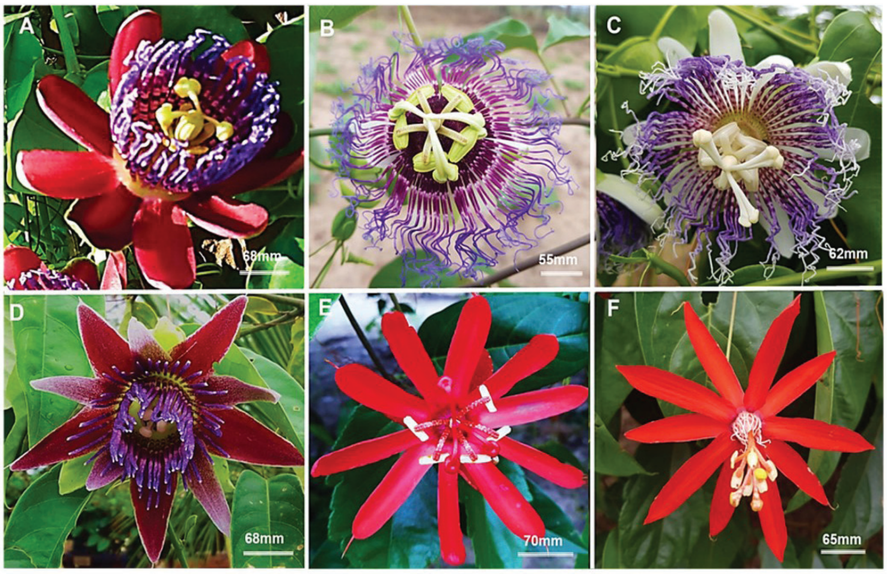

Figure 2. Flowers of species of *Passiflora* with ornamental potential. (A) *P. alata*; (B) *P. cincinnata*; (C) *P. nitida*; (D) *P. capparidifolia*; (E) *P. miniata*; (F) *P. cristalina.*

Passiflora hybrids for ornamental purposes

In view of the ornamental potential of wild *Passiflora*, research centers have selected species with adequate morphological and adaptive traits for the generation of ornamental hybrids (Cerqueira-Silva et al., 2014). The first *Passiflora* hybrid developed for ornamental purposes was reported in 1819 by English breeder Thomas Milne, who made a cross between *P. caerulea* and *P. racemosa* and obtained the sexual hybrid named '*P. violacea*' (Vanderplank, 2000). Currently, passion flower hybryds; e.g. *Passiflora 'Albo-nigra'*, *P. 'Amethyst'*, *P. 'Star of Bristol*', and P. 'Star of Kingston' obtained *P. alata* and *P. caerulea* have been used for decoration of European and American greenhouses (Ulmer and Macdougal, 2004; Conceição et al., 2011).

In Brazil, the Brazilian Agricultural Research Corporation (Embrapa) developed three *Passiflora* hybrids for ornamental use. The first hybrid developed was 'BRS Estrela do Cerrado', obtained from the cross between the wild species *P. coccinea* Aubl. (red flowers), and *P. setacea* DC. (white flowers). Later, another two hybrids were produced from the backcross between 'BRS Estrela do Cerrado' and the parental species, namely 'BRS Roseflora', obtained from the backcross between 'BRS Estrela do Cerrado' and *P. setacea*; and 'BRS Rubiflora', obtained from the backcross between 'BRS Estrela do Cerrado' and *P. coccinea* (Faleiro et al., 2009). The hybrids released by Embrapa and partners have exuberant flowers and are indicated for landscaping of large areas such as walls, fences, and pergolas. In 2016, Embrapa released two new hybrid cultivars for landscaping of large areas. Hybrid 'BRS Céu do Cerrado', with bluish flowers, derived from the cross between the species *P. incarnata* and *P. edulis*; and 'BRS Rosea Púrpura', with pinkish flowers, originating from the triple cross *P. incarnata* × *P. quadrifaria* × *P. setacea* (Fonseca et al., 2017). Other Brazilian institutions also produced hybrids for ornamental purposes; e.g. '*Passiflora alva*', '*Passiflora priscilla*', and '*Passiflora aninha*', developed by the State University of Santa Cruz, Brazil, from the cross between *P. palmeri* and *P. foetida* (Cerqueira-Silva et al., 2014). Fonseca et al. (2017) recently validated morpho-agronomic descriptors used in the protection of cultivars in Brazil and the molecular characterization of ornamental *Passiflora* hybrids developed by Embrapa aiming to differentiate these cultivars obtained from crosses between wild species.

Micropropagation of wild *Passiflora* spp. with ornamental potential

Micropropagation studies in *Passiflora* started in 1966 (Nakayama, 1966) from the culture of nodal segments of an important ornamental species, *P. caerulea*. Since then, a growing number of studies describing in vitro techniques applied to the genus have been published for commercial and wild species with ornamental and phamaceutic propreties (Drew, 1997; Otoni et al., 2013; Ozarowski and Thiem, 2013; Rathod et al., 2014; Pacheco et al., 2016; Rocha et al., 2018; Vieira et al., 2018).

Among the micropropagation systems established for *Passiflora*, organogenesis is the main morphogenetic pathway of regeneration (Otoni et al., 2013) (Table 1), although recent research has demonstrated the occurrence of regeneration via embryogenesis (Silva et al., 2009, 2015; Paim Pinto et al., 2011; Rosa et al., 2015; Ferreira et al., 2015). With only a few exceptions (Moran Robles, 1978), the MS growth medium (Murashige and Skoog, 1962) has been used. However, supplementation with growth regulators varies according to the morphogenetic pathway to be induced. For in vitro organogenesis induction, 6-benzyladenine (BA) at concentration range from 2.2 to 8.8 µM have been adopted for passion fruit shoot bud induction, although thidiazuron (1.1-2.3 µM) alone or in combination with BA has also been reported (Trevisan and Mendes, 2005; Pinto et al., 2010; Garcia et al., 2011; Vieira et al., 2014). On the other hand, somatic embryogenesis has been optimized for several *Passiflora* species from mature and immature zygotic embryos in media supplemented with different combinations of 2,4-D (8.8-72.4 µM) and 4.5 µM BA (Table 1).

Table 1. In vitro regeneration systems for *Passiflora* species with ornamental potential.

Species	Explant source	Regeneration systems	Plant growth regulators (PGRs) (µM)	References
P. morifolia	Zygotic embryo and endosperm	Organogenesis	0.93 µM KIN + 1.0 NAA + 1.0 GA_3	Guzzo et al., 2004
P. palmeri	Zygotic embryo and endosperm	Organogenesis	13.6 2,4-D + 9.3 KIN, 2.25 2,4-D + 1.1 BA + 1.4 IAA	Guzzo et al., 2004
P. nitida	Zygotic embryo and endosperm	Organogenesis	13.6 2,4-D + 9.3 KIN, 0.93 KIN + 1.0 NAA	Guzzo et al., 2004
P. coccinea	Zygotic embryo and endosperm	Organogenesis	13.6 2,4-D + 9.3 KIN	Guzzo et al., 2004
P. "Guglielmo Betto"	Axillary shoots	Organogenesis	2.22 BA	Pipino et al., 2008
P. "Manta"	Axillary shoots	Organogenesis	1.33 BA	Pipino et al., 2008
P. trifasciata	Axillary shoots	Organogenesis	2.32 BA	Pipino et al., 2008
P. foetida "Hastala"	Flower bud	Organogenesis	3.3 BA	Pipino et al., 2008
P. cincinnata	Zygotic embryos	Somatic embryogenesis	18.1 2,4-D + 4.5 BA	Silva et al., 2009
P. alata	Leaf and hypocotyl segments	Organogenesis	2.27 TDZ, 4.43 BA + 2.27 TDZ	Pinto et al., 2010
P. suberosa	Nodal, intermodal and leaf segments	Organogenesis	22 e 44.4 BA	Garcia et al., 2011
P. caerulea	Nodal and internodal segments	Organogenesis	4.44 BA	Severin et al., 2011
P. cincinnata	Root segments	Organogenesis	4.44 BA	Silva et al., 2011
P. foetida	Nodal segments	Organogenesis	8.87 BA + 4.64 KIN	Anand et al., 2012
P. alata	Nodal, internodal and leaf segments	Organogenesis	13.2 e 22 BA	Pacheco et al., 2012
P. foetida	Zygotic embryos	Organogenesis	4.5 BA + 13.6 2,4-D, 4.5 BA + 18.1 2,4-D	Rosa and Dornelas., 2012
P. setacea	Hypocotyl, leaf and root segments	Organogenesis	4.43 BA, 4.43 BA + 2.27 TDZ	Vieira et al., 2014
P. miniata	Zygotic embryos	Somatic embryogenesis	18.1 2,4-D	Ferreira et al., 2015
P. alata, P. crenata, P. foetida, P. giberti	Zygotic embryos	Somatic |embryogenesis	13.6 2,4-D + 4.5 BA	Rosa et al., 2015
P. cincinnata	Synthetic seeds of zygotic embryos and somatic embryos	*In vitro* germination	Sodium alginate + artificial endosperm + ½MS	Silva et al., 2015b

Table 1.cont.

P. suberosa	Root segments	Organogenesis	9.0 BA	Rosa et al., 2016
P. miniata	Zygotic embryos	Organogenesis	3.32 BA	Carvalho et al., 2017
P. cristalina	Leaf segments, hypocotyl, root, cotyledon of zygotic embryo and endosperm	Organogenesis	8.87, 4.43 BA, 2.32 KIN	Faria et al., 2018

Among the ornamental passion flowers, *Passiflora cincinnata* has showing an excellent in vitro performance thanks to its high regenerant formation frequency (Lombardi et al., 2007; Silva et al., 2009; Dias et al., 2009; Silva et al., 2011, Faleiro et al., 2019). Somatic embryogenesis systems have been established for this species from root explants (Reis et al., 2003) and mature zygotic embryos (Silva et al., 2009). However, only with zygotic embryos a reproducible protocol for somatic embryogenesis was obtained (Silva et al., 2009). For *P. cincinnata*, somatic embryogenesis was induced from zygotic embryos cultured in medium supplemented with a high auxin/cytokinin balance (18.1 µM 2,4-D and 4.5 µM BA). Based on this system, other authors have reported successful induction of somatic embryos for other ornamental *Passiflora* species, such as *P. miniata* (Ferreira et al., 2015), *P. alata*, and *P. crenata* (Rosa et al., 2015). However, for *P. suberosa*, only adventitious bud formation was observed when zygotic embryos were cultured in the same conditions established for *P. cincinnata* (Rosa et al., 2015). In vitro regeneration of *P. suberosa* was achieved from nodal segments in the presence of α-naphthaleneacetic acid (NAA), picloram, and 2,4-dichlorophenoxyacetic acid (Garcia et al., 2011), and also from internode (Garcia et al., 2011) and root (Rosa et al., 2016) segments cultured in the presence of BA.

For *Passiflora miniata*, an Amazonian species with great ornamental potential, a regeneration system was observed via organogenesis from zygotic embryos. Regeneration occurred directly and/or from calluses, mainly in the presence of 3.32 µM BA, with an average of 40.0 shoots per explant (Carvalho et al., 2017). Recently, Faria and collaborators (2018) highlighted the high responsiveness of *Passiflora cristalina*, another Amazonian species with ornamental potential. According to the authors, the highest mean number of shoots was observed in hypocotyl segments and in zygotic embryos when cultured in MS medium supplemented with 4.43 µM BA.

Polyploid production of ornamental passion flowers

Ornamental passion flower breeders have used many strategies to obtain polyploids (Fischer, 2004). In general, polyploid individuals have greater vegetative and reproductive vigor as compared with their respective diploids, exhibiting significantly larger floral organs (Stebbins, 1950; Bharadwaj, 2015), despite their reduced fertility. Some allotetraploids have been reported for *Passiflora* spp. species from conventional hybridization: *P. 'Byron Beauty'* (*P. edulis f. flavicarpa* × *P. edulis f. edulis*) × *P. incarnata*), *P. 'Byte'* (*P. edulis* × *P. incarnata*) × (*P. incarnata* × *P. cincinnata*), *P. 'Clear Sky'* (*P. amethystina* × *P. caerulea*) × *P. caerulea*) × *P. caerulea*), *P. 'Fertility'* (*P. incarnata* × *P. cincinnata*), *P. 'Inspiration*' (*P. incarnata* × *P. cincinnata*), *P. 'Ivy Waves'* (*P. coriacea* × *P. suberosa*), *P. 'Jara'* (*P. caerulea* × *P. 'Purple Haze'*), *P. 'Manapany'* [(*P. edulis* × *P. incarnata*) × (*P. incarnata* × *P. cincinnata*)], *P. 'New Amethyst'* (*P. kermesina* × *P. caerulea*), and *P. 'Temptation'* (*P. incarnata* × *P. cincinnata*) (Fischer, 2004).

Because of the triploid nature of the endosperm, the culture of endosperm tissues has been considered a direct method for polyploid production. In *Passiflora*, in vitro endosperm culture was first performed by Mohamed et al. (1996) to obtain triploid *P. foetida* plants. The authors observed the development of shoots via direct organogenesis, with an average of 1.9 shoots per explant in medium supplemented with 2 µM BA and 5 µM NAA. The triploidy of *P. foetida* plants regenerated from endosperms was confirmed (data not published). Triploid *P. foetida* plants also showed greater vegetative vigor and larger leaves and flowers in relation to diploid plants (Figure 3).

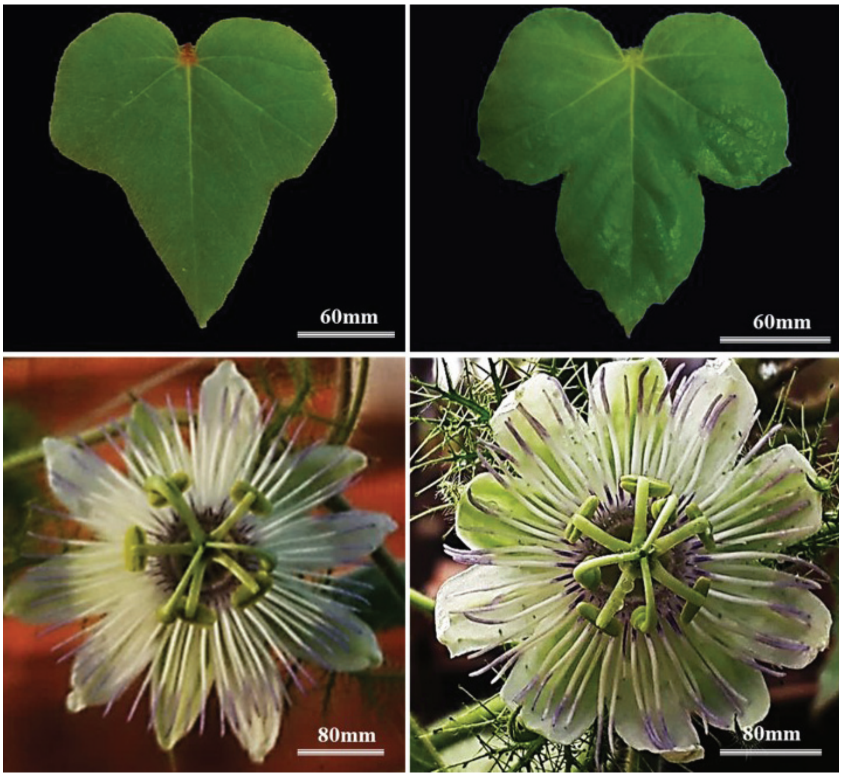

Figure 3. *Passiflora foetida*. (A, B) Leaves of diploid (2n) and triploid (3n) plants. (C-D) Flowers of diploid (2n) and triploid (3n) plants.

The capacity of endosperm tissue to produce triploid and genetically stable plants has also been demonstrated for *P. edulis* (Antoniazzi et al., 2018). The highest number of shoots was obtained when the endosperms were cultured in MS medium supplemented with 9.0 µM TDZ. Faria et al. (2018) also reported plant regeneration from endosperm culture of *P. cristalina* in medium supplemented with 8.87 µM BA. However, the ploidy level of regenerated plants was not evaluated.

Somatic hybridization and genetic transformation

Somatic hybridization by protoplasm fusion could be used to generate new ornamental passion flowers with different shapes, colours and sizes. Protoplast-to-plant regeneration systems have been established for a range passion fruit species and novel interespecific somatic hybrids have been obtained between commercial yellow passion fruit and several wild species (Dornelas et al., 1997; Anthony et al., 1999). However, no hybrids have been produced, by this technique, for ornamental purposes. Considering the diversity of the *Passiflora* species and the relative simplicity of the protocol, the technology has not been used in its full potential (Rocha et al., 2018).

Studies on the genetic transformation of passion fruit are also incipient. The systems alredy stablished have been used for generating disease resistant plants (Vieira et al., 2005; Zerbini et al., 2008). Correa et al. (2015) reported the production of transgenic *P. alata* lines that contain a Cowpea aphid-borne mosaic virus (*CAM V*)-derived coat protein gene fragment via *A. tumefaciens-mediated* transformation. Despite the published reports on genetic transformation of *Passiflora genus*, it is still a way far from being routine, especially for wild species (Rocha et al., 2018).

Conservation and cryopreservation of wild *Passiflora* spp.

The collecting and conserving the diversity of *Passiflora* germplasm is of great importance, no only as a source of genes and natural products, but also for its ecological value (Pacheco et al., 2016). Traditionally, *Passiflora* genetic resources has been conserved in germplasm banks through ex situ conservation strategies (Bernacci et al., 2005; Cerqueira-Silva et al., 2016). It is generally performed inseed banks, although the periodic renewal is limited by the reduction in germination potential, resulting in loss of material. However, some research teams have been focusing in in vitro conservation strategies for the genus (Ávila et al., 2013), seed storage (González-Benito et al., 2009; Ávila et al., 2013) and cryopreservation (Ávila et al., 2013; Pacheco et al., 2016; Simão et al., 2018).

The cryopreservation of passion fruit species has been used to store seeds and in vitro propagules. Due to the difficulties of obtaining seeds from wild populations due to habitat destruction, studies were directed to cryopreservation of shoots and nodal segments. Techniques based on vitrification and encapsulation-vitrification are most often used (Ávila et al., 2013; Pacheco et al., 2016). The plant vitrification protocol has been used for *P. tarminiana*, *P. pinnatistipula* and *P. mollissima* (Ávila et al., 2013). Vianna et al. (2012) observed post-freeze recovery of 60% only with *P. foetida* shoots when cryopreserved using the encapsulation-vitrification protocol. Nodal segments often not chosen for cryopreservation. Nevertheless, a successful cryopreservation protocol for nodal segments of in vitro plants of *P. pohlii* was developed by Merhy et al. (2014) using the vitrification technique. Despite recent advances, future prospects include optimization of existing cryopreservation protocols, as well as innovative approaches to in vitro conservation of *Passiflora* species (Pacheco et al., 2016).

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Author Contribution

A.I.M.[0000-0001-6593-3272]; wrote the paper and design experiments; N.T.S.[0000-0003-1989-1889], C.S.S.[0000-0001-6701-9193] and [MDM0000-0001-5419-7003] designed experiments; W.C.O.[0000-0002-9614-9373], I.F.C.[0000-0003-1657-348X] and D.I.R.[0000-0001-6683-0961] reviewed the paper; M.L.S. [0000-0001-6928-285X] designed the research project and reviewed the paper.

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