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A COMPREHENSIVE STUDY REGARDING MULTIPLICATION OF TWO WORLDWIDE ECONOMICALY IMPORTANT SPECIES: *Solanum tuberosum* AND *Ipomoea batatas* - *IN VITRO* APPROACH

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Abstract

Solanum tuberosum is the third world ranking species in terms of yield and consumption, after rice and wheat. Ipomoea batatas is the seventh world ranking production vegetable and provides food for over 68% of population. They are growing and fructification in hard climate conditions with arid soils or desert (sweet potato), and have important role in biodiversity through culture conditions adaptability, plant diseases and pathogens resistance (potato). Despite all these advantages, infections that are combined on those species reduce production capacity up to 90% in both species and this factor can be controlled to some extent by using micropropagation techniques and thermotherapy. Scientific papers, treaties and communications were studied in order to gather the most relevant dates regarding micropropagation of those two species. The high genetic variability this two species have makes it difficult to standardize micropropagation protocols, such as disinfection, phytohormones combinations and other techniques used in micropropagation regarding the devirosation of the plant material and the production of virus free material. Solanum tuberosum and Ipomoea batatas are two species that are two of the most cultivated worldwide, in poor or in development countries based on their nutritional values and their economic importance, scientists and breeders are focusing on biotechnologies to produce new varieties with high production capacity and promising resistance to pest, diseases and viruses.

Key words: *Ipomoea batatas, micropropagation, Solanum tuberosum, virus free.*

INTRODUCTION

Solanum tuberosum is the one of the world's most important food crop and major food sources for humankind. Potato is asexual propagated, using the tubers, technique which allows the dissemination of pathogens to the new plants and cultures, but also threatens the maintenance of genotypes for commercial or breeding purposes (Golmirzaie and Panta, 2000). Because of its value as a food source - plant is cultivated in over 150 countries (Basera et al., 2018), its important role in biodiversity through culture conditions adaptability, potato plant is a truly studied specie regarding diseases and pathogens resistance (Bamberg et al., 2016). Because potato accumulate several systemic fungi, bacteria and viruses infection (Karyeija et al., 1998), micropropagation offers alternative methods of propagation that provide production and multiplication of plant material with high efficiently (Golmirzaie and Toledo, 1999). *In vitro* conservation of potato facilitates the availability of the material all the time, avoid transfer of major diseases and pests and make possible virus control through meristem culture (Khadiga et al., 2009). Besides clonal multiplication, biotechnology techniques make available material for breeding programs and potato certified seed, and support conservation of germplasm which is a major problem in modern world for this specie, due its high cultivation on small lands, for personal consuming or small commercial purpose. In that situation, *in vitro* multiplication, cryo conservation and storage procedures surge potato plant bioavailability for a sustainable worldwide crop.

Ipomoea batatas, (sweet potato) is the sixth most important food crop worldwide after rice, wheat, potatoes, maize and cassava. In developing countries, however, is the fifth most important food crop and its food importance is rising (Jarret and Florkowski, 1990). More than 105 million metric tons are produced globally each year and 95% of which are grown in developing countries, commonly in Sub-Saharan Africa, some countries from Asia, and the Pacific Islands (Turyagyenda et al., 2015). According to them, sweet potato will empower around 15 million resource-poor households in Asia and Africa by improving the diets nutritional status and enhance crop incomes by 15% by 2023. Despite its benefits, virus diseases have been identified as the main cause of low yield productivity and cultivars degenerate (Wambugu, 1991). *Ipomoea batatas* is usually propagated by shoots tips, stem cuttings or storage roots for asexual propagation but diseases, pests and environmental factors impede sweet potatoes from reaching their maximum potential as a food crop (Guo et al., 2001). Several studies indicate that sweet potato chlorotic stunt virus and sweet potato feathery mottle virus drastically reduced specie yields, losses may often reach 65 to 90% (CABI, 2022; Wilms et al., 2020; Clark et al., 2012; Karyeija et al., 1998). The response could be found in micopropagation and *in vitro* conservation as providing stable clonal material, full time available, fewer pathogens or diseases contamination and virus free (Micheli and Standardi, 2015).

MATERIALS AND METHODS

This review tries to summarise some relevant results regarding culture decontamination, virus eradication, carbon source and growth hormones. We try to make a true listing and we are sure that are important scientific papers that are not find here due the length limitation or missing them from websites. We review over 75 papers for this paper.

In vitro culture decontamination

For *Ipomoea batatas* and *Solanum tuberosum*, micropropagation is one of the tissue culture techniques that provide culture stability and applicability (Leva & Rinaldi, 2012). Starting with 1951 when it was wrote the first scientific paper regarding usage of 2,4D and coconut milk on *in vitro* potato tuberization, several studies tried to establish a relation between virus-meristem and conservation (Steward and Caplin, 1951), using single node cuttings or liquid shaken cultures as are described in CIP documents (Espinoza et al., 1984). Through the years, one of the extended obstacles in the development of in vitro multiplication or storage protocols for potato, remain either fungal, bacterial, or over 40 viruses contamination (Lai et al., 2022).

The first approach is disinfection of the plant material which can be done depending on the type of explant: disc from tubers-potato, tuber pieces-potato, single nodes from sprouts-potato,leaf, shooting node, lateral bud or apex, meristem. The main substance use for both species are Sodium hypochlorite (NaOCl), in concentration varying between 1%-2%-1.5%-3%-3.5%-5.2%-10%-14% and time between 8-10-15-20 minutes, Mercuric chloride ($HgCl_20$) 0.1%-1% for 4 to 8 minutes, ethanol 70% (C_2H_5OH) for 5-15-25-30-40 or 60 sec, depending on explant type and contamination. (Badoni et al., 2010; Alconero et al., 1975; Gudeva et al., 2012; Yang, 2010; Tadda et al., 2021; Zhen, 2001; Hajare et al., 2021; Fufa et al., 2013; Dewir et al., 2020). Almost all studies using fresh material add firs some systemic fungicide to get a higher percentage of sterilization, Bavistine 0.5%-0.9% or Aliette in 0.4%-0.6% concentration.

Virus eliminated methods

Number of researches on potato micropropagation or storage technologies identify and try to control over 30 virus types that are known today (Loebenstein and Gaba, 2012). For endophytic bacteria treatments some studies recommend antibiotics, but the usage of its may produce toxic chemical particles for the plant development. For potato, Schewinski-Pereira (2003) recommend tetracycline, chloramphenicol, streptomycin, and ampicillin in concentration between 32 to 256 mg/l for endophytic bacterial growth inhibit and studies revealed tetracycline and chloramphenicol interfere with *in vitro* potato plant and affect explant survival, in opposition to ampicillin witch has no toxic effect on plant growth.

Since 2004 there are techniques that could applied to potato for eradicate viruses, or limit cross infections (Khurana, 2004). Viral infection of plant base material for commercial usage, infected with one or several viruses, substantially decrease production in the field. Identifying viruses cannot be done by visual report, sequencing of PCR–amplified sections of genomes is required (C. Jeffries and Khurana, 2006).

Micropropagation through meristem culture, thermotherapy, cryotherapy, chemotherapy single but mostly combined impact the viral expression in new plants and could control seed plant material (Green et al., 1989).

Several studies identify and try to eliminate viral contamination through therapies, with successful percent between 0% and almost 100% (Table 1).

Table 1. Main potato viruses and micropropagation methods of eradication

Potato virus type	**Techniques**	**Micropropag ation method**	**Rate of elimination**	**Best results**	**References**
PVS, PVX, PVA, PVY, PVM, PLRV	Meristems culture, shoot-tip cryotherapy by droplet vitrification, chemotherapy combined with thermotherapy	meristem, shoot tips	30-80-100%	Chemotherapy combined with thermmotherapy	Zang et al., 2019
PVM, PVS, PVX, PVY	Chemotherapy/riba virin 100 mg/l, cryotherapy with PVS-2 vitrification protocol	shoot tips	cryotherapy alone and one virus- 38.6%; cryotherapy for 3 viruses- 0%; chemotherapy +cryotherapy and subcultivation - 100%;	Chemotherapy+ cryotherapy	Kushnaren ko et al., 2017
PVX, PVS, PLRV, PVA	Chemotherapy/riba virin 100 mg/l in concentration: 0.75, 100, 150 and 200 mg/l	shoot tips	100% for PVX, PVS, PVA; 33-66% for PLRV	Chemotherapy	Yang et al., 2014
PLRV, PVY,	Electrotherapy, electro+chemothera py, electrotherapy+sub cultivation+antiral+ ASA	shoot tips	46,7%; 40%; 67,2%; 62.8%;	Electrotherapy+ subcultivation+a ntiviral+ASA	Naik et al., 2018
PVA, PVY	Electrotherapy	axillary buds	35 mA (8%/12.5%)	Electrotherapy+ cultivar	Meybodi D.et al., 2011
PVA, PLRV, PVM, PVS, PVX, PVY	Thermo-and/or chemotherapies (ribavirin)	shoots, apex	60%	Combined treatments	Bamberg et al., 2016
PVY, PVX, PVS, PLRV	Thermotherapy, chemotherapy (ribavirin, 5-Azacytidine, 3-Deazauridine) and combined	single nodes	PVY (83.3; 70.0 and 50.0%); PVY (30.0%)	Combined treatments	Nascimento et al. 2003

Regarding sweet potato and its viral infectious status, worldwide production is seriously affected by a range of over 30 viruses, like sweet potato virus disease (SPVD), Sweet potato pakakuy virus (SPPV) or Sweet potato feathery mottle virus (SPFMV), single or combined, the last one cause roots unmarketable (Kapinga et al., 2007). There are still made efforts to eradicate viruses in sweet potatoes to establish a standard for virus-free certificate plant material and produce plant material through *in vitro* techniques (Morais et al., 2018).

Some scientific papers indexed in this work listed meristem culture, thermotherapy, cryotherapy as potential treatment to eradicate viral disease in sweet potato, as they are summarised (Table 2).

Table 2. Main sweet potato viruses and micropropagation methods of eradication

Potato Virus type	Techniques	Micropropagation method	Rate of elimination	Best results	References
SPFMV, SPLV, SPMMV, SPVG, SPMSV, SPCFV, C-6 virus, SPCSV, SPCaLV, CMV	Meristems culture, thermotherapy	meristem, shoot tips	SPFMV-88.89% and SPCSV-100%	combined treatmens	Dugassa and Feyissa, 2011
SPFMV and SPCSV	Meristem culture and cryotherapy	meristem 0.5–1.5 mm	SPFMV- 90–93% faild in 1.5 mm meristem; SPCSV eradicate	cryotherapy	Wang et al., 2008
SPFMV, SPVC, SPMMV, SPCSV and SPLCUV	Repeteating grafting with contaminated I. setosa, controled temperaure and amended soil	meristem culture	none for SPCSV, SPFMV+ SPCSV; SPMMV (100%,20%, 60%) and SPLCUV (80% and 100%)	combined treatments	Ssamula et al., 2018
SPCSV, SPVG, SPVC, SPFMV, SPV2, SPLCV(first report), SPPV	Thermotherapy and meristem tip culture	meristem culture	over 13% for PFMV, SPV2, SPVC, SPVG, and SPLCV except SPPV	combined	Kiemo et al., 2021
SPCSV, SPFMV, SPMMV, and combinations of SPCSV + SPFMV and SPCSV + SPMMV	Thermotherapy 36°C/16 h and 32°C/8 h daily and meristem tip culture	grafted to Ipomoea setosa and micropropagated by meristem 0.5-1.00 mm	SPFMV and SPMMV 97.2%, 80.5%, 69.2%	micropropagation with thermotherapy	Rukarwa et al. , 2011
"non-persistant virus (filamentous particle of 850 nm)" and "virus with filamentous particles of about 1000 nm"	Meristem culture (0.25 to 0.4 mm) and grafting	meristem culture	80%	combined treatments	Frison et al., 1981

Carbon source and tuberization

The success of plant tissue culture is determined by culture media structure and carbon source, most likely sucrose. Sugars are required *in vitro* (they complete life circle of the plant and without them, they will not survive), they cannot be replaced by another element and their action is specific (Fufa and Diro, 2013).

Based on that, sucrose is most used carbohydrate source, among glucose, fructose, galactose, mannose, maltose, lactose, trehalose and raffinose, not all of them used in micropropagation (Yaseen et al., 2012).

Rahman, 2010 research the role of sucrose, glucose and maltose and found that maltose is preferred in terms of multiplication rate and if we are talking about unimodal segments, research results show better response with glucose.
The 30 g/l sucrose could be significant for shoot length in addition with low light and low temperature storage (Pruski et al., 2000).
Sucrose was determined as a necessary external carbon source for induction and micro tuberization and usually increased concentration act better instead of lower concentration used for slow grow techniques and conservation (Lo and Liao, 1993) but both lower or higher concentration can impair with plant development.
The explant response to carbon source vary with cultivar or genotype, presence of hormones, stocking temperature and light intensity, but, generally accepted is that sucrose is the main carbon source for plant micropropagation (Table 3).
Sugar itself or sugar less 20 g/l is not a solution when tuberization is tracked, 40 g/l gave only 75% tuberization, but in case of higher concentration, about 80 g/l, 100% of tuberization achieved (Xu et al., 1998).
Same Xu revealed the relation between sucrose and endogenous gibberellins (GA), sucrose induce the expression of tuberization genes at higher concentration.

Multiplication
Multiplication is part of micropropagation and use an initial explant like source for multiplication stage.

Table 3. Main carbon source in potato and sweet potato micropropagation

Carbon source	**Concentration**		**References**
	Solanum tuberosum	***Ipomoea batatas***	
Sucrose	20 - 25 - 30 - 40 - 50 – 60 - 80 - 100 g/l	15 - 20 - 25 - 30 - 40 - 60 g/l	AlMaarri et.al, 2012; Islam et al., 2017; Zhang et al., 2019; Wang et al., 1982; Rahman et al., 2010; Altindal & Tahsin, 2010; Yoon et al., 2004; Ibrahim, 2019; El-Far, 2007; Dugassa and Feyissa, 2011; Fadaladeen et al., 2022;
Maltose	20 - 30 - 40 - 60 – 80- 120 g/l		Rahman et al., 2010; Altindal & Tahsin, 2010; Yoon et al., 2004
Glucose	30 - 80 g/l	15 - 30 - 45 - 60 g/l	Rahman et al., 2010; Fadaladeen et al., 2022
Fructose		15 - 30 - 45 - 60 g/l	Fadaladeen et al., 2022
Sorbitol		0.2 - 0.4 - 0.6 M	Smith et al., 2019
Sorbitol and manitol		20 g/l	Sriskantharajah & Ketipearachchi, 2012

Taking into account the viral infectious spectre of those two species, first recommendation for micropropagation remains meristem culture (Gudeva et al., 2012) and after that, shoot tips with discussions about optimisation of dimension (Wang et al., 2008). Danci (2011) show that even the meristem is larger and you can produce more plantlets, the presence of leaf primordia is critical for survival rate. Several works show us that meristem culture not only regenerate much rapid then other culture like shoot tips, or organogenesis, but we can manage the viral infections with this approach, both in *Ipomoea batats* and *Solanum tuberosum* (Wang & Hu, 1982; Barka and Feyissa, 2011; Smith et al., 2019; Nascimento et al., 2003).
Sprouts from potato or uninodal segments (stem cuttings from potato) from sweet potato are the next option for micropropagation, and they are used on researches about multiplication, growth rate or tuberization (Ravnikar et al.,1992; Yang et al., 2014; Abubakar et al., 2018; Vettorazzi et al., 2017; Beyene et al., 2020)
One important science direction on sweet potato is salt resistance of this specie and because of its biologically plasticity and food impact on poverty and hunger this important biotic asset need to be exploit.
Studies were made on *in vitro* plants generate through somatic embryogenesis (leaf, petiole and stem explants) and *in vitro* techniques

support plant salt resistance identification (Anwar et al., 2010; Ekanayake and Dodds, 1993).

Growth regulators

Literature suggests hormones regulate explant growth in micropropagation, can induce organogenesis (Nakajima and Kawakami, 1969), callus dedifferentiation, multiplication, rooting and plant wellbeing. Starting with MS medium of Murashige & Skoog (1962) hormones represent the necessary variables that made micropropagation and plant tissue culture possible.

For *Solanum tubeorsum* and *Ipomoea batatas* literature review the major role of hormones for micro tuberization (García-García et al., 2019), virus eradication (Gong et al., 2019; Kiemo et al., 2022) or cryoconservation (Bamberg et al., 2016; Sriskantharajah and Ketipearachchi, 2012).

Table 4. Usual concentration of major hormones used in micropropagation of *Sweet potato* and *Ipomoea batatas*

Hormone	Effect	Concentration		References
		Solanum tuberosum	***Ipomoea batatas***	
Indole-3 -butyric acid (IBA)	regulation of root apical meristem size, root elongation, lateral root development, and formation of adventitious roots;	0.01 - 0.1 - 1 mg/l	0.1 - 2 mg/l	Rabbani et al., 2001; Fadaladeen et al., 2022; Zang, Z. et al., 2019
Indole-3 -acetic acid (IAA)	inducer of cell division and elongation;	0.5 - 1 - 1.5 mg/l	0.2 - 1 - 1.2 mg/l	Alconero et al., 1975; Zhen, H R., 2001; Gudeva, K.L. et al., 2012
1-naphthaleneacetic acid (NAA)	rooting agent;	0.01- 1 mg/l	0.01 - 0.05 - 0.1 - 1 - mg/l	Fadaladeen et al., 2022; Zhen, H R., 2001; Fufa, M. and Diro, M. (2013)
2,4-Dichlorophenoxyacetic acid (2,4-D)	a dedifferentiation (callus induction) hormone	1 - 1.5 - 2.5 - 3 - 4.5 – 5 mg/l	0.01- 1.5 - 2 - 3.5 - 4.5 - 4 mg/l	El Abidine Triqui et al., 2008; García-García et al., 2019; Padmanabhan et al., 2001; Oggema, J. et al., 2007
6 -benzyladenine (BA)	induction of cell division and shoot. differentiation in plant tissue culture	0.5 - 1 - 1.5 – 2 mg/l	0.1 - 0.5 - 1 - 4 - 4.5 - 5 - 6 - 7 - 8 mg/l	Fadaladeen et al., 2022; Zhen, H R., 2001
6-furfurylaminopurine (KIN)	inducing callus (+ auxin), regenerate shoot tissues from callus (- auxins);	0.1 mg/l	0.05 - 0.1 - 0.5 - 1.5 - 2.5 - 3 - 4 - 5 mg/l	Smith, M.S. et al., 2019; Fadaladeen et al., 2022; Zhen, H R., 2001; García-García, J.A. wt al., 2019; Gudeva, K.L. et al., 2012
gibberellic acid (GA3)	essential for the induction of lateral shoots, increasing cell elongation, seed germination, dormancy, reproductive growth, tolerance against various stress types and senescence; essential in callus culture	0.25 - 0.3 - 0.5 mg/l	0.25 - 1 - 2 - 3 - 10 mg/l	Wang, Q.C., JValkonen, J.P.T., 2008; Gudeva, K.L. et al., 2012; Fufa, M. and Diro, M. (2013)
6-benzylaminopurine (BAP)	stimulates the differentiation of the cells generated in meristem and encourage the growth of side shoots, leaves apical dominance and expansion; stimulating cell division	0.1 - 0.5 - 1- 1.5 - 2 - 3 - 4 - 5 mg/l	0.1 - 0.25 - 0.5* - 0.75 - 1 - 2 - 2.5 - 3 - 5 mg/l/*callus proliferation	Wang, Q.C., JValkonen, J.P.T., 2008; Smith, M.S. et al., 2019; García-García, J.A. et al., 2019; Gudeva, K.L. et al., 2012

Micropropagation of potato depends on the genotype, nutrients in the culture medium and plant growth regulators and there is no standard recipe could be applied. Among the usual hormones, most important are Indole-3 -butyric acid - IBA, who can regulate root apical meristem size, root elongation, lateral root development, and promote formation of adventitious roots; Indole-3-acetic acid - IAA, important hormone for inducing cell division and elongation; 1-naphthaleneacetic acid - NAA, prompting rooting agent; 2,4-Dichlorophenoxyacetic acid - 2,4-D, a pesticide who can induce callus formation (dedifferentiation); 6-benzyladenine - BA, who can impact induction of cell division and shoot. differentiation in plant tissue culture; 6-furfurylaminopurine - KIN, an synergic hormone for inducing callus (+ auxin) or regenerate shoot tissues from callus (- auxins); gibberellic acid - GA_3, essential for lateral shoots induction, increasing cell elongation, dormancy seed germination, reproductive growth, supporting different stress types and senescence and essential in callus culture and the last important hormone, 6-benzylaminopurine - BAP, who can stimulates the cells differentiation, generated growth of side shoots, leaves, induce apical dominance and expansion. (El Abidine Triqui et al., 2008; Espinoza et al., 1984; Dewir et al., 2020; Rabbani et al., 2001; Gudeva et al., 2012; Bamberg et al., 2016; Steward et al., 1951). Different concentrations for some of the main hormones are present below (Table 4).

Regarding somatic embryogenesis, growth regulators are key factors for callus induction and plantlet development, studies reveal that for first stages there is a borderline for auxins presence: during initiation is necessary to inhibit auxins but for callus inducing, there is a total request of them (Rabbani et al., 2001) and for the further stages, another hormones are necessary, like 2,4-Dichlorophenoxyacetic acid - 2,4-D , GA_3 or zeatin (El Abidine Triqui et al., 2008; García-García et al., 2019; Padmanabhan et al., 2001).

CONCLUSIONS

Even potato micropropagation is studied since the early 50th, because of its large scale production, domestic or industrial, there are still issues regarding somaclonal variation and genetic stability of this specie. Similar, sweet potato present some issue regarding genetic stability and large scale mass seed production. Even species respond to somatic embryos technique, there is not enough response for this *Solanaceous* plant (potato), and somatic embryogenesis to potato still requiring studies.

Even scientific literature is much bigger than our references, *in vitro* techniques and gene conservation methods remain first option for potato, sweet potato and rest of tubers. Techniques can preserve both species on medium-term time through cryopreservation, and combined chemotherapy with thermotherapy can develop a solution for eradication viral diseases. In the low-income country, there is possible to establish a low-cost protocol for sweet potato micropropagation and that is the major goal for world issue food and hunger for African country.

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