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TISSUE CULTURE APPLIED TO CARNIVOROUS SPECIES

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ABSTRACT - The purpose of the review is to comment on available data on the application of plant tissue culture to carnivorous plants. Thus, the review encompassed publications from 1979 to 2017 along *in vitro* germination studies and micropropagation techniques, such as somatic embryogenesis and organogenesis, which emphasized the responses of plant materials to the stimuli offered during *in vitro* culture. Tissue culture in carnivorous plants is presented as a tool to promote the increase of the population of these plants either for scientific and commercial purposes or for the conservation and reintroduction in their natural habitat, in order to ensure a sustainable exploitation of this nutritional pattern of plants. In general terms, the studies carried out were limited to the following aspects: cultivation technique, explant source, exogenously applied substances and culture medium. The review also revealed the absence of defined protocols for in vitro multiplication of large-scale carnivorous plants.
Keywords: biotechnology, *in vitro* cultivation, insectivorous plants, micropropagation.

CULTURA DE TECIDOS APLICADA A ESPÉCIES CARNÍVORAS

RESUMO - O objetivo da revisão é comentar dados disponíveis sobre a aplicação da cultura de tecidos vegetais para plantas carnívoras. Assim, a revisão englobou publicações de 1979 a 2017 com estudos de germinação *in vitro* e técnicas de micropropagação, como embriogênese somática e organogênese, os quais enfatizam as respostas dos materiais vegetais aos estímulos oferecidos durante o cultivo *in vitro*. A cultura de tecidos em plantas carnívoras apresenta-se como uma ferramenta para promover o aumento da população dessas plantas seja para fins científicos e comerciais ou para a conservação e reintrodução no habitat natural, a fim de assegurar uma exploração sustentável desse padrão nutricional de plantas. Em termos gerais, os estudos realizados limitaram-se aos aspectos: técnica de cultivo, fonte de explante, fitorreguladores e meio de cultura. A revisão revelou ainda a ausência de protocolos definidos para a multiplicação *in vitro* de plantas carnívoras em grande escala.
Palavras-chave: biotecnologia, cultivo *in vitro*, plantas insetívoras, micropropagação.

INTRODUCTION

Carnivorous or insectivorous plants are a group of plants that have a carnivorous habit as an adaptation to low nutrient conditions, complementing the autotrophic pattern. The morphological variation inherent to the carnivore in these plants has attracted the attention of ecologists, biologists, horticulturists and collectors for centuries (ARBER, 1941; HESLOP-HARRISON, 1978; GIVNISH et al.,1984).

Some carnivorous species are economically important, such as *Dionaea muscipula* Ellis. and *Drosera capensis* L., which are sources of pharmacological activity (TENG, 1999; ZIARATNIA; KUNERT; LALLIS, 2009). Another species of the genus *Drosera* is *D. rotundifolia* L., which contains important metabolites with antimicrobial, antifungal and antitumor effects (CROUCH et al., 1990 apud BOBÁK, 1995). Carnivorous plants are also cultivated and appreciated for their ornamental potential such as *Utricularia nelumbifolia* Gardner (PŁACHNO et al., 2017) and *Nepenthes gracilima* Ridl. for displaying jar shaped leaves and the *Cephalotus follicularis* Labill. (KHOSHBAKHT; HAMMER, 2007).

Among the existing carnivorous plants, those of the genera *Dionaea*, *Utricularia*, *Drosera*, *Darlingtonia*, *Sarracenia* and *Nepenthes* received greater notoriety for research, which may be due to the fact that they present greater diversity of their species in the environment. However, with the environmental imbalance directly associated with the increase in environmental degradation, a large part of these carnivorous plants was included in the list of endangered species (GONÇALVES, 2007).

In this sense, the culture of plant tissues plays an important role in the propagation and preservation of germplasm in this group of plants. Most previous research on tissue culture in carnivorous plants highlights nutritional requirements and the search for plant micropropagation protocols. Despite the good results with some species of *Drosera*, the main objective remains the optimization of culture conditions (BONNET et al., 1984a; KUKULCZANKA; CZASTKA, 1988;

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KUKULCZANKA, 1991; BLEHOVÁ et al., 1992; ANTHONY, 1992; JADCZAK; KULPA; ZBROJEWSKA,2017). Another relevant factor in research is the identification of explants that present competence for regeneration, such as for *Drosera rotundifolia* via leaf explant and apical apex, *Cephalotus follicularis* by apical buds and *Pinguicula moranensis* Kunth by leaf segment (ADAMS et al., 1979a; ADAMS et al., 1979b; CROUCH et al., 1990; JANG; PARK 1999). Given the ecological, economic and ornamental importance of carnivorous plants, it is relevant to conduct a review and the objective is to comment on available data on the application of plant tissue culture for this group of plants (Table 1).

TABLE 1 - Historical evolution of tissue culture techniques in species of carnivorous plants.

References	Species	Explants	Countries	Cultivation techniques
Uhnak (2003)	*Darlingtonia californica* Torr. *Sarracenia leucophylla*	Seeds	United States	*In vitro* germination
Gonçalves (2007)	*Drosophyllum lusitanicum* (L.) Link.	Seeds	Portugal	*In vitro* germination
Coelho (2009)	*Drosera intermedia* Haine *Pinguicula vulgaris* L.	Seeds	Portugal	*In vitro* germination
Northcutt et al. (2012)	*Sarracenia leucophylla Sarracenia purpurea* L.	Seeds	Georgia	*In vitro* germination
Carroll (1982)	*Pinguicula lutea, pumila, caerulea, ionantha, planifolia, primuliflora, gypsicola, lilicina*	Apex stem	United States	Micropropagation
Crouch and Standen (1988)	*Drosera natalenses* Diels	Leaves; Flower buds; Flower stems	Republic of South Africa	Micropropagation
Boulay (1995)	*Dionaea muscipula* Elli, *Drosera sp., Serracenia sp., Darlingtonia californica*Torr *Nepenthes sp.*	Seedlings germinated *in vitro*	France	Micropropagation
Jang and Park (1999)	*Drosera rotundifolia* L.	Sprouts	South Korea	Micropropagation
Teng (1999)	*Dionaea muscipula* Ellis.	Flower buds; Peduncle segments; Roots and leaves	China	Micropropagation: Organogenesis
Uhnak (2003)	*Darligtonia californica* Torr.	Seedlings germinated *in vitro*	United States	Micropropagation
Gonçalves and Romano (2007)	*Drosophyllum lusitanicum* L.	Sprouts	Portugal	Micropropagation
Kim and Jang (2004)	*Drosera peltata* Thunb.	Sprouts	South Korea	Micropropagation
Kawiak and Łojkowwka (2004)	*Drosera anglica* Huds. *Drosera binata* Labill	Leaves	Poland	Micropropagation
Gonçalves and Romano (2005)	*Drosophyllum lusitanicum* L.	Sprouts	Portugal	Micropropagation
Rathour et al. (2005)	*Drosera indica* L. *D. burmannii* Vahl	Buds	India	Micropropagation
Jayaram and Prasad (2007)	*Drosera indica* L	Stem segments	India	Micropropagation
Clapa et al. (2009)	*Drosera rotundifolia* L.	Rosettes	Romania	Micropropagation
Putalun et al. (2010)	*Drosera burmanii* Vahl	Stem segments	Thailand, Japan	Micropropagation
Saetiew et al. (2011)	*Pinguicula gigantea* Luhrs.	Leaves	Thailand	Micropropagação: Organogenesis
Devi et al. (2013)	*Nepenthes khasiana* Hook. f.	Nodal stem segments	India	Micropropagação: Organogenesis
Devi et al. (2015)	*Nepenthes khasiana* Hook f.	Node segments	India	Micropropagation
Tkalec et al. (2015)	*Drosera rotundifolia* L.	Rosettes	Poland	Micropropagation
Kukułczanka et al. (1988)	*Dionaea muscipula* Ellis.	Leaves	Poland	Organogenesis
Rathore et al. (1991)	*Nepenthes khasiana* Hook. f.	Node segments	India	Organogenesis
Bobák et al. (1993)	*Drosera spathulate* Labill.	Leaves	Slovakia	Organogenesis
Latha; Seeni (1994)	*Nepenthes khasiana* Hook. f.	Node segments	India	Organogenesis

Continuation of Table 1 - Historical evolution of …

Bobák et al. (1995)	*Drosera rotundifolia* L.	Leaves	Slovakia	Organogenesis
Idei and Kondo (1998)	*Utricularia praelonga* A.St.-Hil.	Apical primordium	Japan	Organogenesis
Antonny (1992)	*Drosera* spp.	Leaves	Texas	Organogenesis
Kawiak et al. (2003)	*Drosera anglica* Huds.; *Drosera binata* Labill.; *Drosera cuneifolia* L.f.	Leaves and stem apex	Poland	Organogenesis
Jadcazak et al. (2017)	*Drosera rotundifolia* L.	Sprouts	Poland	Organogenesis
Ahmad et al. (2005)	*Dionaea muscipula* Ellis.	Leaves	Canada	Organogenesis; Somatic Embryogenesis
Bahadur et al. (2008)	*Nepenthes khasiana* Hook. f.	Sprouts	India	Organogenesis
Wawrosch et al. (2009)	*Drosera rotundifolia* L.	Leaves and Sprouts	Austria	Organogenesis
Ko et al. (2010)	*Cephalotus follicularis* Labill.	Root segments	China	Organogenesis
Jala (2014)	*Dionaea muscipula* Elli.	Leaves	Thailand	Organogenesis
Yanthan et al. (2017)	*Drosera burmannii* Vahl.	Apex stem	India	Organogenesis
Šamaj et al. (1995)	*Drosera rotundifolia* L.	Leaves	Austria	Somatic Embryogenesis
Bobáket al. (1999)	*Drosera rotundifolia* L.	Leaves	Slovakia	Somatic Embryogenesis
Chua and Henshaw (1999)	*Nepenthes macfarlanei* Hemsl.	Leaf Segments, Cotyledon seedlings	Malaysia	Somatic Embryogenesis
Bobák et al. (2004)	*Droseras pathulata* Labill.	Leaves	Slovakia	Somatic Embryogenesis
Bobák et al. (2006)	*Drosera spathulata* Labill.	Leaves	Slovakia	Somatic Embryogenesis

MATERIAL AND METHODS

In vitro germination

Several techniques and methodologies have been used for the propagation of carnivorous plants, not only to increase the germination percentage, but also to achieve a satisfactory initial development of the seedlings obtained. Furthermore, the propagation of plants using *in vitro* techniques is considered an alternative and viable practice and can certainly be applied to carnivorous plants, due to the ecological and economic importance of this group of plants in the ornamental and pharmaceutical sector.

The review highlights that among the most relevant factors associated with the *in vitro* germination and growth of carnivorous plants, those related to the need for nutrients, disinfectants, dormancy breaking methods and exogenously applied substances stand out.

In order to analyze the *in vitro* germination of two species of carnivorous plants, Uhnak (2003) worked with *Darlingtonia californica* and *Sarracenia leucophylla* and tested the efficiency of disinfectants. The author identified hydrogen peroxide and chlorox® as effective for the asepsis process of *D. californica* seeds, while concentrated sulfuric acid worked best for *S. leucophylla*. The author also verified that the use of gibberellin is necessary to stimulate the germination of *D. californica* seeds. When working with *Drosophyllum lusitanicum* in order to develop conservation strategies for this species, Gonçalves (2007) tested methods for breaking seed dormancy and observed that scarification is essential for the rupture or weakening of the seed coat, thus allowing the *in vitro* germination of the species.

In order to establish an *in vitro* propagation protocol, Coelho (2009) observed the germination of the species *Drosera intermedia* and *Pinguicula vulgaris*. In his work the author establishes a simple protocol for *D. intermedia* and observes high germination percentages for *D. intermedia* and very low percentages for *P. vulgaris*. When studying the *in vitro* germination of the genus *Sarracenia*, Northcutt et al. (2012) revealed that scarification with sulfuric acid combined with appropriate concentrations of salts in the MS medium resulted in a satisfactory germination index. The authors found that for the species *S. leucophylla* and *S. purpurea* the best response occurred with 1/3 of the salts of the MS, while for *S. oreophila* it occurred in 1/6 of the salts

It is important to point out that there are few works directly related to the analysis of the germination of carnivorous plants, however, the vast majority of research aims to standardize *in vitro* multiplication, using explants obtained from seedlings germinated *in vitro* thus highlighting the importance of the technique.

Micropropagation

To meet the demand for large-scale production of plants it is necessary to develop suitable and reproducible protocols combined with the choice of species of interest. It is known that the occurrence of carnivorous plants in Brazil is low and, with the increasing environmental degradation caused by human action, several species are in the process of extinction (GONÇALVES, 2007). Thus it is relevant to explore biotechnological techniques that promote the multiplication of carnivorous species and that mitigate the environmental impact with the reintroduction of these species in their natural habitat. However, little is known about the potential for propagation of carnivorous plant species and how these materials will respond to micropropagation.

Plant tissue culture is a sector of biotechnology and has micropropagation as one its most relevant applications. For Fay (1992) through *in vitro* techniques, including micropropagation, several plants listed as endangered have been successful when established under these conditions. Furthermore, according to the same author, the technique favors the movement of species among countries, as the material is sterile there is no urgency in quarantine. According to Clapa et al. (2009), the micropropagation of *Drosera rotundifolia* can facilitate its conservation and the plants obtained at the end of the process can be used to renew the species in its habitat. In addition to it, the technique facilitates large-scale biomass production through bioreactors for the extraction of plumbago, a substance used in the pharmaceutical industry.

Within the scope of ornamental plants, micropropagation also plays an important role in research, such as the *Nepenthes khasiana* studied by Devi et al. (2015), as well as commercially, with species of the genus *Dioneae* (WINKELMANN et al., 2006).

In the micropropagation process it is essential that the applied protocol be suitable for the culture of interest, as it involves several steps that need to be well developed to avoid adversities and losses. One of the main stages of micropropagation is the establishment of the explant *in vitro*, if well executed, it can reduce the chances of contamination and excess oxidation. Crouch and Staden (1988) when working with *Drosera natalenses* observed oxidation of the explants' tissue when they used higher concentrations of sodium hypochlorite (NaClO) in less immersion time. They have also noticed a higher percentage of decontamination when using 1.5% sodium hypochlorite for 3.25 min. when disinfesting flower bud explants.

Jayaram and Prasad (2007) were successful in asepsis of stem segments of *Drosera indica* with 35% ethyl alcohol and 0.05% mercury chloride solution (HgCl2) for 30 to 45 sec., however, when using twice the concentrations for 1 min, tissue deterioration of explants has occurred. Devi et al. (2013) analyzing clonal fidelity in the *Nepenthes khasiana* micropropagation, obtained a satisfactory response when disinfesting the plant material in running water for 30 min., immersion in 5% detergent for 20 min., 1% fungicidal solution for 1 hour and HgCl20.2 solution (w/v).

Another important factor is which part of the plant can be used, that is, the explants. Several authors use the seedling germinated *in vitro* or its stem segments as an explant for the multiplication stage, as described by Boulay (1995) with the genera *Dionaea, Drosera, Serracenia, Darlingtonia* and *Nepenthes*. Kawiak and Łojkowwka (2004) when working with *Drosera anglica* and *Drosera binata*, used leaf explants. Carroll (1982) when studying the *in vitro* cultivation of species of the genus *Pinguicula* used the stem apex. Rathour et al. (2005) worked with buds of *Drosera indica* and *Drosera burmannii* in *in vitro* propagation. In addition to the use of leaf explants, Crouch and Standen (1988) revealed the possibility of using flower buds and flower stems with *Drosera natalensis*.

It is known that the most used medium in *in vitro* cultivation is the MS medium (MURASHIGE; SKOOG, 1962) it meets the nutritional needs of several species of plants, including carnivorous ones. In their works the authors seek better and more efficient use of the medium through adjustments in relation to concentration and/or supplementation. Jang and Park (1999) observed a greater number of sprouts in the multiplication phase using half the salts of MS for the species *Drosera rotundifolia*. Kim and Jang (2004) tested different media in the micropropagation of *Drosera peltata*, such as MS, B5 (GAMBORG et al., 1968), LS (LINSMAIER; SKOOG, 1965) and RN (REINERT; MOHR, 1967) and obtained better results for the characteristics evaluated when the material was grown in MS medium.

The use of half of the salts of MS was also favorable for the multiplication and rooting of *Darligtonia californica* (UHNAK, 2003). In addition to the base medium, which contains nutrients for plant development, growth regulators play a crucial role as they provide the necessary stimulus for the growth of vegetative parts. Saetiew et al. (2011) tested the effect of adding regulators: cytokinin BA (benzyladenine) at concentrations of 0.2; 2.0; 10.0 and 20.0 mg L^{-1} and auxin NAA (naphthalene acetic acid) in concentrations of 0.1; 1.0; 5.0 and 10 mg L^{-1} in obtaining sprouts of the species *Pinguicula gigantea*. Putalun et al. (2010) conducted their experiments with *Drosera burmanii* using benzyladenine and noticed a positive physiological multiplication response. In the case of the species *Drosophyllum lusitanicum*, the cytokinins Kin (kinetin) and zeatin were tested at concentrations of 0.1; 0.2; 0.5; 1.0 and 3.0 mg L^{-1} and it was observed that the concentrations of 0.2 and 0.5 mg L^{-1} were more efficient in the induction of sprouts and the auxins IAA (indolacetic acid) and AIB (acid indolbutyric) at a concentration of 0.2 mg L^{-1} favored the growth of long roots (GONÇALVES; ROMANO, 2005).

Acclimatization is a determining step towards achieving the objective of using the technique, since at the end of it, there is the total number of seedlings produced. In acclimatizing *Drosera rotundifolia*, Clapa et al. (2009) observed better results using liquid medium compared to the use of solid substrates. In contrast, Tkalec et al. (2015) when working with *Drosera rotundifolia* did not perform the gradual process of previous acclimatization in relation to humidity, but the plants received plenty of distilled water and were analyzed in two different environments: one with internal growth through artificial light and the other with external growth through sunlight.

The results showed that the plants were able to adapt well to these two conditions. Gonçalves and Romano (2007) showed excellent results in acclimatizing *Drosophyllum lusitanicum* with *ex vitro* rooting, the base of the sprouts produced in the multiplication phase was immersed in IAA (indolacetic acid) or AIB (indolbutyric acid) solution at a concentration of 0.2 mg L^{-1} for 2 min., then the sprouts were transferred to plastic containers

containing peat and vermiculite in an environment with high humidity, temperature and controlled photoperiod.

Organogenesis

Organogenesis is a technique used for the regeneration of *in vitro* cultures, in which new plant organs are originated from certain cells or plant tissues (vascular exchange, base of the petiole in dicots, leaf bases and scales in monocot bulbs, root segments, among others), by inducing adventitious buds. This process is characterized by being unipolar, that is, it presents a single pole with adventitious development of vegetative primordium, which is connected in vascular terms to the source material (CARVALHO et al., 2006; NAZ, 2014). The technique of organogenesis then allows the development of a new plant through regeneration.

Organogenesis can occur directly, with the formation of adventitious buds directly from the source material, or indirectly, being regeneration preceded by the intermediate callus phase. Numerous external and internal factors influence the success of *in vitro* organogenesis. Among these the following can be highlighted: the type of explant; the genotype of the plant, the nutrients, the exogenously applied substances, the growing conditions, as well as the capacity of the plant tissue to respond to their use (SUGIYAMA, 1999; SHAHZAD et al., 2017).

When studying the *in vitro* regeneration of *Dionaea muscipula* from leaf explants, Kukułczanka et al. (1988) concluded that organogenesis occurred effectively in the RM environment (REINERT; MOHR, 1967), and the use of growth regulators, such as NAA (naphthalene-acetic acid) and kinetin contributed to the efficiency of the regeneration process. Bobák et al. (1993) observed the process of organogenesis with the formation of meristemoids and apical buds in leaf explants of *Drosera spathulata*, by the indirect route, reached 30 days after the formation of the primary callus cultivated in medium with half the salts of MS (MURASHIGE; SKOOG, 1962).

In studies by Bobák et al. (1995), the direct regeneration of *Drosera rotundifolia* was obtained with leaf explants in the presence of concentrations of BA (6-benzyladenine) and NAA (naphthalene-acetic acid) in MS medium, with satisfactory results in cultures with no exogenously applied substances or in presence of 0.01 µM NAA. The authors also verified that the liquid culture medium provided a significant increase in the regeneration capacity of the leaf tissue. Ideie and Kondo (1998) studied the effects of nitrate (NO_3) and BAP (6-benzylaminopurine) on the early apical organogenesis of *Utricularia praelonga* in liquid B5 medium (GAMBORG et al., 1968). They observed that organogenesis was promoted using the source of potassium nitrate (KNO_3), at a concentration of 3.0 mM and 2.0 mg L^{-1} of BAP.

In works carried out with the species *Dionaea muscipula*, Teng (1999) used flower stems and observed that petioles and trap leaves withered when cultivated in the dark. Withered explants showed a significantly higher rate of sprout regeneration than their green counterparts. For the authors, although the method is restricted to the flowering season of the plant, the use of flower stems was considered a good alternative to start the micropropagation of *D. muscipula*.

When investigating the direct regeneration of *Drosera anglica*, *D. binata* and *D. cuneifolia*, through leaf explants and stem apexes, Kawiak et al. (2003) observed that factors such as the concentration of exogenously applied substances and the type of nutrient medium used influenced the species' regeneration capacity. For *D. binata*, the largest number of regenerated plants was obtained in the VW culture medium (VACIN; WENT, 1949), without the presence of exogenously applied substances. In *D. anglica*, the best regeneration rate was obtained in the Fast medium (1981), with the addition of 0.05 µM BA and 0.005 µM NAA. In the case of *D. cuneifolia*, the MS medium with half of the salts supplemented with 0.2 µM of the exogenously applied substances BA and NAA, presented itself as the most effective in the regeneration of the species. The authors also concluded that the liquid culture medium increased the regeneration potential of the *D. anglica* and *D. binata* explants.

Bahadur et al. (2008) analyzed regeneration in *Nepenthes khasiana* aiming to standardize tissue culture techniques for mass multiplication of the species. Sprouts from stem nodes and stem apex and later the rooting of isolated sprouts were tested. In WPM medium (LlOYD; Mc COWN, 1980), combined with auxins and cytokinins or in their absence, the authors observed satisfactory results related to explant survival and regeneration capacity, which were obtained when exogenously applied substances were added to the culture medium.

When working with *Drosera burmannii*, Yanthan et al. (2017) used organogenesis to establish an efficient micropropagation protocol in order to help its conservation and increase its population. The researchers tested the efficacy of substances exogenously applied to stem apexes and noted positive physiological responses by supplementing the culture medium with 4 mg L^{-1} of BAP and 4 mg L^{-1} of NAA. For the authors, the main route of propagation for *Drosera burmannii* is through seeds, however, the work demonstrated that it is possible to successfully obtain multiple sprouts of the species through the organogenesis technique.

In investigations on *in vitro* regeneration of *Nepenthes khasiana*, Rathore et al. (1991) verified the formation of multiple sprouts in nodal segments through induction with BAP and IAA regulators. Latha and Seeni (1994) tested nodal stem segments of the same species in different culture media and obtained, within 7 to 8 weeks, the regeneration of adventitious buds in cultivation with WPM medium combined with 2.2 µM BA.

Ahmad et al. (2005) aiming to standardize a protocol for *in vitro* regeneration of *Dionaea muscipula* clones with the use of leaf explants combined with the exogenously applied substance 2iP, reported that it is possible to promote organogenesis by direct route. Using segments of leaves and sprouts of *Drosera rotundifolia*, Wawrosch et al. (2009) studied the *in vitro* proliferation of

this plant in liquid culture medium and inferred that the propagation can be optimized with the use of liquid media, as they facilitate the cultivation process and promote cost reduction, since it does not use any gelling agent.

Still about liquid media, Ko et al. (2010) investigated the effects of concentrations of the MS medium on the micropropagation of *Cephalotus follicularis* in root segments and concluded that liquid media with 1/5 MS and 1/10 MS were effective in the physiological response of *C. follicularis*, since the rate of explant proliferation occurred much faster when compared to the solid culture medium.

Saetiew et al. (2011) observed the effects of BA and NAA in the multiplication of *Pinguicula gigantea* with leaf explants and noted that the largest number of sprouts was obtained in MS medium with 2 mg L^{-1} of BA and 0.1 mg L^{-1} of NAA. In 2013 Devi et al. used nodal segments of the *Nepenthes khasiana* stem and noted that the highest rate of formation of apical vegetative primordium occurred in medium with half the salts of the DM, 2.5 mg L^{-1} of kinetin and 2.0 mg L^{-1} of BAP. For rooting, the best result was achieved with 2 mgL^{-1} of NAA.

In vitro regeneration in carnivorous plants also involves the use of components such as colchicine, an aromatic alkaloid. It was tested on leaf base explants and leaf blades of *Dionaeamuscipula*, by Jala (2014). The author reports in the study that high concentrations of colchicine, associated with a longer incubation time, resulted in a lower growth rate and a higher rate of mutation in explants. Lower concentrations and shorter incubation times resulted in leaf and root regeneration.

The review of the organogenesis technique for carnivorous species revealed that the complete regeneration of plants through the formation of adventitious buds is successful for the studied species, but it is directly proportional to the type of explant, exogenously applied substances and culture medium. Difficulties for the occurrence of the organogenesis process have also been reported.

Somatic Embryogenesis

In vitro somatic embryogenesis is a process of formation of embryos that convert into seedlings, without the fusion of the male and female gametes. For somatic embryogenesis induction to occur it is essential that the somatic cells of plants regain their totipotency and acquire the necessary competence to respond to embryogenic signals and initiate embryogenesis (PASTERNAK et al., 2002). Embryogenic competence is expressed at the cellular level and these cells are able to differentiate into embryos if they receive differentiation inducers (FEHÉR, 2005).

The appropriate choice of explants or an appropriate ratio of exogenously applied substances to induce somatic embryogenesis in carnivorous plants are the main factors studied and cited in scientific studies. For example, Bobák et al. (2004) tested segments of seedling leaves *in vitro* and concentrations of exogenously applied substances NAA and BAP and observed the formation of somatic embryos through the indirect route, that is, the event presented an intermediate phase of callus formation on the explants of *Droseras pathulata*. In a subsequent study with the same carnivorous species, Bobák et al. (2006) verified the occurrence of somatic embryogenesis by direct route, without callus formation, in *Droseras pathulata*, however, with the use of leaf segments of adult plants in the presence of BAP and NAA.

Previous work also performed by Bobák et al. (1999) with the species *Drosera rotundifolia*, testing leaf explants of plants cultivated *in vitro* and semi-solid induction media were sufficient to promote the development of somatic embryos. Šamaj et al. (1995) concentrated their studies on the extracellular matrix of the development of embryonic cells from *D. rotundifolia*. *Drosera* plants were grown in MS medium with no exogenously applied substances, with leaf segments as explants. The authors verified the formation of embryogenic callus during cultivation with 2,4-D (2,4-dichlorophenoxyacetic acid), with subsequent plant regeneration in a free 2,4-D medium.

As for the induction of somatic embryogenesis in *Nepenthes macfarlanei*, Chua and Henshaw (1999) cultivated immature and mature embryos and leaf segments in different culture media, such as MS, B5, NN (NITSCH; NITSCH, 1969), SH (SCHENK; HILDEBRANT, 1972) and VW (VACIN; WENT, 1949), with the supplementation of 2,4-D or 2,4,5-T (2,4,5-trichloro-phenoxyacetic acid), however, they were not successful. For Ahmad et al. (2005), the induction of somatic embryogenesis was achieved with leaf explants of *Dionaea muscipula*, with a positive result in culture with half of the salts of DM combined with the exogenously applied substance 2iP (2-isopentenyladenine).

The information obtained in the review showed that for the induction of somatic embryogenesis, the use of juvenile explants is recommended, as they have the advantage of being more responsive. It is also indispensable to choose an exogenously applied substance capable of stimulating the somatic embryogenesis induction process and, among the exogenously applied substances, 2,4-D is the most widely applied.

CONCLUSIONS

The tissue culture guidelines applied to carnivorous or insectivorous species are focused on the mass micropropagation of selected species. In the studies presented, *in vitro* germination is the main route to obtain more competent sources of explants for further application of the development of regeneration methodologies, such as organogenesis and somatic embryogenesis.

The review reveals that scientific information on carnivorous plants is presented at a low pace as regards to *in vitro* regeneration methods. Nevertheless, it is important to highlight that tissue culture is a biotechnological tool of great importance for this group of plants that has ecological, ornamental and economic value.

The interest of scholars in applying tissue culture techniques to carnivorous plants is for the perpetuation and sustainable exploitation of these plants once *in vitro* cultivation allows greater biomass production, *in vitro* conservation of genetic resources and production of secondary metabolites of interest to this group of plants.

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