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Yield of five potato varieties in Temporary Immersion Bioreactors (TIB)

Rendimiento de 5 variedades de papa en Biorreactores de Inmersión Temporal (BIT)

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Abstract

The potato (*Solanum tuberosum* L.) is important as both a food and a source of economic activity in Peru. However, potato production is limited by seed quality and other biotic and abiotic factors. We explore a new alternative method for producing prebasic seeds of Peruvian potato varieties known as temporary immersion bioreactors (BIT). The process of producing potato microtubers using BIT consists of two phases: proliferation and microtuberization. During the proliferation phase, we seeded six nodal segments of three nodes in a liquid culture medium with 30 g of sucrose under a photoperiod of 16 light hours and eight dark hours. This phase also included an irrigation cycle of five minutes every three hours for 30 days. During the microtuberization phase, the conditions were changed to a medium with 80 g of sucrose in darkness, and the same irrigation cycle was used for 60 days. Under these conditions we obtained 20, 18.4, 13.4, 13.4, and 4.6 microtubers of the varieties Peruanita, Canchan, Capiro, Unica, and Yungay, respectively.

Key words: *Microtubers, prebasic seed, temporary immersion bioreactors.*

Resumen

La papa (*Solanum tuberosum* L.) es un alimento importante y cultivo de alta importancia económica en Perú. Su producción es limitada debido a la calidad de las semillas y otros factores bióticos y abióticos. Aquí exploramos una nueva alternativa para la producción de semilla prebásica de variedades peruanas de papa llamada biorreactores de inmersión temporal (BIT). La producción de microtubérculos de papa utilizando BIT comprende dos fases: proliferación y microtuberización. Durante la fase de proliferación se utilizó 6 segmentos nodales de 3 nudos sembrados en un medio de cultivo líquido con 30 g de sacarosa bajo un fotoperiodo de 16 horas luz y 8 de oscuridad, además de tener un ciclo de riego de 5 minutos cada 3 horas por 30 días; para la fase de microtuberización se cambió a un medio de 80 g de sacarosa, en oscuridad y con el mismo ciclo de riego por 60 días. Bajo estas condiciones fue posible obtener 20, 18.4, 13.4, 13.4 y 4.6 microtubérculos por biorreactor de las variedades “Peruanita,” “Canchan,” “Capiro,” “Unica” y “Yungay,” respectivamente.

Palabras clave: *Microtubérculos, semilla prebásica, biorreactores de inmersión temporal.*

Introduction

Potatoes are the world’s most important food crop after wheat and rice and are a staple food for 1.3 billion people, with increasing popularity in the developing world. (Stokstad, 2019).

In Peru, growers cultivate four potato species: *Solanum tuberosum* along with three other species exclusive to the Andes. PInstituto de Biotecnología, Universidad Nacional Agraria La Molina, Av. La Universidad S/N, Lima, Perú. mountains and on the coast (Egúsquiza, 2014). Peru ranks 18th among main consumer countries, with an annual consumption of 78.4 kg per capita. Additionally, potato production has increased from 4,704,987 tons in 2004 to 7,704,987 tons in 2014 (FAOSTAT, 2015). Potato cultivation accounts for 25% of Peru’s agricultural gross domestic product and is grown in 19 of the country’s 24 departments. However, certified seed only accounts for 0.2% (1,145 tons) of seed farmers use. In other words, small producers continue to plant seed potatoes acquired through “informal seed systems” that often have a poor sanitary status, leading to significant yield reductions (Egúsquiza, 2014).

Low potato yields in Peru and other countries in the region can be attributed to the use of low-quality seeds and recycling of tubers. Therefore, there is consensus on the need to incorporate effective and efficient seed production technologies that are also consistent with

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the reality of potato cultivation in Latin America. (Del Rio et al., 2017). Among these technologies are certified seed tubers, which allow doubling of yields, although its availability is weather- and season-dependent. Another is the use of potato seedlings in vitro; however, this can be costly for farmers because of the high mortality of plants during the acclimatization phase, a critical stage of in vitro plant production stage, as well as the production of tuber-seeds, which is dependent on weather and season. Another technology that could help overcome these problems is the use of microtubers, which are easy to handle, store, and transport and can be produced throughout the year (Dobránszki, Magyar-Tábori, & Hudák, 2008)

Temporary immersion bioreactors have been used for efficient production of potato microtubers (Rokka, Kämäräinen-Karppinen, Virtanen, & Pirttilä, 2013; Elaleem, Modawi, & Khalafalla, 2015; Pumisutapon & Topoonyanont, 2015; Rahman, Shahinul Islam, Chowdhury, & Sreeramanan Subramaniam, 2015; Tapia, Lorenzo, Mosqueda, & Escalona, 2017; Ali, Khan, Nouroz, Erum, & Nasim, 2018; Naik & Buckseth, 2018; Tapia, Arbizu, Beraún, Lorenzo, & Escalona, 2018).

In Peru the Canchan, Capiro, Unica, Yungay, and Peruanita cultivars stand out for their culinary quality in frying or dry matter content in the tubers. However, there is no history of obtaining microtubers of the last three of these varieties. Our intent is to evaluate the production of Peruvian potato variety microtubers using temporary immersion bioreactors.

Materials and Methods

Experimental place

Our experimental work was carried out in the Institute of Biotechnology (IBT) facilities, area Cultivation of Fabrics of the National Agrarian University La Molina (238 masl).

Plant material

Plant material used as the mother material was in vitro potato seedlings of the Canchan, Unica, Yungay, Capiro, and Peruanita varieties from the International Potato Center.

Culture medium

The culture medium's basal composition was based on the work of Murashige and Skoog (1962). Espinoza et al. (1992) modified the medium with ammonium nitrate (1750 mg l-1), potassium nitrate (2000 mg l^{-1}), calcium chloride (450 mg l^{-1}), phosphate (175 mg l^{-1}), thiamine (0.4 mg l^{-1}), glycine (2.0 mg l^{-1}), nicotinic acid (0.5 mg l^{-1}), pyridoxine (0.5 mg l^{-1}), calcium pantothenate (2 mg l−1), acid folic acid (1 mg l^{-1}), and arginine (4 mg l^{-1}). We also added myo-inositol (100 mg l^{-1}) to the culture medium and adjusted the pH to 5.7 using sodium hydroxide or 1 N hydrochloric acid. To prepare the proliferation medium we added 30 g of sucrose and added 80 g of sucrose per liter of medium to the tubers. The medium was sterilized in an autoclave at 121°C and 1.2 kg cm−2 for 25 minutes.

Temporary Immersion Bioreactors

Two 500 ml vessels were used following the model proposed by Escalona et al. (1999).

Installation of the five varieties to the temporary immersion system We used three to four knot stem segments of Canchan, Capiro, Yungay, Unica, and Peruanita. We seeded six segments in a vessel containing 100 ml of proliferation culture medium, sealed and installed the system on the shelves, and set an immersion time and frequency of 4 min every 3 hours for 28 days with a 16 h light/8 darkness photoperiod using fluorescent lamps (25–30 µmol m-2 s^{-1}). After four weeks, the proliferation medium in the laminar flow chamber was changed to the tuberization medium. After this, the system was sealed and the vessel containing the seedlings was covered with a bag to place the specimens in darkness. We then installed the containers on shelves and set the immersion time and frequency to four minutes every three hours (8F/day). The culture time we set to 60 days in the dark at 22 ± 0.2°C and the specimens were incubated at 24°C for eight weeks.

After the tuberization process was completed, we carefully separated the microtubers from the plant and root residues, rinsed them with running water to remove the culture medium, and placed them in trays on filter paper to remove moisture.

Evaluation system

After harvesting the microtubers we evaluated the number of microtubers obtained per experimental unit, the fresh weight of each microtuber, and the yield per experimental unit.

Experimental design and statistical analysis

We performed a completely randomized design with three replications and processed the resulting data in Minitab 17. We conducted a comparison of means using the Tukey test to observe if there were significant differences between varieties.

Results

Behavior of the five varieties to the temporary immersion system

Figure 1 indicates no significant differences between means of average weight of five varieties of microtubers produced in the bioreactor using the Tukey test with a 5% significance level; however, the Yungay variety obtained an average weight of 0.528 g, which slightly surpasses the minimum weight that microtubers must have for greenhouse development.

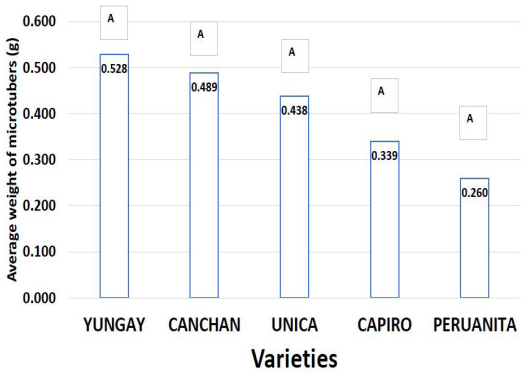

Figure 1. Comparison test of means of average microtuber weight produced by each variety in the temporary immersion system (Tukey α = 0.05)

Figure 2 shows a comparison of the number of microtubers of the five varieties studied produced by the bioreactor and whether the Tukey test with a level of significance of 5% found significant differences between their means. The figure highlights the variety "Peruanita" having the highest yields with 20 microtubers per bioreactor on average; however, this is not significant in comparison with values obtained for varieties Canchan, Capiro, and Unica. In contrast, the Yungay variety produced the lowest number of microtubers with 4.6 microtubers per bioreactor.

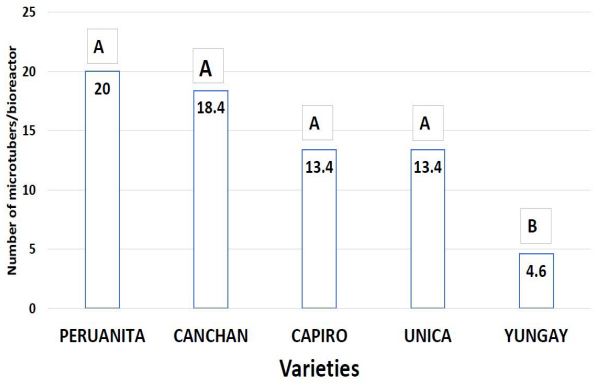

Figure 2. Comparison of microtuber yield means for each variety in the temporary immersion system (Tukey α = 0.05)

Discussion

With respect to average tuber weight, the Yungay variety exceeded the minimum weight needed to be considered as a prebasic seed; however, this weight is lower than the weight that Yu, Joyce, Cameron, and McCown, (2000) obtained for the Russet Burbank cultivar, (1,216 g), that Pérez et al. (2007) obtained for the cv. Atlantic (2,745 g), that Castro (2011) obtained for the Capiro (1.00 g) and Canchan (0.97 g) varieties, and that Araque et al. (2018) obtained for the Capiro variety (123.90 mg). Park et al. (2009) classified microtubers of the Superior potato cultivar in three categories: small (4.0–6.0 mm and 0.18 g), medium (6.1–8.0 mm and 0.29 g), and large (>8.1 mm and >0.54 g). Based on this categorization, the microtubers produced in the bioreactors would mostly be categorized as small to medium. Khalil, El Aal, and Samy (2017) demonstrated that using only sucrose to induce tuberization was favorable for producing Unica variety microtubers, obtaining 89.5 mg per microtuber.

The Unica variety produced fewer tubers per bioreactor than the other varieties evaluated, yielding 0.7 microtubers per explant sown; however, this number is higher than that Yu et al. (2000) obtained for the Russet Burbank cultivar (175 microtubers and 50 stem segments), that Pérez et al. (2007) obtained for the cv. Atlantic cultivar (186 microtubers and 60 stem segments in 4L flasks), that Mani, Mhamdi, Bettaieb, and Hannachi (2014) obtained from seven stem segments in 50 ml of medium with Alaska (3.7 ± 1.1), Safran (6.4 ± 2.1), and Spuntia (13.83 ± 3.8) cultivars, that Elaleem et al. (2015) obtained for the Almera (6.0 ± 0.5) and Diamant (3.0 ± 0.0) cultivars grown in 25 ml of culture medium, and that Castro (2011) obtained in the Capiro (70.5) and Canchan (69) varieties, with 50 stem segments already obtained by Khalil et al. (2017) in the Unica variety (6.4) from five explants. In contrast, Areque et al. (2018) obtained better production with the Capiro variety (49 tubers from 12 explants) and Tapia et al. (2018) obtained better yields with the Canchan (250) and Capiro (270) varieties from 50 explants

These varying potato variety responses are due to plant regeneration in vitro in temporary immersion systems depends on abiotic (e.g., temperature and photoperiod) and biotic (e.g., manipulation of organic and inorganic medium constituents, explant type and species, and nutritional characteristics) factors. Additionally, morphological, organogenic, and nutritional characteristics are determined by genotype, making it possible to improve in vitro investigations. When using the most organogenic genotypes, genotypic variability is used to induce in vitro organogenesis (Casas et al., 1993). Complete regeneration from the explant is often specific to the species, variety, or introduced genotype, which determines its morphogenic expression capacity (Ziv, 2005). Sucrose concentration in the tuberization medium considerably influences microtuberization, with an optimal concentration of 8% (80 gr.l^{-1}) as demonstrated by Mani et al. (2014), Elaleem et al. (2015), Salem and Hassanein (2016), Islam, Roni, Jamal, and Shimasaki (2017), Khalil et al. (2017), and Ali et al. (2018). García and Azofeifa (2017) showed that exposure to light during the tuberization phase is favorable to microtuber production and size, although in our case light exposure inhibited production and induced budding in the microtubers formed.

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Conclusions

Producing microtubers in temporary immersion bioreactors is influenced by genotype. Additionally, we observed that native potato varieties such as Peruanita adapt very well to this technique of prebasic seed production.

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