

APPLICATION OF TISSUE CULTURE TECHNIQUES IN POTATO

APLICAÇÃO DA CULTURA DE TECIDOS VEGETAIS EM BATATA

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ABSTRACT: Potato is the world's most important non-cereal food crop, and therefore, it is considered one of the major food sources for humankind. Its conventional propagation is asexual, by using the tuber, which allows the accumulation and dissemination of pathogens to new cultivation areas. This fact not only impairs the yield of this solanaceous plant, but also threatens the maintenance of genotypes for commercial or breeding purposes. Due to the impossibility of using botanical seed, conservation and exchange of germplasm of this species by means of conventional methods are not feasible. In all potato-producing regions, the demand for high-quality tubers has been paramount to ensure crops production. Thus, biotechnological techniques based on tissue culture are very important. Plant tissue culture offers alternative methods of propagation by *in vitro* techniques that provide production and multiplication of material with high sanity. Thus, this literature review summarizes the history and current situation of tissue culture techniques applied to potato crop. Besides clonal multiplication, this biotechnological tool makes available initial indexed material to breeding programs and certified seed potato, and facilitates the exchange and conservation of germplasm. For all these reasons, the use of these techniques in potato production chain directly benefits producers by providing high-quality propagules.

KEYWORDS: *Solanum tuberosum* L. *In vitro* cultivation. Seed potato. Pathogen elimination.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual solanaceous plant from the Andes (MENEZES et al., 1999). Its commercial product, the tuber, is the underground stem adapted to work as reserve source and reproduction mean (FORTES; PEREIRA, 2003b). It is a culture of outstanding importance worldwide for having socio-economic repercussions, and for being one of the major food sources for humankind. Potato is cultivated in about 150 countries (BASERA et al., 2018), and is the most important non-cereal food crop in the world (BAMBERG et al., 2016; NIKITIN et al., 2018). Last harvest season, Brazil produced 4.3 million tons of potato, in an area of 803,900 hectares (IBGE, 2017).

On a commercial scale, potato has vegetative propagation. However, this type of multiplication can accumulate several systemic fungi, bacteria and virus infections that provoke degeneration in the plants, culminating with yield and vigor losses (NIKITIN et al., 2018). Thus, the use of virus-free propagative material, with high phytosanitary, physiological and genetic quality is of great importance to ensure that the plant expresses its maximum yield potential.

In this scope, plant tissue culture can be applied to potato production chain in order to efficiently propagate the material of interest, maintain germplasm banks, facilitate genetic

exchange, provide the study of this species and of its interactions with biotic and abiotic factors, and to produce genetically modified plants and pathogen-free seed potatoes. This literature review summarizes some relevant results obtained with tissue culture in potatoes from 1977 to 2018. We apologize in advance for the papers not included in this review, due to the limited length of our manuscript, and encourage more publications on this subject to contribute to or innovate existing knowledge.

TISSUE CULTURE IN POTATOES

Micropropagation

In vitro establishment

Among plant tissue culture techniques, micropropagation has the greatest practical application (reviewed in SHAHZAD et al., 2017). Several studies have been reported in the literature, aiming at the optimization of potato micropropagation protocols (ÁVILA et al., 1994; PEREIRA; FORTES, 2000, 2004; DUTRA et al., 2010; VENKATASALAM et al., 2013). However, one of the greatest difficulties in the development of these protocols is the *in vitro* establishment of plants, mainly due to fungal, viral and bacterial contaminations.

Fungi and yeasts can be controlled by eliminating the contaminated material (LEIFERT; WOODWARD, 1998), or by previously sanitizing

the explants. In this case, potato shoots are disinfested by immersion in 70% alcohol for 15 seconds, followed by immersion for 15 minutes in 1.5% sodium hypochlorite solution with 0.1% Tween-80, and rinsed three times with distilled and autoclaved water (PEREIRA; MATTOS; FORTES, 2003).

Viruses can be physically (heat) or chemically (by antiviral compounds) eliminated. In a study developed by Nascimento et al. (2003), potato microcuttings with positive reaction to Potato virus Y (PVY) were subjected to thermotherapy (37°C) and chemotherapy [Ribavirin (RBV), 5-Azacitidine (AZA) and 3-Deazauridine (DZD)]. The thermotherapy, during 30 and 40 days, eliminated 20 and 37.5% of PVY, respectively. Among the chemotherapeutic agents, RBV presented the best eradication rates, and obtained 55.5% of healthy plants. These authors also verified that simultaneous treatments of thermotherapy and chemotherapy showed higher efficiency in virus elimination, reaching percentages of 83.3, 70.0, and 50.0 of healthy plants, with RBV, AZA, and DZD, respectively.

For the control of bacterial contamination, the use of substances antagonistic to these microorganisms is required. The application of antibiotics in the decontamination process may be beneficial since bacteria compete with plants for the nutrients of the culture medium, and may excrete toxic substances to explants (SCHERWINSKI-PEREIRA, 2010). Antibiotics may be incorporated into the culture medium or directly used on contaminated explants (SALEHI; KHOSH-KHUI, 1997). In potato tissue culture, Pereira, Mattos and Fortes (2003) reported that the antibiotics ampicillin, chloramphenicol, streptomycin and tetracycline in concentrations varying from 32 to 256 mg L⁻¹ inhibited the growth of endophytic bacteria. Besides acting on microorganisms, these antibiotics can also affect explants survival. The addition of tetracycline and chloramphenicol to the culture medium adversely affects the *in vitro* development of potato explants; conversely, ampicillin has no phytotoxic effects on growth and multiplication rate, and may be indicated for *in vitro* decontamination of this species (PEREIRA; FORTES, 2003b).

In vitro established plant material is more readily used as an explant donor than field-grown material (as surface disinfestation treatments are no longer required), once the established material is carefully handled under aseptic conditions (FORTES; PEREIRA, 2001; PEREIRA; FORTES, 2003a, b, 2004a; GONÇALVES et al., 2009).

Multiplication

Several parts of the mother plant can be used as explant source at the multiplication stage. *In vitro* potato regeneration protocols have been reported using immature zygotic embryos (PRETOVA; DEDICOVA, 1992), stem segments (GARCÍA; MARTÍNEZ, 1995; SANTIAGO et al., 2012; KAUR; REDDY; KUMAR, 2017; ALI et al., 2018; SILVA FILHO et al., 2018), leaves (CEARLEY; BOLYARD, 1997; KAUR; REDDY; KUMAR, 2017), tuber discs (ESNA-ASHARI; VILLIERS, 1998), meristems (BADONI; CHAUHAN, 2009), and shoot tips (DUTRA et al., 2010).

Due to the high susceptibility of potato to diseases, especially to viruses, several studies have started micropropagation by using meristems and shoot tips as explants in order to produce virus-free seed potato. However, some aspects may compromise the success of this technique, such as the delay in the differentiation of the meristematic tissue in the culture medium, the possibility of contamination (FORTES; PEREIRA, 2003a; PEREIRA; MATTOS; FORTES, 2003), and explant survival difficulty due to its small size.

The relationship between explant size, survival rate and pathogen elimination were observed by El-Fiki et al. (1992). In this work, seven potato cultivars were evaluated to determine the ideal size of the shoot tip and the efficiency in PVX eradication (Potato virus X). Higher seedling survival and growth rates were obtained by using larger explants (0.4 mm), although they presented smaller percentage of healthy plants (21.2) when compared with the explants of 0.2 mm (47.4).

Despite the proven efficiency of meristem culture in pathogens eradication, the combination of this technique with antiviral treatments to ensure pathogen elimination is often necessary. Wang et al. (2006) reported higher frequency in the regeneration of PLRV-free (Potato leafroll virus) and PVY-free (90 and 93%, respectively) potato seedlings by applying thermotherapy, followed by meristem culture. The *in vitro* technique itself provided the regeneration of only 56% of healthy plants for PLRV, and of 62% for PVY.

The sanity of the micropropagated material is guaranteed by indexing tests. The ELISA test (Enzyme-Linked Immunosorbent Assay) has been widely adopted for this purpose, since it is fast and allows detecting virus persistence in a large number of regenerated plants (NAIK; BUCKSETH, 2018). Molecular techniques based on the use of primers for specific regions of the virus genome, such as RT-PCR, are also largely used (LORENZEN et al.,

2006; 2008; MUÑOZ et al., 2018; NIKITIN et al., 2018). In relation to the use of polyclonal antibodies, the technique involving RT-PCR has the advantage of differing strains and genetic variants, distinguishing the types of viral isolates. The indexation is of fundamental importance in the production chain of seed potatoes, as it ensures the quality of the material obtained by means of meristem culture, reflecting in field yield, which can be at least twice as high as the yield of plants derived from tubers (NAGIB et al., 2003).

The chemical composition and physical state of the culture medium may also interfere with potato *in vitro* multiplication. Regarding the chemical composition, the type of carbohydrate added to the culture medium affects explants survival, as verified by Fortes and Pereira (2001). In this work, higher survival rate was obtained when the carbon source was sucrose in detriment to the use of mannitol. Moreover, several studies mention that 3% of sucrose is the ideal concentration for the optimal micropropagation of potato, which, in addition to promoting greater biomass accumulation, favors the synthesis of sucrose, glucose and fructose, which may play an important role during potato plants acclimatization (WANG; HU, 1982; DONNELLY; COLEMAN; COLEMAN, 2003; BADR; ANGERS; DESJARDINS, 2011; BANDINELLI et al., 2013).

Although it has been reported that the use of growth regulators in potato meristem culture is dispensable (ROSELL et al., 1987; GOPAL; MINOCHA; DHALIWAL, 1998), the addition of indole-butyric acid, indole-acetic acid, and naphthalene-acetic acid, even at low concentrations, increases and accelerates the production of virus-free seedlings (GHAFFOR; SHAH; WASEEM, 2003). These results corroborate those observed by Badoni and Chauhan (2009), who reported greater development and multiplication of potato seedlings when the culture medium was supplemented with 0.01 mg L⁻¹ of naphthalene-acetic acid and 0.25 mg L⁻¹ of gibberellic acid.

Among the classes of plant regulators that are not widely used in *in vitro* cultivation, jasmonic acid, salicylic acid, ethylene, polyamines and brassinosteroids deserve attention. It is postulated that supplementation of the culture medium and/or the pretreatment of potato mother plants with jasmonic acid promotes *in vitro* tuberization and improves the quality of microtubers (PRUSKI; ASTATKIE; NOWAK, 2002; SARKAR; PANDEY; SHARMA, 2006). Moreover, this growth regulator confers higher rates of potato micropropagation, although not always a

quantitative effect was observed (RAVNIKAR; GOGALA, 1990; RAVNIKAR; VILHAR; GOGALA, 1992; ZHANG et al., 2006; KUMLAY, 2016).

Salicylic acid, in concentrations from 30 to 120 mg L⁻¹, has been used in association with mannitol (87.6 mM) for the *in vitro* preservation of potato (FORTES; PEREIRA, 2001). In addition, ethylene is associated with the formation of hyperhydric structures (PARK et al., 2004), and the supplementation of the medium with ethylene synthesis inhibitors, such as silver thiosulfate, is often necessary (PERL; AVIV; GALUN, 1988; MÖLLERS; ZHANGM; WENZEL, 1992; SARKAR; KAUSHIK; NAIK, 1999; ZOBAYED; ARMSTRONG; ARMSTRONG, 2001; ROSTAMI; EHSANPOUR, 2010). Similarly to jasmonic acid, polyamines favor *in vitro* potato tuberization (PROTACIO; FLORES, 1992; FERAY et al., 1993; MADER, 1995), while brassinosteroids have been used in the induction of embryogenic calli (MORE et al., 2001), acclimatization (AGRAMONTE et al., 1996; PAVLOVNA et al., 2015), and proliferation (BASERA et al., 2018). Although researchers have already taken the first steps for the study of these regulators, there are gaps regarding the best concentrations and combinations at different stages of potato micropropagation.

In relation to the consistency, the liquid culture medium is more efficient in potato *in vitro* multiplication when compared with the semi-solid (PEREIRA; FORTES, 2003a). According to the same authors, higher biomass production is obtained when the material is cultivated in liquid medium composed of MS salts at full concentration, supplemented with pantothenic acid (5.0 mg L⁻¹), thiamine (1.0 mg L⁻¹), gibberellic acid (0.25 mg L⁻¹), and sucrose (20 g L⁻¹) under constant shaking. Similar result was observed for the organogenesis of potato meristematic apices (PEREIRA; FORTES, 2004b), in which the liquid culture medium had a higher multiplication rate when compared with the semi-solid medium.

However, a disadvantage of the cultivation in liquid medium is the occurrence of hyperhydricity. Hyperhydric structures were reported by Rosell et al. (1987) and Park et al. (2004) when studying potato micropropagation. Nevertheless, the authors observed that the formation of these structures can be avoided by transferring the culture to solid medium. Media are generally polymerized with agar or phytagel, though many alternative gelling agents, as *Lallemantia royleana* seeds, are proposed to reduce the costs of potato *in vitro* micropropagation (JABEEN;

KAUSAR; SHAH, 2017). Besides the consistency, the concentration of salts, the type and the dose of cytokinins present in the medium also affect the formation of hyperhydric structures (PALMA et al., 2011).

Important aspects concerning incubation of micropropagated potato plants are addressed in some research. Those range from light sources (ROCHA; OLIVEIRA; SCIVITTARO, 2015), growth room ventilation (VENKATASALAM et al., 2015), and culture vessels (SHUKLA et al., 2017) until changes in plantlets profiles (GUPTA; PATTANAYAK, 2017; LOYOLA-VARGAS; OCHOA-ALEJO, 2018).

Tuberization

The transition between *in vitro* multiplication and the establishment of this solanaceous in the field is known as microtuberization (NISTOR et al., 2010). In this case, microtubers have been used as an alternative method for national and international distribution of potato germplasm and as pathogen-free starting material for seed multiplication programs (MUÑOZ, 1996). These small tubers have some advantages in relation to *in vitro* seedlings, such as: easy distribution and handling of the material due to its reduced size, greater resistance to transport conditions, and possibility of dark storage under low temperatures for longer periods than that of micropropagated seedlings (TOVAR et al., 1985; McCOWN; JOYCE, 1991). In addition, microtubers can be used as an explant source in studies on genetic transformation (SNYDER; BELKNAP, 1993).

Several protocols to induce *in vitro* tuberization have already been developed, such as that of Wang and Hu (1982), which is adopted by the International Potato Center (*Centro Internacional de la Papa - CIP*) (TOVAR et al., 1985; DODDS; SILVA-RODRIGUEZ; TOVAR, 1992). This protocol consists of adding 6-benzylaminopurine, chlorocholine chloride and sucrose to the liquid culture medium. Tuberization can also be achieved in bioreactor systems, even resulting in greater yields and less hyperhydricity rates compared to the liquid culture (RAHMAN et al., 2015). The *in vitro* microtuber, subjected to proper stimuli, can further be directly seeded in open field (SHIN et al., 2018).

Microtubers ideal growing conditions (photoperiod, temperature, and nutrient source and dose) may vary according to the genotype under study. In this context, Gopal, Minocha and Dhaliwal (1998) evaluated 22 potato genotypes and verified,

for most of the materials, the formation of microtubers under short photoperiod conditions (10 h photoperiod with irradiance of $12 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low temperature ($20 \pm 2^\circ\text{C}$ during the day and $18 \pm 2^\circ\text{C}$ at night). In relation to the source and dose of carbon in the culture medium, 6% of sucrose and 4% of maltose were considered suitable for the potato *in vitro* tuberization, for cultivars Agria and Justine, respectively (ALTINDAL; KARADOĞAN, 2010). Thus, genotype x environment interactions indicate the importance of developing specific protocols for each potato cultivar in order to maximize micropropagation, resulting in the availability of sufficient and high quality material for farmers.

Production of indexed seed potato

In Brazil, the official production process of pathogen-free seed potatoes begins with the *in vitro* production of plants from the shoot tip culture. The methodology for shoot-tip propagation depends, as in any micropropagation process as previously described, on asepsis, on the genotype of the plant material, on the explant itself, and on the composition of the culture medium in which the material will be inoculated.

Shoot tips are isolated and inoculated in medium containing more cytokinin than auxin for differentiation into aerial parts. Subsequently, these multiplied shoots are transferred to the medium containing only cytokinin (or not). A shoot tip can generate from 500 to more than 1000 plants, depending on the cultivar or even on the shoot tip. Bandinelli et al. (2013) reported that the 50% reduction in the MS salts concentration during multiplication increases the *ex vitro* survival rate in Asterix, Macaca, and SMINIA793101-3 potato clones. At the establishment and multiplication stages, plants are kept in a growth chamber, with light (14-16 h photoperiod) and temperature ($25-27^\circ\text{C}$) control (PEREIRA; FORTES, 2004b). Seedlings can be transferred to a medium richer in sucrose for *in vitro* tuberization. However, this procedure is not widely adopted. Instead, tuberization in a tray or plate with sterile substrate under anti-aphid conditions has been more frequently used. Bandinelli et al. (2013) recommend the mineral substrate (coarse sand), as it favors the acclimatization of the plants, increasing the survival rate in the field. However, according to Daniels, Pereira and Fortes (2000), the production of seed tubers in solid matrix presents low productive efficiency, since three to five tubers per plant are produced.

Soil-free (or non-solid) technologies have gained attention and replaced the conventional production system of seed tubers. Factor et al. (2007) reported that these technologies prevent the contact of seed tubers with soil phytopathogens, facilitates cleaning after the production cycle, enables staggered harvesting, standardizes the tubers, and guarantees high phytosanitary quality and higher multiplication rate. Soil-free technologies include hydroponics, nutrient film technique, deep flow technique, and aeroponics. Among them, aeroponic systems have emerged as a possibility in many developing countries, such as Brazil (FACTOR et al., 2007), Peru (OTAZÚ, 2010), China (WANG et al., 2017), and Uganda (KAKUHENZIRE et al., 2017). Briefly, it consists of spraying a nutrient solution to the suspended plant roots and draining it back to the reservoir tanks until the next cycle of misting.

After cultivation, seed potatoes are stored at ambient conditions for 10 to 14 days for peel suberification and further stored for six to eight months, at 3 to 4°C, for dormancy overcome and for the sprouting of the largest number of eyes. After this period, tubers are able to produce seed potatoes in the field, in isolated areas with low incidence of insect vectors, meeting certification standards (DUTRA et al., 2010).

The production and commercialization of seed potatoes within the Brazilian territory follow rules that aim at ensuring the genetic identity and health of this plant material, which are determined by the Ministry of Livestock and Food Supply (MAPA). According to the Normative Instruction No. 32 of November 20, 2012, of MAPA, seed potatoes should be produced in the following categories: genetic seed potatoes; basic seed potato (G0, G1, G2 and G3); certified seed potato of first generation (C1); certified seed potato of second generation (C2); S1 and S2. The basic seed potato G0 should be obtained from the genetic seed potato or from the basic potato seedling, cultivated in protected environment. The basic seed potato G1 should be obtained from the basic seed potato G0; the basic seed potato G2 should be obtained from the basic seed potato G1; and basic seed potato G3 should be obtained from the basic seed potato G2. Certified seed potatoes of first generation (C1) should be obtained from basic or certified seed potatoes, whereas certified seed potatoes C2 can be obtained from certified seed potatoes C1, basic seed potatoes, or from certified seedlings. S1 seed potatoes should be obtained from certified seed potatoes C2 or above; and S2 seed potato should be

obtained from S1 seed potatoes, or from higher categories (MAPA, 2012).

In addition to the tuber, the shoot can also be used as propagative material in the production of basic and certified seed potatoes (MOREIRA, 2008), reducing costs with the production or acquisition of seed potatoes (SILVA et al., 2011; VIRMOND; KAWAKAMI; DIAS, 2011). The Normative Instruction No. 32 of November 20, 2012 establishes that certified seedlings in the form of sprouts can only be produced using basic seed potatoes (MAPA, 2012). In production of seedlings, minitubers and basic seed potatoes, their indexation by the ELISA test is mandatory, in order to confirm the absence of viruses.

Somatic embryogenesis

Several studies have reported the formation of potato somatic embryos in solid culture medium from tuber discs (BRAGDØ-AAS, 1977; NASSAR et al., 2011), nodal segments (GARCÍA; MARTÍNEZ, 1995; SEABROOK; DOUGLASS; TAI, 2001; VARGAS; GARCIA; OROPEZA, 2005; VARGAS et al., 2008; SHARMA et al., 2007, 2008b), and leaf tissues (JAYASREE et al., 2001; KAUR; REDDY; KUMAR, 2018). The formation of these structures in liquid medium was first described by Fiegert, Mix-Wagner and Vorlop (2000).

Growth regulators content and ratio are key factors on callus induction and somatic embryos initiation and development. The molecular and biochemical mechanisms behind these phenomena however are yet to be elucidated. For some information, consult Nassar, Kubow and Donnelly (2015). At the first stage, auxins are required to induce callusing but must be removed during embryo initiation due to their suppressive effect. The most frequent used is the 2,4-dichlorophenoxyacetic acid (2,4-D) (BORDALLO et al., 2004; VARGAS; GARCIA; OROPEZA, 2005; VARGAS et al., 2008; SHARMA; MILLAM, 2004; SHARMA et al., 2007, 2008a, b; NASSAR et al., 2011; SABETI; ZARGHAMI; EBRAHIM, 2013). Embryo formation is later induced by a range of growth regulators, including zeatin and gibberellin (GA₃) (FIEGERT; MIX-WAGNER; VORLOP, 2000; JAYASREE et al., 2001; SEABROOK; DOUGLASS, 2001; SEABROOK; DOUGLASS; TAI, 2001; VARGAS; GARCIA; OROPEZA, 2005; VARGAS et al., 2008; SHARMA; MILLAM, 2004; SHARMA et al., 2007; SHARMA; SARKAR; PANDEY, 2010; NASSAR et al., 2011).

Jayasree et al. (2001) established an efficient protocol to induce somatic embryos and regenerate potato seedlings, cv. Jyothi, from leaf segments. Initially, leaves are grown in MS medium supplemented with 2,4-D and benzyladenine (BA). The embryogenic calli, obtained from the borders of the leaf segments, are transferred to MS medium containing zeatin and BA, resulting in high formation of somatic embryos. Embryos mature in the same induction medium, and are transferred to MS medium without growth regulators for seedling regeneration when they reach the cotyledonary stage.

Seabrook and Douglass (2001) also reported the *in vitro* induction of somatic embryos of 18 tetraploid potato cultivars from several explants (nodal segments, leaves, microtubers, and roots). These authors observed differences in induction capacity depending on the genotype of the evaluated materials, varying from 2.2 to 44.5 somatic embryos.

This technique, when applied to potato crop, is a potentially effective method in the production of synthetic seeds. Synseeds produce virus-free, true-to-type materials that are easy to handle and transport (POND; CAMERON, 2003; BORDALLO et al., 2004; SHARMA et al., 2007). However, there has been relatively little progress on this application due to problems of somaclonal variation and requirement of bioreactors for large-scale production of somatic embryos followed by their encapsulation and field planting (FIEGERT; MIX-WAGNER; VORLOP, 2000; NYENDE et al., 2002; SCHAFER-MENUHR; MIX-WAGNER; VORLOP, 2003; BAMBERG et al., 2016). Thus, the development of reliable systems of somatic embryogenesis for this solanaceous plant is still incipient (SHARMA; MILLAM, 2004), and few studies are found in the literature regarding the occurrence and performance of somaclones in greenhouse (SEABROOK; DOUGLASS, 2001) and in field (NASSAR, 2009; NASSAR et al., 2014) and the genetic mechanisms that regulate this process (SHARMA et al., 2008a, b).

Protoplast culture

Protoplast technology has focused especially on the generation of interspecific hybrids between wild and cultivated species that cannot be produced via conventional hybridization (DAVEY et al., 2005). In potato culture, wild *Solanum* species are commonly used to introduce desirable traits to commercial genotypes.

In 1987, Fish, Karp and Jones developed a protoplast culture technique applicable to several

dihaploid potato genotypes. The protoplasts were isolated from *S. tuberosum* mesophilic cells and from the wild diploid species *S. brevidens*, and fused together at high pH and high calcium concentrations. Putative hybrids were then selected by the phenotype from regenerated shoots, and of these, 11 somatic hybrids were identified in function of their isoenzymatic patterns and morphological traits.

By means of somatic hybridization, several resistance genes from wild species have already been introduced into potato, including those that confer tolerance to herbicide (BINDING et al., 1982), resistance to leafroll virus (AUSTIN; BAER; HELGESON, 1985), tolerance to frost (ROKKA et al., 1998), tolerance to late blight disease (SHI et al., 2006), and resistance to insects (CHENG; SAUNDERS; SINDEN, 1995; BUTLER et al., 2011; DIAZ-MONTANO et al., 2014). Specific reviews of somatic hybridization in potato can be found in Pandey et al. (2010), Cho and Park (2014) and Rokka (2015).

In vitro preservation and cryopreservation

The maintenance of *in vitro* collections has been considered an alternative method to the conservation of germplasm, especially for vegetative propagated species. In the case of potato, the minimum *in vitro* growth occurs by the control of incubation conditions, such as low temperatures, low light intensity, and mixed photoperiod (WESTCOTT, 1981a; GOPAL; CHAUHAN, 2010), or by the addition of substances to the culture medium, especially growth retardants and osmotic stress inducers (WESTCOTT, 1981b; FORTES; PEREIRA, 2001; GOPAL; CHAUHAN, 2010).

Sarkar and Naik (1998) studied the effect of simultaneous manipulation of incubation conditions and culture medium on the *in vitro* preservation of four potato genotypes. The combination of 40 g L⁻¹ sucrose, 20 g L⁻¹ mannitol, and 16 h photoperiod provided better conservation of potato microplants for up to 30 months without the need for subcultivation. Alternatively to mannitol, ancimidol - a growth retardant - can be used (SARKAR; CHAKRABARTI; NAIK, 2001). At CIP, more than 4,000 potato accessions are kept in culture medium with 40 g L⁻¹ sorbitol and under low temperature (6-8°C) (NIINO; ARIZAGA, 2015), as similarly observed at the US Potato Genebank (30 g L⁻¹ sorbitol, 8-10°C) (BAMBERG et al., 2016). In the case of liquid culture medium, González-Benito, Ramírez and Aranda (2004) reported that supplementation of MS medium with 2% sucrose

and incubation at 10°C allows the maintenance of potato seedlings for about 2-3 years.

The choice of the conservation method to be used should consider the occurrence of somaclonal variation, which may also be associated with the nature of the plant material and to the substances employed (WITHERS; ENGELMANN, 1998). This genetic variation needs to be evaluated at the end of the whole process in order to detect possible threats to the genetic stability of plant material. As an example, Harding (1994) detected hypermethylation of potato microplants' DNA maintained for long periods under osmotic stress promoted by the use of mannitol. Conversely, Sarkar, Chakrabarti and Naik (2001) detected no genetic instability after 16 months of conservation by means of Random Amplified Polymorphic DNA (RAPD) markers, when evaluating the effects of ancimidol on the conservation of potato microplants.

Long-period *in vitro* preservation may not guarantee the genetic stability of the micropropagated material. Thus, cryopreservation is a more reliable alternative. In addition to long-term storage of potato germplasm, cryotherapy can simultaneously be applied as an alternative method for virus elimination and production of indexed plants (WANG et al., 2006; KACZMARCZYK; ROKKA; KELLER, 2011).

The first report on the use of this technique in potatoes was in the late 1970s (BAJAJ, 1977), and from that work, different methods have been developed using slow or ultrafast freezing (TOWILL, 1984; BENSON; HARDING; SMITH, 1989). In 1995, CIP started to test a cryopreservation method by vitrification. The process consisted of dehydrating potato meristems in concentrated sugar solutions and cryoprotectors and rapidly freezing them using liquid nitrogen (GOLMIRZAIE; PANTA, 2000). Other techniques of vitrification have already been tested for several potato cultivars, such as the encapsulation-vitrification method (HIRAI; SAKAI, 1999; HIRAI, 2011), the droplet-vitrification (KIM et al., 2006; YOON et al., 2006), and the V-cryo-plate (YAMAMOTO et al., 2013).

The viability of potato shoot tips maintained for long periods in liquid nitrogen has also been reported (MIX-WAGNER; SHUMACHER; CROSS, 2003). These authors compared the regeneration of 51 cultivars in long-term (3 to 8 years) and short-term preservation. Only for three

genotypes the cryopreserved shoot tips presented a significant decrease in regeneration.

CONCLUSIONS

Plant tissue culture techniques have been widely used as an alternative to solve the lack of propagating material of potatoes with high phytosanitary quality. Among these techniques, micropropagation stands out, whose clonal multiplication protocols have already been well established, and whose process is considered the main route of production of pathogen-free seed potatoes.

The use of somatic embryos has long been considered promising alternative to conventional potato propagules; however, little has been achieved in the development of synthetic seeds for this solanaceous plant. Thus, aspects concerning the application of somatic embryogenesis in this species still require further studies. In addition, more studies should address the viability of potato genetic transformation via culture and protoplast fusion.

In addition to large-scale clonal production of indexed plants, *in vitro* techniques can be employed for medium-term preservation and safe exchange of potato germplasm. Thus, cryopreservation can be considered an additional tool for genetic material conservation.

Finally, the use of the products generated by scientific research by the different segments in the potato production chain is directly dependent on regular investments in the sector, and on the constant technical training of the professionals involved in research. Only then will potato be assisted with technology capable of guaranteeing highly productive crops.

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RESUMO: A batata é a cultura não-cereal mais importante do mundo e, portanto, uma das principais fontes de alimento para a humanidade. Sua multiplicação convencional é assexuada utilizando o próprio tubérculo, o que permite o acúmulo e a difusão de patógenos para novas áreas de cultivo, comprometendo a produtividade desta solanácea e ameaçando a manutenção de genótipos de interesse comercial ou para fins de melhoramento. Devido à inviabilidade de utilização das sementes botânicas, a conservação e o intercâmbio de germoplasma dessa espécie por meio de métodos convencionais torna-se inviável. Em todas as regiões produtoras de batata, a demanda por tubérculos de alta qualidade tem sido primordial para garantir a produção das lavouras. Dessa forma, técnicas biotecnológicas baseadas na cultura de tecidos são de suma importância. A cultura de tecidos vegetais oferece métodos alternativos de propagação através das técnicas *in vitro* que proporcionam a produção e multiplicação de material com alta sanidade. Dessa maneira, esta revisão visa sumarizar o histórico e panorama atual das aplicações da cultura de tecidos em batata. Além da multiplicação clonal, essa ferramenta biotecnológica fornece material inicial indexado para programas de melhoramento e de produção certificada de batata-semente e facilita o intercâmbio e a conservação de germoplasma. Por tudo isso, o emprego destas técnicas na cadeia produtiva da batata proporciona benefícios diretos aos produtores, uma vez que fornece material propagativo com elevada qualidade genética e fitossanitária.

PALAVRAS-CHAVE: *Solanum tuberosum* L. Cultivo *in vitro*. Batata-semente. Erradicação de patógenos.

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