ALTERNATIVE INPUTS FOR MICROPROPAGATION OF *Solanum tuberosum*, *Ullucus tuberosus* AND *Oxalis tuberosa* IN SEMISOLID AND LIQUID MEDIUM AND TEMPORARY IMMERSION SYSTEM †

[USO DE INSUMOS ALTERNATIVOS PARA LA MICROPROPAGACIÓN EN MEDIO SEMISÓLIDO Y SISTEMA DE INMERSIÓN TEMPORAL DE *Solanum tuberosum*, *Ullucus tuberosus* Y *Oxalis tuberosa*]

Gilmar Peña-Rojas*[1], Héctor Sánchez-Sotomayor[3], Indira Roel Barahona[3], Vidalina Andía-Ayme[2], Martha Segura-Turkowsky[3] and Rolando Estrada-Jiménez[3]

[1]Laboratorio de Biología Celular y Molecular. Universidad Nacional de San Cristóbal de Huamanga, Ayacucho. Dirección postal: Ciudad Universitaria 05001. Ayacucho – Perú. Email: gilmar_p@yahoo.com
[2]Laboratorio de Microbiología de Alimentos. Universidad Nacional de San Cristóbal de Huamanga, Ayacucho. Dirección postal: Ciudad Universitaria 05001. Ayacucho – Perú.
[3]Laboratorio de Recursos Genéticos y Biotecnología. Universidad Nacional Mayor de San Marcos – Lima, Perú. Dirección postal: Padre Urraca 296 –Lima 32 – Perú
**Corresponding author*

SUMMARY

Background. Micropropagation allows high rates of clonal multiplication of plants in short periods of time. However, despite being a simple technique, its implementation can be costly for regions and producers with limited resources. One of the main limiting factors is the lack of access to the inputs (QP) for the basic media that is widely published and disseminated in the literature. Other limiting factors include high costs, logistical difficulties and timely access to such inputs. **Objective.** In order to address these problems, the present work proposes the use of alternative inputs in the composition of the culture media for micropropagation of potatoes (*Solanum tuberosum*), ulluco (*Ullucus tuberosus* Caldas), and oca (*Oxalis tuberosa* Molina) in semi-solid and liquid media, as well as in temporary immersion systems (SIT). **Methodology.** All the macro and micronutrients of the basic media were replaced with inputs available at low cost in any local market. **Results.** The best results for micropropagation rate, plant vigor and rooting were obtained in the medium with substituted and accesible inputs (EP medium). These results were validated for micropropagation by nodal cutting in liquid and semi-solid médium and SIT. **Implications.** The basic medium (EP), formulated herein may be used by any laboratory and resource-poor farmers to apply micropropagation and improve revenues. **Conclusion.**The production cost of the EP medium, with easily accessible inputs in low-cost local markets, is significantly cheaper than MS medium used as a control, without differences in micropropagation.

Key words: Plant culture media; Micropropagation; Temporary Immersion System; *Solanum tuberosum; Oxalis tuberosa; Ullucus tuberosus*

RESUMEN

Antecedentes. La micropropagación permite altas tasas de multiplicación clonal de plantas en cortos períodos de tiempo. Sin embargo, a pesar de ser una técnica simple, su implementación puede ser costosa para regiones y productores con recursos limitados. Uno de los principales factores limitantes es la falta de acceso a los insumos químicamente puros (QP) para los medios básicos que se publican y difunden ampliamente en la literatura. Otros factores limitantes incluyen los altos costos, las dificultades logísticas y el acceso oportuno a dichos insumos. **Objetivo.** Para abordar estos problemas, el presente trabajo propone el uso de insumos alternativos en la composición de los medios de cultivo para la micropropagación de papa (*Solanum tuberosum*), ulluco (*Ullucus tuberosus* Caldas) y oca (*Oxalis tuberosa* Molina) en medio semisólido y líquido, así como en sistemas de inmersión temporal (SIT). **Metodología.** Todos los macro y micronutrientes de los medios básicos fueron reemplazados por insumos disponibles a bajo costo en cualquier mercado local. **Resultados.** Los mejores resultados para la tasa de micropropagación, el vigor de la planta y el enraizamiento se obtuvieron en el medio con insumos alternativos y

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accesibles (medios EP). Estos resultados fueron validados para la micropropagación por corte nodal en medio líquido, semisólido y SIT. **Implicaciones.** El medio básico (EP), formulado en este documento, puede ser utilizado por cualquier laboratorio y agricultores de bajos recursos para aplicar micropropagación y mejorar sus ingresos. **Conclusión.** El costo de producción del medio EP, con insumos de fácil acceso en mercados locales a bajo costo, es significativamente más barato que el medio MS utilizado como control, sin diferencias en la micropropagación.
Palabras clave: medios de cultivo de plantas; micropropagación; sistema de inmersión temporal; *Solanum tuberosum*; *Oxalis tuberosa*; *Ullucus tuberosus*

INTRODUCTION

Since the last three decades of the 20th century, the use of plant tissue culture techniques has increased greatly. This has helped improving and increasing food productivity, as well as promoting marketing, sustainable use and the conservation of biodiversity (germplasm Banks). Furthermore, it has improved secondary metabolites production, basic studies of plant biology, development of molecular markers (Bornman et al., 2004; Botha et al., 2004; Okole et al., 2004; Berjak et al., 2011) along with plant regeneration through genetic engineering. Due to the threats to diversity derived from climate change, in the last two decades this technique has expanded to *in vitro* conservation of plants in germplasm Banks, and to using micropropagation for the mass production of basic seed of commercial species (Berjak et al., 2011).

Micropropagation provides numerous advantages over conventional propagation. One example is the mass production of elite and disease-free plants that can grow in small spaces, under controlled conditions, and independent of the season. The micropropagation protocols were standardized for the commercial production of species of commercial importance, now they are also applied to non-food plant biotechnology research, as for the conservation of medicinal and ornamental species (Fennell et al., 2004), remaining as a promising technique (Shahzad et al., 2017).

The composition of the culture medium is crucial in micropropagation. The most widely used media (Vasil 2008; Rout et al., 2000; George et al., 2008; Husain et al., 2010; Niedz et al., 2007, Azofeifa et al., 2008) was developed by Murashige and Skoog (1962) and modified by Linsmaier and Skoog (1965). The differences in nutrients and concentrations in the culture medium are a determining factor for the *in vitro* regeneration and morphogenesis of the plant (Samson et al., 2006; Niedz et al., 2007; George et al., 2008: Kothari-Chajer, et al., 2008).

There is a permanent search for substitutes of the culture medium components to reduce costs of plant micropropagation. The main focus is to replace the macro and micronutrients QP, sugars and gelling agents with nutritive solutions from cheaper sources (Montenegro et al., 2014: Azafeita et al., 2008). There are reports on the use of foliar fertilizer in *in vitro* culture of *Catleya* sp., seed culture of *Arabidopsis thaliana*; fertilizers for micropropagation of *Laelia anceps* and several commercial foliar fertilizers in the micropropagation of *Solanum tuberosum* (Montenegro et al., 2014). Agar is one of the most used gelling agents in the preparation of culture media, but has high demand and cost in the market. However, alternatives such as starches and gums have reportedly been used to create economical and accessible media for the *in vitro* commercial propagation of plants (Jain et al., 2011; Romay et al., 2006; Mohamed et al., 2010). The alternative gelling agents used include cassava starch, wheat flour, semolina, potato starch, rice powder, sagu, and others (Romay et al., 2006, Mbanaso, 2008, Maliro et al., 2004, Prakash et al., 2004; Mohamed et al., 2010; Sharifi et al., 2010; Martin et al., 2013).

In the Andean zone, tubers such as potatoes (*Solanum tuberosum*), Olluco (*Ullucus tuberosus* Caldas), Oca (*Oxalis tuberosa* Molina), and others are of great importance for the diet and economy of local and native communities; therefore, it is a priority to develop technologies that favor their production (Lyon, 1992). The micropropagation of potatoes (Roca et al., 1978, Espinoza et al., 1984), olluco and oca (Estrada, 1993) allows the production of plants of high phytosanitary and genetic quality. However, the production of basic seeds of these and other native species is restricted in developing countries due to the limited availability of the commercial media produced by specialized laboratories in local markets, its high costs and the lenghty time to import.This paper reports the use of alternative inputs available in local markets for the preparation of culture media for micropropagation of the tuber crops: potato, olluco, and oca by nodal cuttings, using both semi-solid and liquid media in a temporary immersion system.

MATERIALS AND METHODS

Samples

This research was carried out in the Genetic Resources and Biotechnology Lab. (LRGB) of the School of Biological Sciences – Universidad Nacional Mayor de San Marcos, Lima-Peru. Three varieties of potato from two species (*S. tuberosum* and *S. goniocalix*), three morphotypes of olluco

(*Ullucus tuberosus* Caldas) and 4 of oca (*Oxalis tuberosa* Molina) from the *in vitro* germplasm bank of the LRGB, were evaluated. (Table 1).

Table 1. Varieties of potatoes and Ulluco and Oca tested.

Potato	Olluco	Oca
Mariva	UHY-051	OCU-772
Revolución	UHY-08	G
Amarilla (*S. goniocalix*)	UHY-041	B *H1*

The samples were obtained from *in vitro* plantlets previously cultivated in the Murashige and Skoog medium (1962) supplemented with 2% sucrose, 100 mg of myo-inositol, 2 mg of calcium pantothenate and 0.2% of gelrite®.

Culture media

In order to compare the variability of the components in the different basic media used for micropropagation, Table 2. Shows the composition of the different culture media evaluated. Murashigue and Skoog (1962) basic medium is used as a control; and the proposed medium is formulated with fertilizers for agricultural use and water-soluble vitamins B complex as a vitamin supplement. Three other media were also included: Linsmaier and Skoog (1965), White (1934) and B5 of Gamborg (1968) media.

Table 2. Culture media composition.

***2.A. MACROELEMENTS* (mg/L)**					
Culture media (mg/L)	**CONTROL**	**PROPOSED**	**COMPARATIVE MEDIA FORMULA (NOT EVALUATED)**		
	Murashige and Skoog (MS)	New formula (EP)	Linsmaier y Skoog (LS)	Gamborg (B5)	White
Ammonium nitrate (NH_4NO_3)	1.650.000	117.000	1.650.000	---------	--------
Calcium Chloride ($CaCl_2.2H_2O$)	440.000	22.000	332.200	150.000	--------
Calcium Nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$)	--------	--------	--------	--------	208.500
Ammonium sulfate $(NH_4)_2SO_4$	---------	--------	---------	134.000	--------
Magnesium Sulfate hepta hydrated ($MgSO_4.7H_2O$)	370.000	21.000	180.690	250.000	720.000
Potassium Nitrate (KNO_3)	1.900.000	100.000	1.900.000	2.500.000	80.000
Potassium phosphate monobasic (KH_2PO_4)	170.000	8.500	170.000	---------	--------
Potassium Chloride (KCl)	--------	--------	--------	--------	65.000
Sodium Phosphate Monohydrate ($NaH_2PO_4.H_2O$)	--------	--------	--------	150.000	--------
Sodium Phosphate Dihydrate ($NaH_2PO_4.2H_2O$)	--------	--------	--------	--------	18.700
Sodium Sulfate (Na_2SO_4)	--------	--------	--------	--------	200.000

2.B. MICROELEMENTS (mg/L)					
Culture media (mg/L)	**CONTROL**	**PROPOSED**	**COMPARATIVE MEDIA FORMULA (NOT EVALUATED)**		
Boric acid (H_3BO_3)	6.200	--------	6.200	3.000	15.000
Boro *	--------	0.00625	--------	--------	--------
Cobalt Chloride Hexadidrate ($CoCl_2.6H_2O$)	0.025	--------	0.025	0.025	--------
Copper Sulphate Pentahydrate ($CuSO_4.5H_2O$)	0.025	--------	0.025	0.025	0.001
Copper *	--------	0.01875	--------	--------	--------
EDTA (Ethylene-diaminetetraacetic acid, Disodium salt) dihydrate	37.250	--------	37.300	37.300	--------
Ferrous Sulfate heptahydrate ($FeSO_4.7H_2O$)	27.850	--------	27.800	27.000	--------
FeNaEDTA	--------	--------	--------	--------	4.590
Iron *	--------	0.05000	--------	--------	--------
Manganese Sulfate (Tetra Hydrated ($MnSO_4.4H_2O$)	22.300	--------	---------	---------	7.000
Mono Manganese Sulphate hydrated ($MnSO_4.H_2O$)	-------	--------	16.900	---------	--------
Manganese Sulfate ($MnSO_4$)	--------	--------	--------	10.000	--------
Manganese *	--------	0.05000	--------	--------	--------
Molybdic acid (sodium salt) di hydrated ($Na_2MoO_4.2H_2O$)	0.250	--------	0.213	0.250	--------
Molybdenum oxide (MoO_3)	--------	--------	--------	--------	0.0001
Molybdenum *	--------	0.00125	--------	--------	--------
Potassium Iodide (KI)	0.830	--------	830	0.750	0.750
Zinc Sulfate heptahydrate ($ZnSO_4.7H_2O$)	8.600	--------	8.600	2.000	3.000
Zinc *	--------	0.01875	--------	--------	--------

* Fertrilon Comb1 ® (Quelated)

2.C. VITAMINS (mg/l)					
Culture media (mg/L)	**CONTROL**	**PROPOSED**	**COMPARATIVE MEDIA FORMULA (NOT EVALUATED)**		
Myo-Inositol	100.000	--------	100.000	100.000	--------
Thiamin Hydrochloride	0.100	--------	0.400	10.000	0.100
Thiamin Hydrochloride * (B1)	--------	0.800	--------	--------	--------
Glycine	2.000	--------	--------	--------	3.000
Nicotinic acid	0.500	--------	--------	1.000	0.500
Nicotinic acid * (B3)	--------	4.000	--------	--------	--------
Pyridoxine HCl (B6)	0.500	--------	--------	1.000	0.100
Pyridoxine HCl * (B6)	--------	0.050	--------	--------	--------
Hemicálcic Salt of Pantothenic Acid *	--------	0.800	--------	--------	--------
Riboflavin * (B2)	--------	0.800	--------	--------	--------

* Complex B. Capsules from the Induquímica Laboratory

Culture media preparation

a. ***Selection of gelling agents.*** Preliminary tests included three gelling agents at different concentrations and physical chemical conditions: cornstarch (at 50, 65, 75 and 100 g/L); colapez (at 25, 50 and 100 g/L) and potato starch (potato chuño at 80, 90 and 100 g/L). Cornstarch at 65g/L was selected for this study due to its stability during the sterilization process and its low cost.

b. ***Preparation of Murashige and Skoog medium*.** Four stock solutions (A, B, C and D) were prepared for the basic Murashige and Skoog medium according to the protocol of Espinoza et al. (1984), adapted in the LRGB, as described below.
Stock B (x 100) is constituted by magnesium ($MgSO_4.7H_2O$), PM 246.5 (3.7gr was dissolved in 100 ml of distilled wáter). While in the case of Stock C (x 200) that is constituted by $FeSO_4.7H_2O$, PM 336.2, and Na_2EDTA, PM 278 (0.746 gr and 0.556 gr were weighed respectively). Each one was dissolved separately in 20 ml. of distilled water, then mixed and brought to a volume of 100ml with distilled water.

Once the preparation of the medium was done, the pH was brought to 5.6. In case of semi-solid medium 2.0 g of gelrite was added.

Table 3. Constituents of the *Stock A. Sales (x10) of the Murashige and Skoog media.

Reagent	MW	g/200mL of H_2O
KNO_3	101.1	38
NH_4NO_3	80.04	33
$CaCl_2.2H_2O$	147.0	8.80
KH_2PO_4	136.1	3.40
$MnSO_4.H_2O$	169.01	0.3380
$ZnSO_4.H_2O$	179.45	0.1228
H_3BO_3	61.83	0.1240
KI	166.01	0.0166
$Na_2Mo0_4.2H_2O$	241.95	0.0050
$CoCl_2.6H_2O$*	237.93	0.0005
$CuSO_4.5H_2O$*	249.7	0.0005

* Pre-stocks are prepared, dissolving 5 mg of each in 10 ml of distilled water. From this solution, an aliquot of 1 ml was taken for a final 200 ml solution. All dissolved reagents are mixed to complete 2000 ml of stock A.

Table 4. Constituents of * Stock D (x1000) Vitamins for a 50 mL solution.

Composition	Quantity (mg)
Thiamine HCl	0.1
Pyridoxine HCl	0.5
Nicotinic acid	0.5
Glycine	2.0

All stocks (A, B, C and D) were kept under refrigeration (5 ºC).

Table 5. Quantity of stock solutions for 1 liter of MS medium.

Stocks	Volume (ml)
*A. Salts	100
*B. Magnesium	10
*C. Iron	5
*D. Vitamins	1
Calcium Panthotenate	2
Sucrose	20 (g)
Myo-inositol	0.10 (g)

Table 6. Composition and necessary quantity required to prepare one liter of culture medium with alternative inputs (EP)

Stock	Composition	Quantity (g/L)
A	Ammonium nitrate	23.40
	Monopotassium	1.70
	phosphate	4.20
	Magnesium sulphate	20.00
	Potassium nitrate	
B	Calcium chloride	4.40
C	Fetrilon® combi	0.25
D	B Complex ® (Vitamins)	50 (ml)

c. ***Preparation of stock solutions (A, B, C and D) of the formulated medium (EP)***

Stock C (micronutrients) was prepared from Fertrilon Combi® foliar fertilizer and stock D (vitamins) from Complex B-capsules of Induquimica Laboratory S.A.; with the concentrations described in table 7.

The preparation of one liter of EP culture medium included: 5 ml of stock A; 5 ml of stock B, 5 ml of stock C, 20 ml of stock D and 20 g of sugar; in the case of semi-solid medium 65 g of cornstarch were added. The pH of the culture medium in all cases was 5.6 and the medium was sterilized at 121 ° C and 15 psi for 15 minutes.

Table 7. Composition of stock C in 1 liter of solution and D in 50 ml of solution, for the preparation of EP medium.

Stock C		Stock D	
Composition	**Quantity (mg)**	**Composition**	**Quantity (mg)**
Manganese	10	Thiamine monohidrate	2
iron	10	Rivoflavin	2
Copper	3.75	Nicotinamide	10
Zinc	3.75	Pyridoxine hydrochloride	0.125
Boron	1.25	Calcium pantothenate	2
Molybdenum	0.25		

Micropropagation of plants in semi-solid and liquid médium

The explants of olluco, oca and potato were seeded in semi-solid medium in test tubes containing 10 ml of EP or control media. In the semi-solid medium, 2 nodal cuttings were planted per tuve, while in the liquid medium 2 explants of 5 nodal cuttings were planted in each sterilizable plastic containers. The conditions of the growth chamber were 18 ±2 °C and 16 light hours. The formation of nodal cuttings, roots, growth and vigor of the seedlings was evaluated at 7, 14, 21, 28 and 35 days after sowing.

Micropropagation by using temporary immersion system

We used a comercial label (RITA®) for the temporary immersion system. 30 mL of the media to be compared (MS and EP) was added to each vessel respectively. The media were sterilized by autoclave at 121 °C and 15 psi for 15 minutes. Five explants with 4 nodes each, were planted in a bottle in a laminar flow chamber each one of olluco, oca and potato; on a polyurethane sponge disk of each container. Immersion cycles of 15 minutes were programmed every 6 hours. The parameters evaluated in the three crops (olluco, oca and potato) were the number of nodes, root, growth and vigor of the seedlings until 35 days of cultivation.

The results were subjected to statistical analysis with a completely randomized design. The analysis of variance was performed using the F test. The quantitative variables were the number of nodes, leaves and root formation, growth and vigor. These results were evaluated using the "Limit Difference test of Significance" with an allowable error of 5%, in order to determine if the difference is significant in each of the media tested. Twenty explants were used for each treatment. The data were analyzed through ANOVA, and the mean values of the treatments were compared with the Turkey test (P = 0.05).

RESULTS AND DISCUSSION

Alternative gelling agents in the preparation of the EP medium.

The success of the micropropagation depends on the components of the culture medium and the gelling agent used. The best response in micropropagation of olluco, oca and potato was obtained when using cornstarch at 65 g/L as a gelling agent. Chirinos et al. (1998) evaluated three gelling agents (sweet potato flour, chuño and cornstarch) at different concentrations, finding that in 15 potato varieties the best growth response was obtained by using sweet potato flour at 9% when compared to phytagel. Studies on gelling agents for the preparation of culture media are very extensive. Zsabados et al. (1993) used different gelling agents in the culture of callus and cellular suspensions of *Stylosanthes* Sp, meristem culture and micropropagation of *Manihot* and also in the culture of rice anthers; concluding that the best gelling agents were agarose, gelrite and agar. However, these products are chemically pure and significantly increase the value of the seedlings produced by tissue culture.

Cornstarch as a gelling agent shows stability to variations in temperature and pH in the culture medium. Dufour et al. (2000) indicate that this starch support acidic pH, autoclaving temperatura, and cooling. It is likely that the functions of starch under *in vitro* conditions go beyond that of being a gelling agent, and are nutritional and osmotic in nature as pointed out by Chirinos et al. (1998). Romay et al. (2006) evaluated modified starch in the micropropagation of cassava, concluding that there were no significant differences in the growth and multiplication rate of plants grown in either media gelled with Phytagel® or with starch. Similarly, Kuria et al. (2008) used the gelled medium with cassava starch mixed with agar for the micropropagation of *Solanum tuberosum* L., achieving better results in nodes and biomass. Furthermore, the use of 10% cassava starch reduced the cost by 42.5% compared to the use of agar alone.

Martín et al. (2013) point out that the partial substitution of 40% of agar by weight with potato starch as gelling agent does not have the same solidifying characteristics. However, this does not alter the development of the explants in the micropropagation of *Solanum quitoense*. Mohamed et al. (2010) determined that the mixtures of agar with potato and corn starch was an alternative gelling agent for micropropagation of *Solanum tuberosum*. Mengesha et al. (2012) studied the starch efficiency of *Ensete ventricosum* in the micropropagation of *Vanilla planifolia*, achieving a new alternative of gelling agent. Similarly, Jain et al., (2011) used mixtures of gelling agents in the micropropagation of *Albizia lebbeck*.

Micropropagation in semi-solid medium

The results obtained in the micropropagation of oca, olluco and potato after 35 days of culture show no significant difference between the control medium (MS) and the medium with alternative inputs (EP - Table 2.) in the nodal cuttings growth, (Figure 1) in the formation of roots; as well as in the growth of olluco, oca and potato seedlings up to 35 days of culture. In the case of the three varieties of potatoes (mariva, amarilla and revolucion) they reached an average growth of 10 cm at 28 days of cultivation; while Ruiz (1990) reported growths of 5.5 and 5.6 cm in the potato varieties "Desiree" and "Tomasa Condemayta" after 22 days of cultivation. Our results show up to 14 nodes per seedling at 28 days, while Ruiz (1990) reported achieving 5 nodes at 22 days.

Although the Murashige and Skoog medium is used for a group of ornamental herbaceous plants of commercial interest, there is little work on the use of alternative inputs; as is the case of Chirinos et al. (1998) and Ruiz (1990). Le et al. (1999) found favorable results in the production of potato microtubers using hydroponic solutions; while Rigato et al. (2016) reported the efficient multiplication of potato seedlings using micropropagation techniques in a system similar to those used in hydroponics.

Micropropagation in liquid medium

Initially, no significant differences were found between the EP and MS liquid media for the three crops tested; as shown in Figure 2 and Figures 3 and 4. However, there are significant differences both in the development of the seedlings of the different clones and varieties, along with the development of the seedlings for all crops as time passes. The potato seedlings that were planted in the EP medium achieved a growth of up to 9 cm. at 35 days, which coincides with the work of Ruiz (1990), who reports a growth of up to 7.8 cm 35 days after sowing 5 potato varieties using fertilizers.

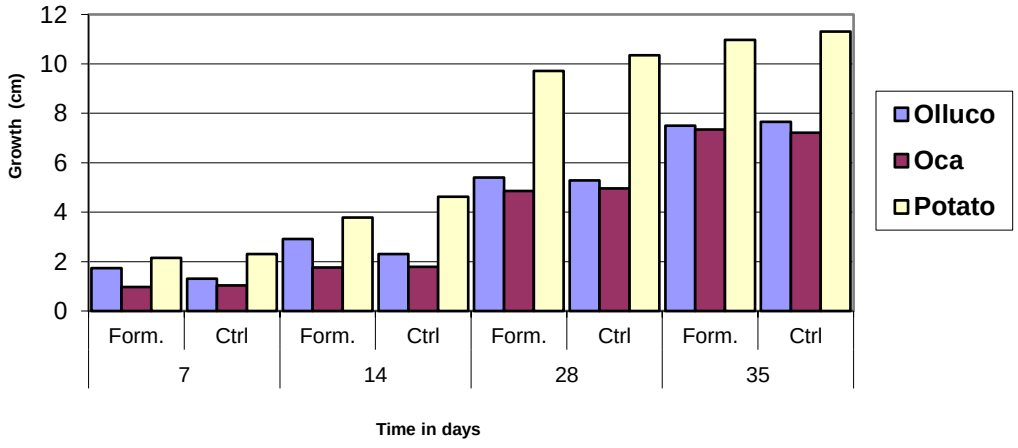

Figure 1. Olluco, oca and potato seedling size in semisolid medium (EP) and control after 7, 14, 28 and 35 days.

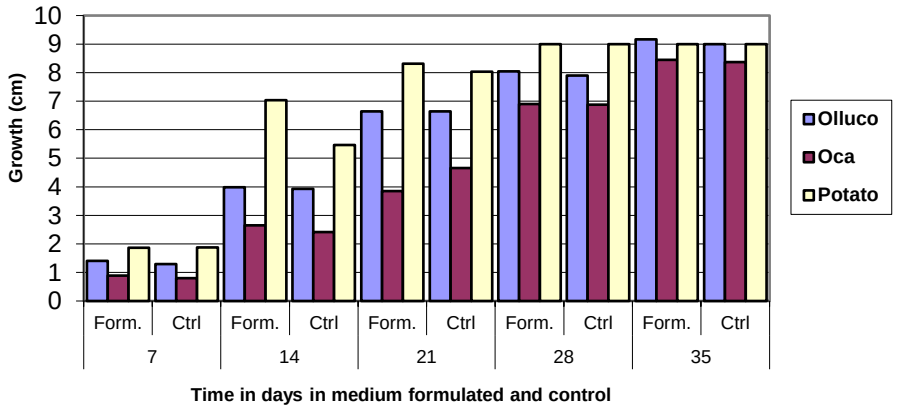

Figure 2. Size of olluco, oca and potato seedlings in liquid EP medium and control at 7, 14, 21, 28 and 35 days.

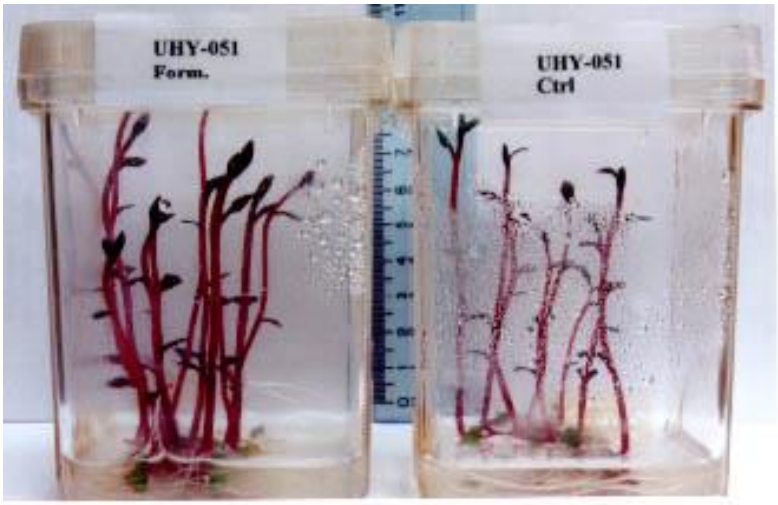

Figure 3. Growth of olluco seedlings (UHY-051) in EP medium (left) and control-MS (right) after 28 days of planting.

Olluco, oca and potato seedlings developed vigorously in both the EP and control media; with formation of leaves, nodes and roots (figure 5). The culture in liquid medium in agitation allows the efficient absorption of nutrients by the explants under study, while adding the advantage of significantly reducing the cost of micropropagation. These results are similar to those of Del Avila et al. (1996) who indicate that the liquid medium increased the growth of the potato sprouts without affecting the number of nodes; moreover, the higher growth rate of the sprouts was correlated with increases in their organic content of nitrogen and sugar. Alcaraz-Meléndez et al. (2002) mention that the culture in liquid medium of *Turnera diffusa* facilitates the absorption of nutrients by the tissues while decreasing the time required for the development of the explants.

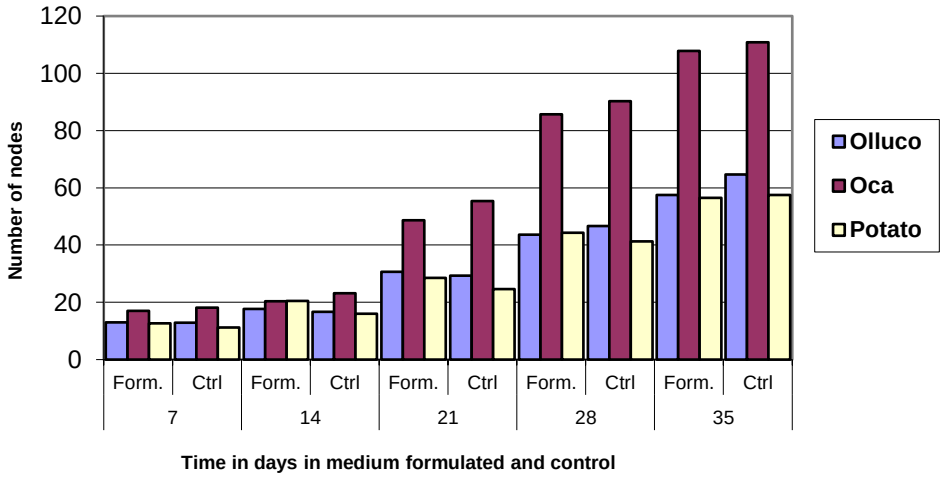

Figure 5. Olluco, oca and potato node formation in liquid medium (EP) and control at 7, 14, 21, 28 and 35 days.

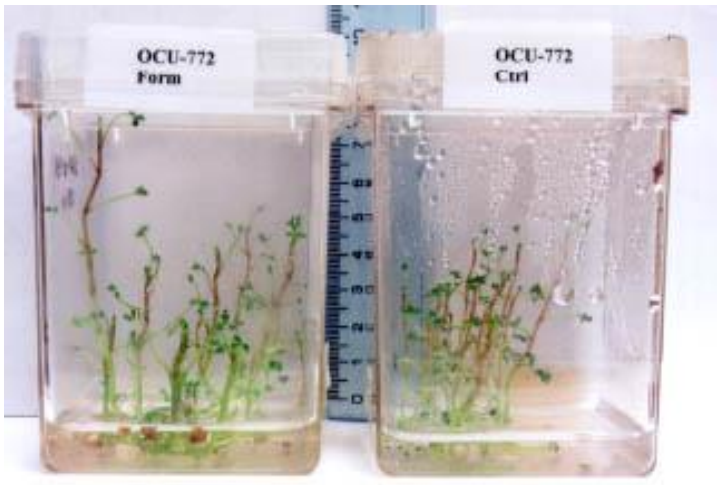

Figure 4. Growth of oca seedlings (OCU-772) in EP medium (left) and control - MS (right) after 28 days of planting.

Multiplication in temporary immersion system (RITA®)

Research to increase efficiency in *in vitro* production is constant. We look for development of practical, economical and simple methodologies; easy to implement in any laboratory. Alvard et al. (1993) studied five different methods of cultivation in liquid medium compared to a solid one for the propagation of banana meristems. From this and other investigations, a new system of *in vitro* culture in liquid médium emerged, known as temporary immersion system (SIT)

Using the vessels of the RITA® system containing the EP medium, with dives of 15 minutes every 6 hours, up to 116 buds were obtained in olluco, 324 in oca and 128 in potatoes after 35 days of incubation. In contrast, the vials of the system SIT with the control medium produced 118 nodal cuttings in olluco, 313 in oca and 142 in potato respectively (Figure 6 and Figure 7). Statistically, there is no significant difference between the EP medium and control in the development of olluco, oca and potato seedlings in the SIT. However, there was a significant difference between the clones sown in the buds formation in all three crops, while in the case of potatoes there was a significant difference in both the formation of buds and of nodal cuttings, as well as the size of the buds and seedlings.

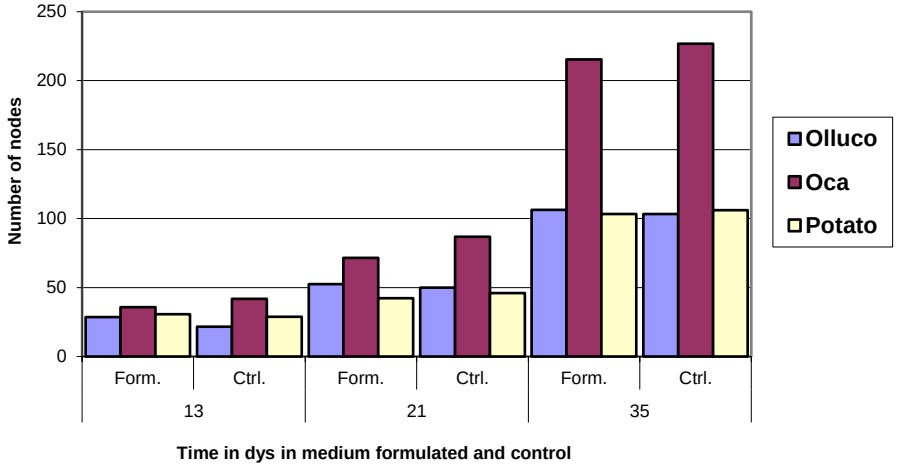

Figure 6. Olluco, oca and potato node formation in SIT system with EP medium and control at 13, 21 and 35 days.

Figure 7. Growth of the Oca and olluco seedlings in EP medium using the temporary immersion system (RITA®) after 35 days of planting.

Seedlings developed vigorously with a high rate of nodes formation, probably due to the increased gas exchange and nutrient availability of the culture medium in the SIT. Quiala et al. (2012) obtained high numbers of shoots of *Tectona grandis* L. when the explants grew in the SIT with MS medium containing BA. González et al. (2013) stated that there is a better morphological response in the temporary immersion system due to factors such as water relations, gaseous exchange and the increase in nutrient intake. Igarza et al. (2011) achieved the highest number of potato microtubers with an immersion frequency of every four hours. González et al. (2013) point out that the number of shoots per explant, the length of the shoots, and the number of leaves per shoot were significantly higher in the explants immersed in the culture medium for one minute.

The SIT allowed to obtain vigorous seedlings with greater number of shoots in oca, olluco and potato, compared with production systems using conventional liquid or semi-solid medium. The SIT is successfully used in the micropropagation of different species of economic interest, such as in the intensive production of potato microtubers (Jimenez et al., 1999); for the *in vitro* propagation of pineapple (Escalona et al., 1999); banana meristems (Alvard et al., 1993), to improve the efficiency of the *in vitro* propagation process of sugarcane (Montes de Oca, 2010); the production of *in vitro* plants and yam microtubers (Jova, 2009), among others.

In addition, the SIT has advantages over cultures in semi-solid media; overcoming some of the most

frequent problems of the culture in liquid medium such as asphyxia and vitrification, improving nutrition and gas exchange. Supplying culture media in short periods of time promotes the seedlings to be autotrophic. Compared to the conventional system, the SIT produce larger amounts of seedlings, it is automatable, not very laborious and improves production at a low cost. In this regard Vegas et al. (2015) mention that the development of the SIT has allowed the optimization of the multiplication of plant species and the propagation at commercial scale. By reducing operating costs through the use of liquid media and better handling, the time for initial establishment of *in vitro* explants is decreased and multiplication rates are increased. In comparison with the conventional forms of *in vitro* propagation in semi-solid media or semi-submerged liquid, SIT yields plants with greater vigor. In addition, Jiménez et al. (1999) mention that this system showed several advantages in comparison with solid cultures; increasing three times the length of the sprout, giving more internodes per plant and improving vigor in the potato.

Cost of the culture medium with alternative inputs (EP)

Micropropagation is a technique that allows large quantities of selected plants in a short time. However, to produce seedlings for commercial purposes, the technique must be simple, maintaining the quality of the seedlings and reducing production costs. In the present work, a significant difference in the cost of one liter of the EP culture medium compared to the control was determined. Using the alternative inputs for the preparation of the formulated medium, the cost was reduced significantly for the micropropagation of olluco, oca and potato.

Table 8. Cost analysis (US $) of the constituents of the Murashige and Skoog medium (1962) for one liter of culture medium. The prices were quoted in the national market and include all the corresponding taxes.

Description	Quantity per bottle (g)	Unit cost per bottle	Cost for gram	Quantity neccesary in g/L of medium	Cost (Liter of medium)
Potassium Nitrate	500	278.00	0.55600	1.9	1.05640
Ammonium nitrate	500	220.87	0.44174	1.65	0.728871
Calcium chloride dihydrate	500	79.06	0.15812	0.44	0.069573
Potassium phosphate monobasic	100	61.95	0.61950	0.17	0.105315
Manganese Sulfate	100	62.00	0.62000	0.0223	0.013826
Sulfato de zinc	100	85.20	0.85200	0.0086	0.007327
Boric acid	500	95.88	0.19176	0.0063	0.001208
Potassium iodide	100	98.23	0.98230	0.00083	0.000815
Sodium molybdate	100	110.00	1.10000	0.00025	0.000275
Cobalt sulfate	100	72.27	0.72270	0.000025	1.81E-05
Copper sulphate	250	84.66	0.33864	0.000025	8.47E-06
Magnesium sulphate	500	189.76	0.37952	0.37	0.140422
Na_2EDTA	100	83.48	0.83480	0.0373	0.031138
Iron sulphate	250	63.24	0.25296	0.0278	0.007032
Thiamine hydrochloride	100	112.39	1.12390	0.0001	0.000112
Pyridoxine hydrochloride	100	168.74	1.68740	0.0005	0.000844
Nicotinic acid	100	58.50	0.58500	0.0005	0.000293
Glycine	100	71.86	0.71860	0.002	0.001437
Myo inositol	100	88.90	0.88900	0.0001	8.89E-05
Calcium pantothenate	100	170.50	1.70500	0.002	0.00341
Sucrose	1000	261.30	0.26130	20	5.22600
Gelrite	1000	474.20	0.47420	2	0.94840
				Cost of 1 liter of culture medium (US $)	**8.34271**

Table 9. Cost analysis (US $) of the constituents of the EP for one liter of culture medium. The prices were quoted in the national market and include all the corresponding taxes.

Description	Quantity per bottle (g)	Unit cost per bottle	Cost for gram	Quantity neccesary in gram/ liter of medium	Cost (Liter of medium)
Monopotassium phosphate	1000	2.81	0.00281	0.17	0.000478
Ammonium nitrate	5000	2.55	0.00051	2.34	0.001193
Magnesium sulphate	1000	0.89	0.00089	0.42	0.000374
Potassium nitrate	1000	1.30	0.00130	2	0.002600
Calcium chloride	5000	5.90	0.00118	0.44	0.000519
Fetrilom combi®	0.250	7.80	0.03120	0.025	0.000780
Complex B	Capsule	0.50	0.50000	1	0.500000
Sugar	1000	0.71	0.00071	20	0.014200
Cornstarch	1000	0.90	0.00090	65	0.058500
				Cost of 1 liter of culture medium (US $)	**0.578644**

Many researchers agree that the cost of the culture medium involves approximately 20% of the total cost of production. The results obtained show that the use of alternative inputs of the EP medium, available in local markets and in the evaluated systems, allows for high propagation rate, plant vigor and a significant reduction in production costs. Ogero et al. (2012) mention that they developed a low-cost medium for the regeneration of sweet potato in order to increase its production not only in sub-Saharan Africa, but also throughout the world. The low-cost developed medium can be used to increase production of affordable and disease-free sweet potato seedlings. Similarly, Kodyn et al. (2001) point out that by using natural light and alternative consumables, the costs of banana micropropagation decreased up to 90%.

CONCLUSION

The components of the Murashige and Skoog medium (1962) were replaced by low cost alternative inputs available in the local markets of Lima, Peru to evaluate the *in vitro* behavior of the crops: Oca, Olluco and potato. Our results showed no significant differences in the number of nodes, roots or vigor between the seedlings that grew in either media (EP and MS as control). However, the cost analysis of both media shows us that the production costs of medium EP (low cost inputs) is significantly lower; which ultimately allows for low-income farmers to access this technology and increase their income.

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Compliance with ethical standards. It has been carried out following the bioethical principles and clauses, applicable to crops; which are of wide use in the Andean region.

Conflict of interest. The authors declare no conflict of interest including academic institutions from which they are part, nor any other public or private institution.

Data availability. Data is available from Gilmar Peña Rojas (email: gilmar_p@yahoo.com), upon reasonable request.

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