OPTIMIZATION OF SWEET POTATO (*IPOMOEA BATATAS* L.) *IN VITRO* CULTIVATION BY USING THE CONTAMINATED CULTURES FOR OBTAINING NEW SHOOTS IN GREENHOUSE CONDITIONS

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Abstract

The artificial media used for plant tissue cultures contains numerous nutrients that can create favorable conditions for the development of pathogens. As antibiotic use is not encouraged, a new strategy has been tried to reduce the losses caused by the presence of microbial infections in *"in vitro"* cultivation of sweet potato. This consisted in the transplantation in greenhouse conditions of sweet potato plantlets from contaminated *"in vitro"* cultures, in order to obtain new shoots. Five sweet potato varieties were used in this study, and two types of substrate for planting: pearlite and a mixture of peat and pearlite (1:1). The survival rate of the plantlets was 100% on the substrate consisting only of pearlite. The surviving sweet potato plants had a rapid growth rate, the greenhouse conditions being favorable for this culture. Approximately two months after transplantation, the obtained shoots could be used as a source of explants to initiate new *"in vitro"* cultures. Regarding the number of shoots the highest value was obtained by the Yulmi variety. The length of the shoots varied according to the variety, thus the highest value was recorded by the KSC1 variety (98.30 cm) . The number of buds/shoot is strongly influenced by the variety. In some sweet potato varieties the distance between buds is smaller, and in others larger, this being a characteristic of the variety. Regarding this trait the best results were obtained in Juhwangmi variety. By applying this method, the process of sweet potato *"in vitro"* multiplication becomes more economically efficient. After only a few weeks under greenhouse conditions, involving minimal costs, many shoots can be obtained.

Key words: plant tissue culture, microbial contamination, plantlets, greenhouse, sweet potato

Tissue culture has many advantages such as production of disease-free planting materials in large numbers hence permits rapid dissemination of healthy and improved plants within and among countries, as the materials are readily certified as disease-free (Ogero *et al*., 2012; Rahman *et al.*, 2017; Naik and Karihaloo, 2007). Plant tissue culture is uniquely suited for obtaining and maintaining mass propagation of specific pathogen-free plants (Mervat, 2007). Traditionally, cuttings are obtained from the shoots grown from tubers buried in warm, humid soil (25–28 °C) or from plants grown in greenhouses (Ching, 2000; Novac *et al*. 2007; Doliński and Olek, 2013). A solution which makes it possible to produce larger numbers of uniform, healthy plants is ”*in vitro”* culture.

Sweet potato can be propagated by stimulating the development of apical and axillary buds, by means of adventitious buds and by somatic embryogenesis (Gosukonda *et al*., 1995; González *et al*., 1999; Mukherjee, 2002, Doliński and Olek, 2013). This technique is based on the principle that appropriate culture conditions induce the growth of a pre-existent terminal or axillar buds, resulting in a new plantlet. The nutritional and hormonal conditions of the medium break the bud dormancy and promote its rapid growth (Vollmer, 2010). However, sweet potato is highly recalcitrant in generation and response to tissue culture (Abubakar *et al*, 2018). A number of factors including genotypes, nature and doses of different growth regulators are found to determine the rate and nature of regeneration of sweet potato (Shaibu *et al*, 2016).

The use of nodal explants may promote direct regeneration of plantlets (Yadav, 2009). However, microbial contamination would be very high due to large size of the explant (Amissah *et al*, 2016). Studies indicate that ”*in vitro”* cultures of sweet potato are prone to microbial contamination emanating from both endogenous and exogenous sources, which leads to culture mortality (Jena and Samal, 2011; Rojas, 2010).

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MATERIAL AND METHOD

The study was conducted at the National Institute for Research and Development for Potato and Sugar Beet Brasov, within the Research Laboratory for Plant Tissue Cultures. As a biological material, 5 korean sweet potato varieties were used: Juhwangmi, KSP1, KSC1, Yulmi and Hayanmi. The initial material consisted in plantlets from *"in vitro"* cultures contaminated with microbial infections (*figure 1*). Sweet potato plantlets were planted in pots placed in the greenhouse, on two types of substrate: peat and pearlite (1:1) and pearlite. They were about 10-11 cm high, with well developed leaves and roots (f*igure 2*).

Figure 1 **Sweet potato plantlets from "*in vitro*" contaminated culture**

Before planting the roots were carefully cleaned, removing the culture media, without damaging the roots. It is important to remove the culture media because it prevent the absorption of nutrients from the culture substrates by roots. Each pot contained a single sweet potato plantlet. The substrate was kept moist, and two weeks after planting a complex fertilizer NPK 16:16:16+sulfur (12 g/l) was applied. The survival rate of sweet potato plantlets after transplantation was higher when the pearlite substrate was used, compared to the peat and pearlite mixture. Pearlite is a horticultural substrate, an inorganic material, recommended for improving soil quality. Pearlite is obtained by thermal processing of siliceous volcanic rocks. The mineral extracted from the deposits contains 2-5% bound water; heated to 1200 °C, it expands, becoming a porous material, granulated with a bulk density of 130-180 kg/m^3 (Atanasiu, 2007). Substrates containing pearlite are well drained and aerated. The pearlite granules have good physical stability and are chemically inert. The pearlite granules are also used for the preparation of mixed culture substrates by mixing with some organic materials (peat).

Figure 2 **Sweet potato plantlets transplantation in greenhouse conditions**

The surviving plants had a fast growth rate, the greenhouse conditions being favorable for this culture. Approximately two months after transplantation, the obtained shoots could be used as a source of explants to initiate new *"in vitro"* cultures (f*igure 3*).

Figure 3 **Sweet potato shoots obtained in greenhouse conditions starting from *"in vitro"* plantlets (one month after transplantation – top; two and a half months after transplantation – bottom)**

After detaching the shoots from the mother plant, they are washed with tap water to remove soil and other impurities, then the leaves are removed, keeping a small part of the petiole, which has the role of protecting the buds during sterilization. The shoots are fragmented into uninodal stem cuttings. Sterilization consists of immersing explants in sodium hypochlorite solution (1%) for 15 minutes followed by immersion in ethyl alcohol (70%) for 5 minutes. After sterilization, the cuttings are rinsed in 3-4 rounds with sterile distilled water and then inoculated on the culture

media. After approximately 3-4 weeks of cultivation under controlled conditions (24-26 °C; photoperiod of 16 hours light and 8 hours dark) new sweet potato plantlets can be obtained (f*igure 4*).

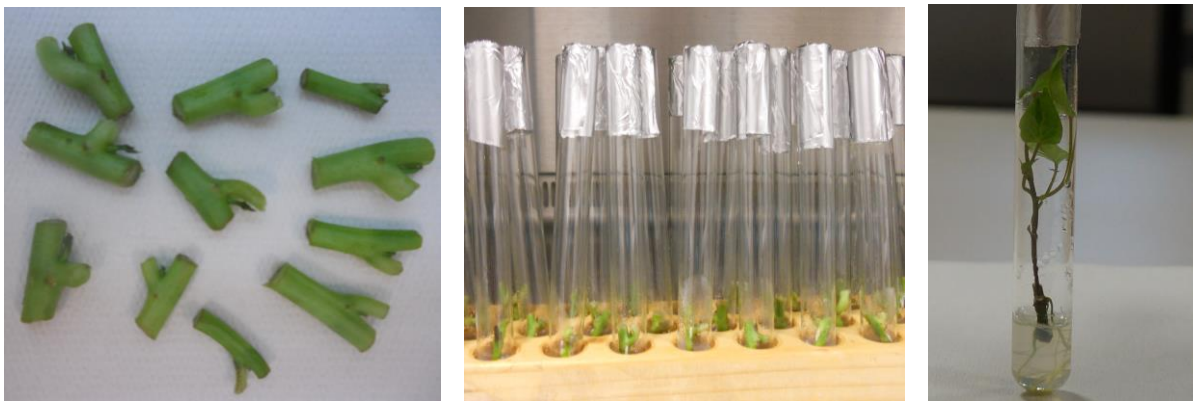

Figure 4 **Inoculation of explants on culture media and obtaining healthy sweet potato plantlets from shoots grown in greenhouse conditions**

RESULTS AND DISCUSSIONS

In order to optimize the sweet potato micropropagation technology, a cheap and easy method of obtaining shoots under greenhouse conditions was tested starting with plantlets from *"in vitro"* cultures contaminated with microbial infections. Thus, the losses caused by microbial contamination can be reduced by using these plantlets, which would otherwise have been eliminated, in order to obtain a source of explants.

In this study several aspects were pursued: the percentage of survival, on the two types of substrate, of plantlets from contaminated *"in vitro"* cultures, following their transplantation under greenhouse conditions, as well as the number of shoots obtained according to variety, shoots length and number of buds.

The survival rate of sweet potato plants was 100% in the case of using pearlite as substrate, for all 5 studied varieties. Regarding the use as substrate of peat and pearl mixture, for some varieties (Yulmi, Hayanmi and KSC1) the survival rate was 50%, while for other varieties (KSP1 and Juhwangmi) the survival percentage was 100% (*table 1*).

Table 1

The survival rate of sweet potato plantlets after tranplantation in greenhouse conditions

Variety	The survival rate of plantlets (%)	
	Peat+pearlite (1:1)	Pearlite
Yulmi	50	100
Hayanmi	50	100
KSC1	50	100
KSP1	100	100
Juhwangmi	100	100

In addition to the survival rate of plantlets after transplanting under greenhouse conditions, other growth parameters were followed: the number of shoots, shoots length and the number of buds/shoot. The average of the five varieties was considered the control variant. In *tables 2, 3* and *4* are presented the results regarding the evolution of these traits, three and a half months after planting. Regarding the number of shoots, there were no significant differences between the analyzed varieties (*table 2*). However, the highest number of shoots was obtained by the Yulmi variety, and the lowest was recorded in the KSC1 and KSP1 varieties.

Table 2

Effect of sweet potato variety on shoots number/pot

Variety	Shoot number		
	Average number	Diff.	Signif.
Mean (Ct)	1.47	-	-
Yulmi	2.33	0.86	ns
Hayanmi	1.33	-0.14	ns
KSC1	1.00	-0.47	ns
KSP1	1.00	-0.47	ns
Juhwangmi	1.67	0.20	ns

LSD5% = 0.59 shoots; 1% = 0.83 shoots; 0.1% = 1.18 shoots

Regarding the length of the shoots, there were significant differences between the analyzed varieties (*table 3*). Thus, the best results were recorded by the KSC1 variety (98.30 cm), which presented a distinct positive difference (35.72 cm) compared to the control, and the lowest values were recorded by the Hayanmi variety (32.98 cm), which presented a distinct negative difference (-29.60 cm).

Table 3

Effect of sweet potato variety on shoots length

Variety	Shoots length (cm)		
	Average length (cm)	Diff. (cm)	Signif.
Mean (Ct)	62.58	-	-
Yulmi	61.09	-1.49	ns
Hayanmi	32.98	-29.60	oo
KSC1	98.30	35.72	**
KSP1	57.80	-4.78	ns
Juhwangmi	62.75	0.17	ns

LSD 5% = 18.07 cm; 1% = 25.37 cm; 0.1% = 35.82 cm

Measurements on the number of buds/shoot were also made (*table 4*). In this case there were a significant positive difference between Juhwangmi variety (7.94 buds/shoot) and control, and a significant negative difference for Yulmi variety (-6.28 buds/shoot).

Table 4

Effect of sweet potato variety on number of buds/shoot

Variety	Number of buds/shoot		
	Average number	Diff.	Signif.
Mean (Ct)	16.89	-	-
Yulmi	10.61	-6.28	o
Hayanmi	22.33	5.44	ns
KSC1	13.67	-3.22	ns
KSP1	12.67	-4.22	ns
Juhwangmi	24.83	7.94	*

LSD5% = 5.68 buds; 1% = 7.98 buds; 0.1% = 11.26 buds

Regarding the number of buds/shoot, this trait is strongly influenced by the variety. In some sweet potato varieties the distance between the buds is smaller, and in others larger, this being a characteristic of the variety. In the case of the varieties analyzed in this study, in Juhwangmi and Hayanmi the buds are arranged closer to each

other, while in Yulmi, KSP1 and KSC1 the buds are farther apart.

Plantlets from contaminated *"in vitro"* cultures, which would otherwise have been eliminated, are used to obtain new sweet potato plants. In this way the technology of "in vitro" micropropagation of the sweet potato can be optimized successfully).

CONCLUSIONS

The application of plant tissue culture technology has an important contribution to *"in vitro"* production, multiplication and conservation of healty sweet potato plants for commercial, research and other purposes. Sweet potato offers strategic opportunities to improve nutrition and rural incomes in several countries and regions all over the world. It is already an important component of the cropping systems because of its robustness to produce under difficult conditions and it will become more important in the face of a changing climate. Thus, it is very important to make the *"in vitro"* multiplication technology of sweet potato more efficient.

Microbial contamination could occur at any stage of *"in vitro"* micropropagation systems. Sweet potato plantlets from contaminated *"in vitro"* cultures, which can no longer be used in the micropropagation process, can be used to obtain new sweet potato plants by transplantation in greenhouse, using pots with different substrates. Due to the fact that the sweet potato is characterized by the rapid growth and the active formation of the roots that gives a higher survival rate of the plantlets, the process of microplants acclimatization is quick and easy. "*In vitro"* formed roots have an important role in acclimatization and survival of sweet potato plantlets. These not only survived transfer to the substrate but elongated, formed secondary and tertiary roots, are functional during acclimatization and contributes significantly to the early growth of transplants "*ex vitro"*.

After only a few weeks of cultivation under greenhouse conditions, involving minimal costs, many shoots can be obtained which, after being removed from the mother plant, are sterilized and reintroduced into the micropropagation process under *"in vitro"* conditions, as nodal cuttings. Only one plantlets was planted in each pot, but three or more plantlets/pot can be planted. Thus, the number of shoots and respectively the number of buds/shoots that can be obtained is higher, and the process becomes more efficient. Mother plants can remain in pots, generating new shoots, until we get the desired number of explants. By applying this method, the process of *"in vitro"* sweet potato multiplication becomes more economically efficient.

Considering the results obtained in this study, the *"in vitro"* sweet potato multiplication process can be optimized by applying this method.

REFERENCES

Abubakar A.S., Yahaya S.U., Shaibu A.S., Ibrahim H., Ibrahim A.K., Lawan Z.M., Isa A.M., 2018 - *In vitro propagation of sweet potato (Ipomoea batatas (L.) Lam.) cultivars*. Agric. Sci. Digest., 38 (1) 2018:17-21, Print ISSN:0253-150X/Online ISSN:0976-0547.

Amissah S., Coleman Priscilla A., Sintim H.Y., Akromah R., 2016 - *In vitro control of microbial contamination of sweet potatoes cultured with nodal explants*. Anual Reasearch & Review in Biology, 9(3): 1-8.

Atanasiu N.E., 2007 - *Culturi agricole fără sol*; ediţia a II-a revizuită şi adăugită. Editura ATAR, Bucureşti.

Ching A., 2000 - *The effect of transplant container cell shape on vegetative growth and root yield of sweet potato*. Acta Hort. 616:163–166.

Doliński R., Olek Ana, 2013 - *Micropropagation of sweet potato (Ipomoea batatas (L.) Lam.) from node explants*. Acta Sci. Pol., Hortorum Cultus 12(4):117-127.

González R.G., Sánchez D.S., Campos J.M., Vázquez E.P., Guerra Z.Z., Quesada A.L., Valdivia R.M., González M.G., 1999 - *Plant regeneration from leaf and stem explants from two sweet potato (Ipomoea batatas L. Lam.) cultivars*. Biotecnol. Aplic. 16, 1:11–14.

Gosukonda R.M., Prakash C.S., Dessai A.P., 1995 - *Shoot regeneration in vitro from diverse genotypes of sweetpotato and multiple shoot production per explant*. HortSci. 30(5):1074–1077.

Jena R.C., Samal K.C., 2011 - *Endogenous microbial contamination during in vitro culture of sweet potato [Ipomoea batatas (L.) Lam]: Identification and prevention*. J. Agric. Technol. 2011;7:1725–1731.

Mervat M.M. EL Far, 2007 - *Optimization of growth conditions during sweetpotato micro-propagation*. African Potato Association Conference Proceedings, Vol. 7, Egypt, p. 204 - 211.

Mukherjee A., 2002 - *Effect on NaCl on in vitro propagation of sweet potato (Ipomoea batatas L.)*. App. Biochem. Biotech. 102/103:431–441.

Naik P.S., Karihaloo, J.L., 2007 - *Micropropagation for production of quality potato seed in Asia-Pacific* - Asia-Pacific Consortium on Agricultural Biotechnology, New Delhi, p. 47.

Novac B., Žutić I., Toth N., Dobričević N., 2007 - *Sweet potato (Ipomoea batatas (L.) Lam.) yield influenced by seedlings and mulching*. Agric. Conspec. Sci. 72, 4:357-359.

Ogero K.O., Mburugu G.N., Mwangi M., Ngugi M.M., Ombori O., 2012 - *Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (Ipomoea batatas (L) Lam)*. Research Journal of Biology (2012), Vol. 02, Issue 02:51-58, ISSN 2049-1727.

Rahman M.Z., Shahinul Islam S.M., Chowdhury A.N., Subrama S., 2017 - *Identification and prevention of microbial contaminants of potato culture in temporary immersion bioreactor (TIB) system*. Malaysian Journal of Microbiology, 13(4): 289-297.

Rojas L., 2010 - *Elimination of bacterial contaminants from sweetpotato in in vitro cultures - OP53*. Head Genebank-CIP; 2010.

Shaibu A.S., Abubakar A.S., Lawan Z.M., Ibrahim A.K., Rabiu H.M., Muhammad A.I. et al, 2016 - *Media Optimization and Effect of Surface Sterilization Timing on In Vitro Propagation of Sweet Potato*. Proceedings of the 2nd International Conference on Drylands.

Vollmer R., 2010 - *In vitro multiplication of sweetpotato*. International Potato Center (CIP), Lima, Peru – OP59, p.1.

Yadav R, Arora P, Kumar D, Katyal D, Dilbaghi N, Chaudhury A., 2009 - *High frequency direct plant regeneration from leaf, internode, and root segments of Eastern Cottonwood (Populus deltoides)*. Plant Biotechnol. Reports. 2009, 3:175-182.

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