USE OF RITA® TEMPORARY IMMERSION SYSTEM TO OBTAIN MICROTUBERS OF SEVERAL MASHUA (*Tropaeolum tuberosum* Ruiz & Pavón) MORPHOTYPES †

[EMPLEO DE SISTEMAS DE INMERSIÓN TEMPORAL RITA® PARA LA OBTENCIÓN DE MICROTUBÉRCULOS DE VARIOS MORFOTIPOS DE MASHUA (*Tropaeolum tuberosum* Ruiz & Pavón)]

Gilmar Peña-Rojas*[1], Roxana Carhuaz-Condori[1], Vidalina Andía-Ayme[2] and Juan Z. Dávalos-Prado[3]

[1]*Laboratorio de Biología Celular y Molecular. Universidad Nacional de San Cristóbal de Huamanga, Ayacucho. Ciudad Universitaria 05001. Ayacucho – Perú. Email: gilmar_p@yahoo.com*

[2]*Laboratorio de Microbiología de Alimentos. Universidad Nacional de San Cristóbal de Huamanga, Ayacucho. Ciudad Universitaria 05001. Ayacucho – Perú.*

[3]*Instituto de Química-Física "Rocasolano"- CSIC, c/Serrano 119, 28006, Madrid, España.*

** Corresponding author*

SUMMARY

Background. The Temporary Immersion System (TIS) is an effective strategy to obtain quality mashua microtubers through the *in vitro* propagation of seedlings in a small space and in a short period of time. **Objective.** Obtain microtubers of 27 mashua morphotypes (*Tropaeolum tuberosum*) using the TIS-RITA®. **Methodology.** The seedlings were previously propagated in MS (Murashige & Skoog) solid medium, supplemented with 3% sucrose, at 5.6 pH and 0.7% agar. After fifteen days of cultivation in this medium, the seedlings were transferred to a MS liquid. For the tuberization process, seedlings with an average size of 10 cm were transferred to TIS-RITA® systems for ten weeks, under conditions of complete darkness, at 19 ± 2 ° C, an immersion frequency of 2 minutes every 3 hours in a MS liquid medium supplemented with 8% sucrose and 2 mg.L^{-1} benzylamino purine (BAP). **Results.** The best results of microtubers were obtained with different morphotypes, which are respect to i/size, MAC 06A (1.9 cm) and MAC 056 (1.8 cm); ii / fresh weight, MAC 06A (0.2g) and MAC 092 (0.18 g); iii / quantity, MAC 08A (51 units) and MAC 048 (56 units). **Implications**. TIS-RITA® can be used to obtain quality and virus-free seeds of a wide variety of commercially important crops. **Conclusion**. The SIT-RITA® based on the intermittent contact of the explants cultivating medium, is an efficient system to obtain mashua microtubers.

Keywords: Microtubers; TIS temporary immersion system; mashua; Tropaeolum tuberosum; in vitro propagation.

RESUMEN

Antecedentes. El sistema de inmersión temporal (SIT) es una estrategia eficaz para la obtención de microtubérculos de mashua de calidad mediante la propagación *in vitro* de plántulas en un espacio reducido y en corto periodo de tiempo. **Objetivo.** Obtener microtubérculos de 27 morfotipos de mashua (*Tropaeolum tuberosum*) utilizando el SIT- RITA®. **Metodología**. Las plántulas fueron previamente propagadas en medio MS (Murashige & Skoog) suplementado con 3% de sacarosa, pH 5.6 y 0.7% de agar. Transcurridos 30 días de cultivo en MS semisólido, las plántulas fueron transferidas a un MS líquido. Para el proceso de tuberización, plántulas con tamaño promedio de 10 cm fueron transferidas a SIT-RITA® durante 10 semanas bajo condiciones de completa oscuridad, a 19 ± 2 °C, una frecuencia de inmersión de 2 minutos cada 3 horas en un medio MS líquido suplementado con 8% de sacarosa y 2 mg.L^{-1} de benzilamino purina (BAP). **Resultados.** Los mejores resultados de microtubérculos se obtuvieron con algunos morfotipos; así respecto, i/ del tamaño con MAC 06A (1.9 cm) y MAC 056 (1.8 cm); ii/ del peso en fresco con MAC 06A (0.2g) y MAC 092 (0.18 g); iii/ de la cantidad con MAC 08A (51 unidades) y MAC 048 (56 unidades). **Implicaciones.** SIT-RITA® puede

† *Submitted June 22, 2020 – Accepted July 19, 2020.*
ISSN: 1870-0462.

ser empleado para la obtención de semilla de calidad y libre de virus de una amplia variedad de cultivos comercialmente importantes. **Conclusión**. SIT-RITA® basado en el contacto intermitente del medio de cultivo de explantes, es un sistema eficiente para obtener microtubérculos de mashua.
Palabras claves: Microtubérculos; sistema de inmersión temporal SIT; mashua; *Tropaeolum tuberosum*; propagación *in vitro*.

INTRODUCTION

The mashua (*Tropaeolum tuberosum*) is an ancestral crop tuber, domesticated by native pre-Inca peoples (Valle *et al*., 2018) and considered as a source of genetic reserve and potential functional food worldwide (Campos *et al*., 2006). Originally from the Andean region of the South American continent (León, 1964, Guevara *et al*., 2018, Manrique *et al*., 2013), it is grown mainly in Peru, Bolivia, Ecuador, Venezuela and Colombia (Grau *et al*., 2003; Pissard *et al*., 2008) and currently extended to countries such as Argentina, Chile, New Zealand, Canada, United States and United Kingdom (National Research Council, 1989, Guevara *et al.*, 2018). The cultivation of mashua is considered the fourth most important of Andean tubers, after potatoes, oca and olluco (Pissard *et al.*, 2008). The greatest diversity of mashua occurs between the central regions of Perú and Bolivia at altitudes between 3 500 and 3 800 masl; it has the peculiarity of growing in poor soils with little use of fertilizers and/or pesticides (Arbizu *et al.*, 1986, Ortega *et al.*, 2007, Manrique *et al.*, 2013). These conditions would favor in mashua the presence of a wide variety of bioactive compounds (glucosinolates, polyphenols, anthocyanins, carotenoids, flavonoids, etc.) with multiple nutritional, ecological, pharmaceutical, antibiotic and therapeutic benefits (Grau *et al.*, 2003; Chirinos *et al*., 2007). One of these evidences is *e.g*. the reduction of benign prostatic hyperplasia (Aire *et al.*, 2013). It is important to mention that glucosinolates, by the action of the enzyme mirosine, are converted into isothiocyanates, sulfurans, nitriles, and thiocyanates with antibiotic, insecticidal, nematicidal, anticancer, neuroprotective and diuretic properties (Chirinos *et al*., 2007; Manrique *et al.*, 2013).

The mashua traditional cultivation for productive purposes is not precisely the most effective, partly because virus infection in propagation tubers is not avoided (Grau *et al*., 2003, Guimarães *et al*., 2005). Biotechnological methods for the production of quality seed, with the appropriate use of strategic inputs, allow improving their productivity and profitability (Doria, 2010). In this context, the temporary immersion system (TIS) is one of the fastest and most efficient propagation strategies that allows obtaining quality microtubers in a controlled space, in a short period of time, at any period of the year, free of pathogens and with the possibility of automation (Igarza *et al*., 2012). In this context, the TIS is an important strategy for the production of microtubers which constitutes the base material of a program of production of seed of certified quality, free of pathogens (Salauces *et al.*, 1998) when they are obtained under aseptic and controlled conditions. The microtubers thus produced are transferred to the greenhouses to obtain minitubers, which in turn are used for seed production (Igarza *et al*., 2012).

TIS is determinant in the micropropagation of outbreaks, microtuberization and somatic embryogenesis. In this process, the frequency is one of the most important parameters for the effectiveness of the system (Etienne *et al*., 2002). The growth and development of the seedlings can be controlled by modifying the frequency and duration of the immersion (Jäger *et al.*, 1993; Cabrera *et al*., 2011). The operation of the TIS consists to apply a flow of air under pressure that allows to raise the liquid *medium* of the crop to put in contact with the explants for intermittent times. Once the injection of air is suppressed, the medium will descend by gravity. The process generates changes in the internal atmosphere of the system facilitating greater growth and development of the seedlings. In addition, this process reduces the problems of hyperhydration which are frequent in growing systems in liquid media (Maldonado et al., 2003, Escalona et al., 1999, Tirado et al., 2005). The TIS-RITA® is a bioreactor TIS, with automation possibility, whose principle of operation is the alternation of immersion periods of the seedlings. It is currently used for example in the production of herbaceous and forest species (Werner *et al.*,., 2018, Jiménez *et al.*,., 1999, Montoya *et al.*,. 2008). The use of liquid media in the automated TIS-RITA® is an effective tool for micropropagation since it increases the multiplication coefficient, improves the quality of the *in vitro* material regenerated and achieves the strength of the seedlings (Alvarenga *et al.*,. 2015; Mehrotra *et al.*,. 2007; Ziv, 2005).

Finally, it is worth mentioning the importance of the genotype in the production of microtubers. In this regard, Rivera A, *et al*. (2008) find that the *in vitro* microtuberization of Colombian potatoes is

determined, among other variables, by the genotype and the different concentrations of hormones benzilaminoapurina (BAP) and chlorine chloride choline (CCC).

MATERIALS AND METHODS

The study has been carried out in the Cellular and Molecular Biology Laboratory of Faculty of Biological Sciences of the Universidad Nacional San Cristóbal de Huamanga (UNSCH), Ayacucho (13° 08'43 "S 74° 13'16"W, 2790 masl). It has been considered twenty-seven mashua morphotypes coming from Peruvian regions such as Ayacucho and Apurímac (Table 1). The growing conditions by micropropagation of the seedlings and their tuberization, under TIS-RITA® systems, were standardized and optimized conveniently.

Table 1. Indicative data (passport) of the diversity of 27 mashua (*Tropaeolum tuberosum* Ruiz & Pavón) morphotypes.

Morphotype	Region	Province	District	Location	Altitude/ m	S.E. Lat.	S.W. Long.
MAC 001	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC002	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 003	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 006A	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 007	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 008A	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 008B	Ayacucho	Huamanga	Vinchos	Yaruca	3 739	555290.51	8524274.99
MAC 010	Ayacucho	Huamanga	Vinchos	Yaruca	3 739	555290.51	8524274.99
MAC 011	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 013	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 017	Ayacucho	Cangallo	Morochucos	Condorccocha	3 609	586371.04	8513193.58
MAC 019	Ayacucho	Huanta	Uchuraccay	Iquicha	3 802	601807.25	8582772.00
MAC 042	Ayacucho	Huanta	Uchuraccay	Iquicha	3 802	601807.25	8582772.00
MAC 048	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 051	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 056	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 057	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 058	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 066	Ayacucho	Cangallo	Morochucos	Codorccocha	3 609	586371.04	8513193.58
MAC 080	Apurímac	Andahuaylas	Huayana	Patahuasi	3 868	657128.19	8451202.10
MAC 083	Apurímac	Andahuaylas	Uripa	Uripa	4 060	646838.51	8500799.01
MAC 084	Apurímac	Andahuaylas	Uripa	Uripa	4 060	646838.51	8500799.01
MAC 090	Apurímac	Andahuaylas	Uripa	Uripa	4 060	646838.51	8500799.01
MAC 092	Ayacucho	Huamanga	Acocro	Pumapuquio	3 680	601865.03	8530546.20
MAC 094	Ayacucho	Huamanga	Acocro	Pumapuquio	3 680	601865.03	8530546.20
MAC 098	Ayacucho	Cangallo	Morochucos	Condorccocha	3 610	586371.04	8513193.58
MAC 120	Ayacucho	La Mar	Chiquintirca	Osccoccocha	3 669	634555.00	8558368.76

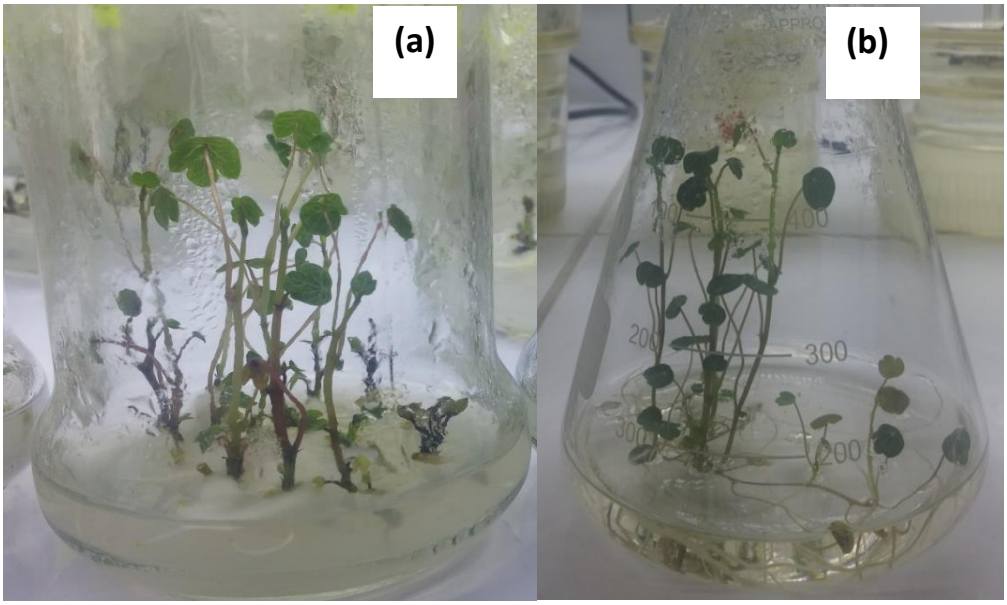

Figure 1. *In vitro* propagation of mashua, in (a) solid and (b) liquid *medium.*

***In vitro* propagation of mashua seedlings**
(Figure 1)

As source of the explants, it has been used *in vitro* sprouts of mashua twenty-seven morphotypes from the Germplasm Bank (Laboratory of Cellular and Molecular Biology) of the UNSCH. Seedlings were obtained by micropropagation in flasks containing 100 mL of MS (Murashige & Skoog 1962) solid *medium*, supplemented with 3% sucrose, at 5.6 pH, gelled with 0.7% agar. Optimum growing conditions were found maintaining the system at 19 ± 2 ° C; 16 hours of light, 8 hours of darkness, and relative humidity between 60 and 70% during the multiplication phase. After 15 days of growing, the seedlings were transferred to a liquid *medium* in constant agitation to strengthen them.

Tuberization in the temporary immersion system (TIS -RITA)

RITA® containers were used in the TIS tuberization process, according to the one described by Etienne *et al.* (2002). In these containers have been prepared MS liquid cultivation media supplemented with 2 $mg.L^{-1}$ benzylamino purine and 8% sucrose, at 5.6 pH. The system was sterilized at 121 °C for 15 minutes under 15 pounds of pressure. Subsequently these devices received 20 seedlings with average size of 10 cm from the micropropagation flasks in liquid *medium* (Figure 2a). The most effective TIS-RITA® process involves an immersion frequency of 2 minutes every 3 hours for 10 weeks of treatment, under 19 ± 2 ° C in complete darkness (protocol previously standardized in other research). In order to harvest the microtubers (Figure 2b), the containers were opened and carefully separated from the residues of plants and roots. Then, the microtubers were harvested from the cultivation flasks and then rinsed with drinking water, to remove the culture medium, and placed in trays on filter paper to remove moisture. Measurements of weight (in g) and size (in cm) of each fresh microtuber, were done using an analytical balance and Vernier caliper, respectively.

Data analysis

The data obtained from completely randomized statistical design, with two replications, were performed with the Kruskal Wallis test and processed with the InfoStat statistical program version 2018.

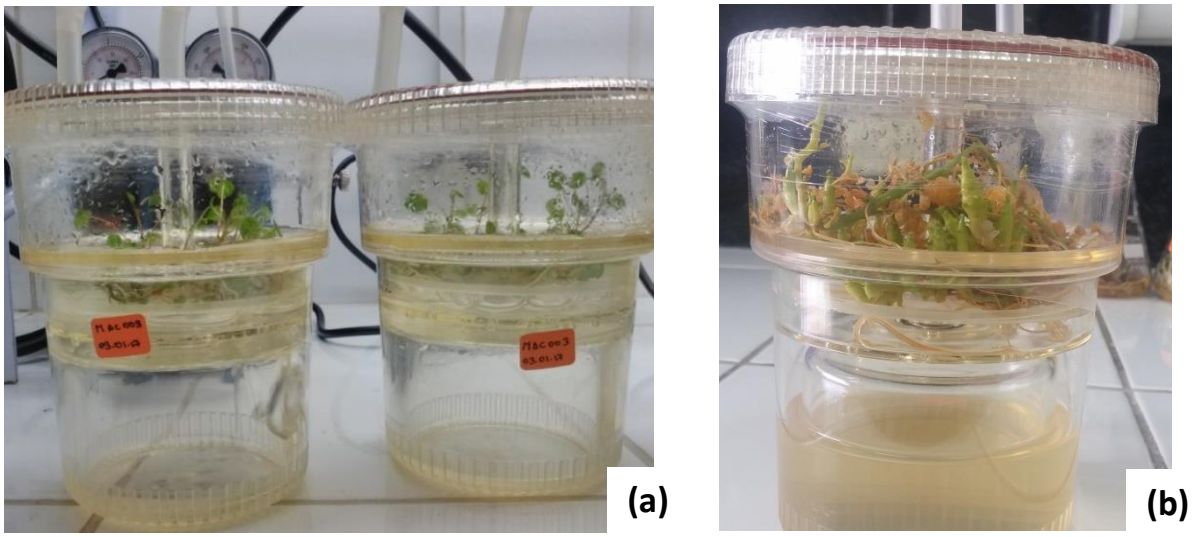

Figure 2. (a) SIT RITA® tuberization systems and (b) microtubers obtained.

RESULTS AND DISCUSSIONS

Size of mashua microtubers

Previous standardization and optimization of both seedling-cultivation and tuberization-process (described in the before section), microtubers have been obtained with an average of 1.0 cm of the size of the total of mashua morphotypes considered (Figure 3). The best results corresponded to the MAC 06A and MAC 056 morphotypes with sizes of 1.9 and 1.8 cm respectively (see Figure 4). However, MAC 19 is one of the morphotypes that formed the smallest size and weight of the microtubers studied. This result would be due to a genotypic response of the morphotype in the tuberization process, such as mentioned by Morales-Fernández *et al.* (2011) in obtaining potato minitubers. It is important to highlight the adjustment of parameters such as dive times and immersion frequencies in TIS. In this regard, Pérez *et al.* (2011) mentioned that the cultivation medium, immersion frequency and number of explants have a significant influence on the production of *Musa* spp; Montoya *et al*. (2008) achieved the largest quantity and size of *Solanum tuberosum* shoots with immersion frequencies of three hours; Escalona *et al.* (1999) in the pineapple (*Ananas commosus* (L) Merr) cultivation reported that with a time of immersion of two minutes and a frequency of three hours increased the multiplication coefficient of seedlings and also facilitated greater efficiency in the assimilation of nutrients by the explants; Igarza *et. al*. (2011) obtained on average between five and seven microtubers of 0.4 and 1.6 cm of potato of the "Andinita" variety; Cabrera *et al.* (2011) obtained the best results in the formation of *Discorea alata* L microtubers using an immersion time of 15 minutes after 18 weeks; Aguilar *et al.* (2016) achieved between 1.04 and 1.17 cm microtuber sizes of cultivated potato "Burren" and mentioned that the induction and development of these can be influenced by the concentration of sucrose in the cultivation medium, temperature, photoperiod, intensity of the light, formulation of the cultivation medium and the genotype of the potato.

On the other hand, when 8% sucrose is used in the MS medium, microtubers are obtained for all the mashua morphotypes studied. In this regard, Montoya *et al.* (2008) using this concentration of sucrose in MS-SIT, achieved a greater number and weight of *Solanum tuberosum* L microtubers, Diacol Capiro variety. Khuri *et al.* (1995) reported that sucrose plays an important role *in vitro* tuberization process as a carbon source for the absorption of seedlings, and the use of 8% sucrose provides a favorable osmolarity in the development of the *Solanum tuberosum* microtubers. Dodds *et al*. (1992) using the same concentration of sucrose in MS also reported good results in obtaining *Solanum tuberosum* microtubers. García *et al.* (2004) also highlight the importance of sucrose in the development of microtubers as a carbon source easily assimilated by seedlings that becomes starch during the development of microtubers. These

authors reported that the use of sucrose concentrations different to 8% slows down the *in vitro* tuberization process of *Dioscorea alata* L, in addition to obtaining few and small microtubers.

Figure 3. Typical microtubers of mashua obtained in this work.

Weight of microtubers

In respect of weight of microtubers, 0.10 g of the average fresh weight of the total morphotypes studied was obtained. The MAC 06A and MAC 092 morphotypes present the best results with average weights of 0.2 and 0.18 g respectively (Figure 5). It is also important to mention the importance of the use of TIS systems for the optimization of fresh weight and quantity of microtubers. In this regard, working with *Solanum tuberosum* L potatoes, Higgins *et al.* (2017) obtained microtubers with fresh weight of 19.8 g, length of 8.1 cm; Akita *et al*. (1994) described the procedure to obtain microtubers containing approximately 18% (w/w) dry matter and a weight of more than 0.2 g; Pérez *et al.* (2011) achieved an average of 2.6 tubers/plant *in vitro* with a diameter of 11.4 mm and fresh-weight averages of 1.27g; Igarza *et al.* (2014) achieved an average 8.5 to 9.5 microtubers/plant and fresh weight less than 3.5 g. Aguilar *et al*. (2016) achieved microtubers with a diameter greater than 10 mm and between 0.69 and 0.75 g of "Burren" potato microtubers in a medium containing gibberellic acid and BAP.

Microtubers quantity

The amount of microtubers obtained for the twenty-seven mashua morphotypes studied is shown in Figure 6. The best results were obtained with the MAC 08A and MAC 048 morphotypes with, respectively 51 and 56 microtubers.

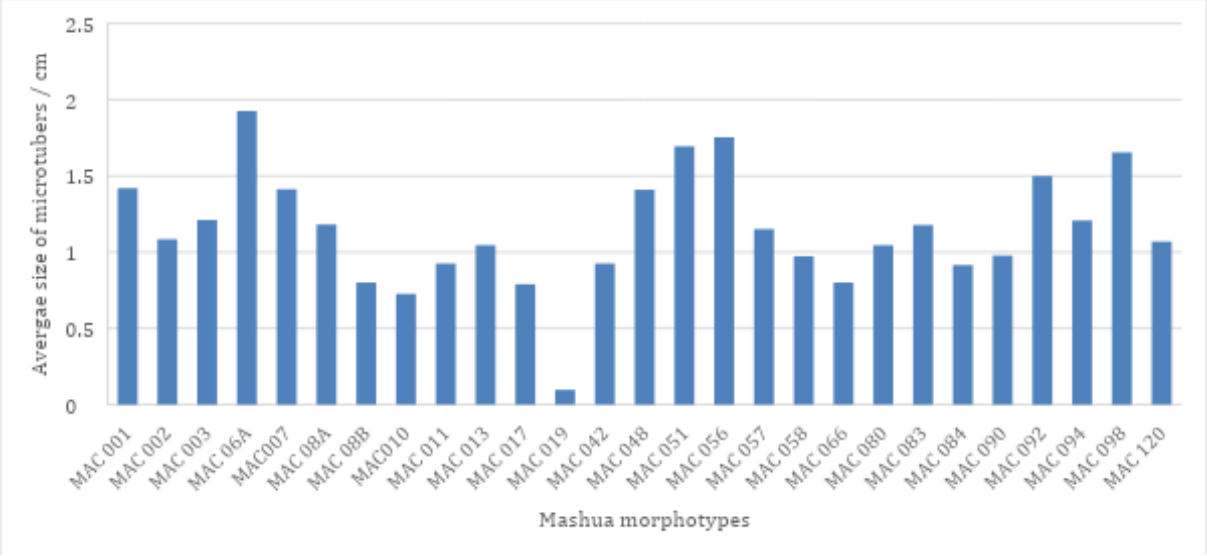

Figure 4. Average size of microtubers of twenty-seven mashua (*Tropaeolum tuberosum* Ruiz & Pavón) morphotypes obtained with SIT RITA® temporary immersion system with an immersion frequency of two minutes every three hours ($p < 0.05$).

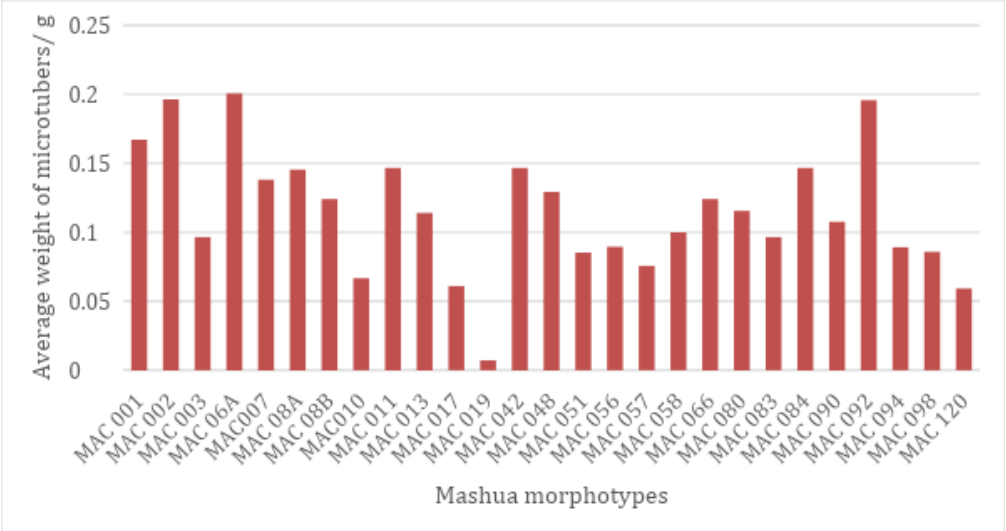

Figure 5. Average weight of microtubers of twenty-seven mashua (*Tropaeolum tuberosum* Ruiz & Pavón) morphotypes obtained with SIT RITA® temporary immersion system with an immersion frequency of two minutes every three hours ($p < 0.05$).

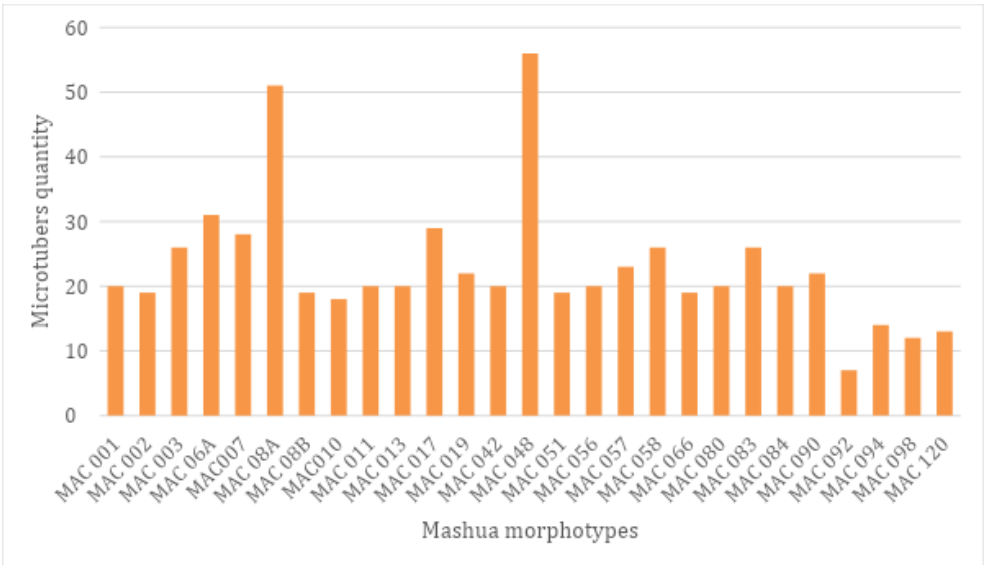

Figure 6. Microtubers quantity of twenty-seven mashua (*Tropaeolum tuberosum* Ruiz & Pavón) morphotypes obtained with SIT RITA® temporary immersion system with an immersion frequency of two minutes every three hours ($p < 0.05$).

CONCLUSION

Microtubers of twenty-seven mashua (*Tropaeolum tuberosum* Ruiz & Pavón) morphotypes were obtained using explants -previously propagated- in TIS-RITA® temporary immersion systems with immersion frequency of 2 minutes every 3 hours (for 10 weeks), in an MS (Murashige and Skoog) solid medium supplemented with 8% sucrose, 2 ppm BAP, under complete darkness at 19 ± 2 °C. The most effective morphotypes were; in size, MAC06A with 1.9 cm and MAC056 with 1.8 cm; in weight, MAC06A with 0.2 g and MAC 092 with 0.18 g; in quantity, MAC 08A with 51 units and MAC 048 with 56 units. These results show that TIS-RITA®, based on the intermittent contact of the cultivating medium of the explants, is an effective system to obtain mashua microtubers.

Acknowledgment

Funding. This work was developed within the scope of the Peruvian MINEDU/CONCYTEC project 199-2015-FONDECYT – UNSCH "Obtención de microtubérculos de *Tropaeolum tuberosum* mashua negra en sistema de inmersión temporal automatizado y caracterización de sus moléculas bioactivas (fenólicos y glucosinolatos)". We thank to MSc. Reynan Cóndor for his contribution in the data statistical analysis.

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 (gilmar_p@yahoo.com), upon reasonable request.

REFERENCES

Aguilar, M., Cruz, R., Ortiz, M., Zeledón, J. 2016. Producción de microtubérculos de papa (*Solanum tuberosum* L.), cultivar burren, en biorreactrores económicos de inmersión temporal. La Calera, 16(27), 75-80.

Akita, M., Takayama, S. 1994. Stimulation of potato (Solanum tuberosum L.) tuberization by semicontinuous liquid medium surface level control. Plant Cell Reports: 13(3-4): 184-187. DOI: https://doi.org/10.1007/BF00239889

Aire-Artezano, G., Charaja-Vildoso, R., De La Cruz-Santiago, H., Guillermo-Sánchez, B., Gutarra-Vela, M., Huamaní-Charagua, P., Pari-Ñaña, R. 2013. Efecto de Tropaeolum tuberosum frente a la hiperplasia prostática benigna inducida en ratas Holtzman. CIMEL Ciencia e Investigación Médica Estudiantil Latinoamericana, 18(1), 1-13.

Alvarenga, S. 2015. Micropropagación masiva de *Stevia rebaudiana* Bertoni en sistemas de inmersión temporal. Cultivos Tropicales, 36(3), 50-57.

Arbizu, C., Robles, E. 1986. Catálogo de recursos genéticos de raíces y tubérculos. Universidad Nacional de San Cristóbal de Huamanga, Facultad de Ciencias Agrarias, Programa de Investigación en Cultivos Andinos, Ayacucho, Perú, p 82.

Cabrera, M., Gómez, R., Espinosa, E., López, J., Medero, V., Basail, M., Santos, A. 2011. Yam (*Dioscorea alata* L.) microtuber formation in Temporary Immersion System as planting material. Biotecnología Aplicada, 28(4), 268-271.

Campos, D., Noratto, G., Chirinos, R., Arbizu, C., Roca, W., Cisneros-Zevallos, L. (2006). Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: native potato (Solanum sp.), mashua (Tropaeolum tuberosum Ruiz & Pavón), Oca (Oxalis tuberosa Molina) and ulluco (Ullucus tuberosus Caldas). Journal of the Science of Food and Agriculture, 86(10), 1481-1488. DOI: 10.1002/jsfa.2529.

Chirinos, R., Campos, D., Arbizu, C., Rogez, H., Rees, J. F., Larondelle, Y., Cisneros-Zevallos, L. 2007. Effect of genotype, maturity stage and post harvest storage on phenolic compounds, carotenoid content and antioxidant capacity, of Andean mashua tubers (*Tropaeolum tuberosum* Ruiz & Pavón). Journal of the Science of Food and Agriculture, 87(3), 437-446.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. InfoStat versión 2018. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Dodds, J. H., Silva-Rodriguez, D., Tovar, P. 1992. Micropropagation of potato (Solanum tuberosum L.). In High-Tech and Micropropagation III (pp. 91-106). Springer, Berlin, Heidelberg. DOI: https://doi.org/10.1007/978-3-662-07770-2_6

Doria, J. 2010. Generalidades sobre las semillas: su producción, conservación y almacenamiento. Cultivos tropicales, 31(1), 74-85.

Escalona, M., Cid, M., Lezcano, Y., Capote, I., Yánez, E., González, J. 1999. Propagación de la piña (*Ananas comosus* (L.) Merr.) en biorreactores de inmersión temporal. Efecto de la frecuencia de

inmersión y el paclobutrazol. BioVeg, 99, 28.

Etienne, H., Berthouly, M. 2002. Temporary immersion systems in plant micropropagation. Plant Cell, Tissue and Organ Culture, 69(3), 215-231. DOI: https://doi.org/10.1023/A:1015668610465

García M. G., Escalona, M., & Meneses, S. 2004. Efecto de la concentración de sacarosa y de reguladores del crecimiento en la tuberización in vitro de *Dioscorea alata* L. variedad Cartagena. Biotecnología vegetal, 4(4), 243-246.

Guevara-Freire, D. A., Valle-Velástegui, L., Barros-Rodríguez, M., Vásquez, C., Zurita-Vásquez, H., Dobronski-Arcos, J., Pomboza-Tamaquiza, P. 2018. Nutritional composition and bioactive components of mashua (*Tropaeolum tuberosum* Ruiz and Pavón). Tropical and Subtropical Agroecosystems, 21(1), 53-68.

Guimarães, R. L., Flores, H. 2005. Tropaeolum mosaic potyvirus (TropMV) reduces yield of Andean mashua (*Tropaeolum tuberosum*) accessions. HortScience, 40(5), 1405-1407.

Grau, A., Ortega, R., Cabrera, C., Hermann, M. 2003. Mashua (*Tropaeolum tuberosum* Ruíz & Pav.). Promoting the conservation and use of underutilized and neglected crops. 25. International Potato Center, Lima, Peru. International Plant Genetic Resources Institute, Rome, Italy. IPGRI.

Higgins, S., Francis, R. D., Smith, A. 2017. Production of *Solanum tuberosum* L. Microtuber Using Temporary Immersion System. Eur Exp Biol, 7(6), 38. DOI: 10.21767/2248-9215.100038

Igarza J., Agramonte D., De Feria M., Jaime, J., Pérez, M., San Roman, M. 2011. Obtención de microtubérculos de papa cv. 'Andinita' en Sistemas de Inmersión Temporal. Biotecnología Vegetal 11 (1): 59-62.

Igarza, J., de Feria, M., Alvarado-Capó, Y., Pugh, T., Pérez, M., San Roman, M., Agramonte, D. 2014. Caracterización morfo-agronómica de plantas de papa cv.Andinita'a partir de la siembra en campo de microtubérculos obtenidos en sistemas de inmersión temporal. Biotecnología Vegetal, 14(2).

Igarza, J., Agramonte, D., Alvarado-Capó, Y., De Feria, M., Pugh, T. 2012. Empleo de métodos biotecnológicos en la producción de semilla de papa. Biotecnología vegetal, 12(1), 3-24.

Jager, A., Schottlander, B., Smitt, W., Nyman, U. 1993. Somatic embryogenesis in cell cultures of *Thapsia garganica*: correlation between the state of differentiation and the content of thapsigargins. Plant Cell Rep, 12, 517-520. DOI: https://doi.org/10.1007/bf00236099.

Jiménez, E., Pérez, N., De Feria, M., Barbón, R., Capote, A., Chavez, M., Quiala, E., Perez, J. 1999. Improved production of potato microtubers using a temporary immersion system. Plant Cell, Tissue and Organ Culture, 59(1), 19-23.DOI: https://doi.org/10.1023/A:1006312029055

Khuri, S., Moorby, J. 1995. Investigations into the role of sucrose in potato cv. Estima microtuber production in vitro. Annals of Botany, 75(3), 295-303. DOI: https://doi.org/10.1006/anbo.1995.1024

León, J. 1964. Plantas Alimenticias Andinas. Bol. Tech 6. Instituto Interamericano de Ciencias Agricolas Zona Andina. Boletín Técnico. Lima, Perú.

Maldonado, E., Rodríguez de Francisco, O., Alvarado M., Cárdenas, C. 2003. Diseño y construcción de un sistema de inmersión temporal. Cent. Agríc. 30: 69-72

Manrique, I., Arbizu, C., Vivanci, F., González, R., Ramírez, C., Chávez, O., Elís, D. 2013. *Tropaeolum tuberosum* Ruíz and Pav. Colección de germoplasma de mashua conservada en el Centro Internacional de la Papa (CIP). Primera Edición. Centro Internacional de la Papa, La Molina, Perú.

Mehrotra, S., Goel, M. K., Kukreja, A., Mishra, B. 2007. Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization. African Journal of Biotechnology, 6(13). 1484-1492.

Montoya, N., Castro, D., Díaz, J., Ríos, D. 2008. Tuberización in vitro de papa (*Solanum*

tuberosum L.), variedad Diacol Capiro, en Biorreactores de Inmersión Temporal y evaluación de su comportamiento en campo; 2008; 16(3), 288-295.

Morales-Fernández, S. D., Mora-Aguilar, R., Rodríguez-Pérez, J. E., Salinas-Moreno, Y., Colinas-León, M., Lozoya-Saldaña, H. 2011. Desarrollo y rendimiento de papa en respuesta a la siembra de semilla-tubérculo inmadura. Revista Chapingo. Serie horticultura, 17(1), 67-75.

National Research Council. 1989. Lost crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation. National Academies Press.

Ortega, R., Duran, E., Arbizu, C., Ortega, R., Roca, W., Potter, D., Quiros, C. 2007. Pattern of genetic diversity of cultivated and non-cultivated mashua, Tropaeolum tuberosum, in the Cusco region of Perú. Genetic resources and crop evolution, 54(4), 807-821.

Pérez, M., Vega, V., Gálvez, E., Delgado, M., Jova, M., Torres, J., García, Y. 2011. Multiplicación in vitro de 'FHIA-25'(*Musa spp*., AAB) en sistemas de inmersión temporal. Biotecnología Vegetal, 11(1), 27-31.

Pissard, A., Arbizu, C., Ghislain, M., Bertin, P. 2008. Influence of geographical provenance on the genetic structure and diversity of the vegetatively propagated Andean tuber crop, Mashua (*Tropaeolum tuberosum*), highlighted by Intersimple Sequence Repeat Markers and Multivariate Analysis Methods. International journal of plant sciences, 169(9), 1248-1260.

Rivera Calderón, Á., Valbuena, R., Hidalgo, R., Moreno, J. 2008. Microtuberización *in vitro* de siete accesiones de papa de la colección central colombiana. Acta agronómica, 57(3), 175-180.

Salauces, R., Rocabado, C., Blanc, D. 1998. La producción de semilla Prebásica. Cochabamba, BO. (Unidad de Producción de Semilla de Papa); 1998: 57 p.

Tirado, J. M., Naranjo, E. J., Atehortúa, L. 2005. Propagación in vitro de Phalaenopsis (Orchidaceae) a partir de protocormos, mediante el sistema de inmersión temporal" RITA". Revista Colombiana de Biotecnología, 7(1), 25-31.

Valle-Parra, M., Pomboza-Tamaquiza, P., Buenaño-Sanchez, M., Guevara-Freire, D., Chasi-Vizuete, P., Vásquez-Freitez, C., Pérez-Salinas, M. 2018. Morphology, phenology, nutrients and yield of six accessions of *Tropaeolum tuberosum* Ruiz y Pav (mashua). Tropical and Subtropical Agroecosystems, 21(1), 131-139.

Werner, S., Maschke, R., Eibl, D., Eibl, R. 2018. Bioreactor Technology for Sustainable Production of Plant Cell-Derived Products. Bioprocessing of Plant In Vitro Systems, 413-432. DOI: https://doi.org/10.1007/978-3-319-54600-1_6

Ziv, M. 2005. Simple bioreactors for mass propagation of plants. In Liquid culture systems for in vitro plant propagation (pp. 79-93). Springer, Dordrecht. DOI: https://doi.org/10.1007/1-4020-3200-5_5