

Agronomic Performance of Temporary Immersion Bioreactor – Derived Potato Microtubers in a Peruvian Low Input Cropping Agriculture System

María de Lourdes Tapia y Figueroa

Universidad Nacional Agraria La Molina

José Faustino Beraún Tapia

Universidad Nacional Agraria La Molina

Elliosha Hajari

Agricultural Research Council

Maritza Escalona

Bioplant Centre, University of Ciego de Avila

Hervé Etienne

CIRAD

José Carlos Lorenzo Feijoo (✉ jclorenzo@bioplantas.cu)

Universidad de Ciego de Avila <https://orcid.org/0000-0003-3610-1789>

Research Article

Keywords: agronomic traits, micropropagation, potato microtuber, *Solanum tuberosum* L., temporary immersion bioreactors

Posted Date: June 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-513992/v1>

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Abstract

Potato cultivation is limited by a lack of access to quality propagation material. The application of micropropagation techniques combined with the diagnosis and sanitation of the main pathogens of the crop, has contributed to increased production efficiencies. In this regard, the use of temporary immersion bioreactors (TIBs) has improved the quality of microtubers micropropagated along with savings in costs of production. With the final goal of applying these technologies for commercial production, the current study investigated the agronomic performance of Peruvian Canchan potato microtubers derived from TIBs (basic agamic seed 1 and 2) under low-input agro-technology in the coastal zone of Peru. The results indicated that following 75 d of growth, plants derived from microtubers produced in TIBs displayed slower vegetative growth than those from conventional tubers. However, at harvest, these differences were no longer apparent. Although plants raised from conventional tubers produced the highest fresh mass of tubers, significantly more propagules were produced by plants regenerated from basic agamic seed 1 and 2 derived from micropropagation in liquid media. These results demonstrate that much more planting material (seed tubers) can be obtained from microtubers in the field (basic agamic seed 1) than from the conventional commercial seed tubers.

Main Text

Cultivation of potato (*Solanum tuberosum* L.) makes a valuable contribution to food security with production levels steadily increasing in the last 20 years (Devaux et al. 2020). However, the industry faces several challenges including pests, diseases, increasing soil salinity, drought and susceptibility to high temperatures. A major difficulty faced by producers worldwide is access to high quality planting material. In many developing countries seed tubers are not regularly renewed. This leads to the accumulation of endophytic pathogens (Wasilewska-Nascimento et al. 2020).

Alternative propagation technologies have been investigated to promote production efficiency. One avenue of investigation has been the use of micropropagation techniques. This, when combined with methods for disease detection, has allowed for the production of seedlings with significantly improved phytosanitary status with efficient multiplication rates (Yagiz et al. 2020). Moreover, a range of bioreactors have been developed (Valdiani et al. 2019) with various applications. In this context, TIBs have become one of the most useful bioreactor technologies to improve propagation efficiency and reduce costs. The TIBs have been successfully used to produce microtubers in potato (Jiménez et al. 1999). However, it is imperative that the agronomic performance of bioreactor-derived microtubers be investigated under local conditions before TIBs can be scaled-up and applied for roll out in potato agamic seed production schemes. To this end, it is important to characterize the physiological development and morphological parameters of plants in the field with due consideration of the resource status of local farmers, prevailing environmental conditions and testing of this planting material in the most common agrosystem in the country. This stage of participatory research is essential to guarantee further adoption of this new planting material by farmers. Furthermore, the variety cultivated needs to be considered because different genotypes are known to have varying agronomic responses (Radouani and Lauer

2015). In Peru, the Peruvian Canchan is often grown by local farmers in low input systems. Therefore, the current study investigated the agronomic characteristics of the Peruvian Canchan potato, propagated from microtubers regenerated from TIBs, in on farm field trials in the coastal zone of Peru and under the low-input agro-technology.

Tubers were sourced from the germplasm bank of the IPC and *in vitro* plants were obtained via meristem culture. Tubers were planted in greenhouses under controlled conditions allowing the material to be free of pests and diseases. After 60 d of growth, apical cuttings were harvested and disinfected with 2.5% (v/v) sodium hypochlorite for 15 min. Meristems (0.1 - 0.3 mm) from apical and lateral buds were excised and placed onto culture medium in a growth chamber at 25°C with a photosynthetic photon flux density of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a photoperiod of 16 h light/8 h dark. Meristems were transferred weekly onto fresh medium. After 6 - 8 weeks of growth, seedlings were obtained, which were micropropagated for indexing. The plants were evaluated at the IPC for any persistent virus infection. Pathogen-free plants from meristems were sectioned into nodal segments and placed in culture flasks with semi-solid medium for further multiplication.

The microtubers (pre-basic agamic seed) were generated in 4 L-TIBs (Jiménez et al. 1999) in two stages, the first targeted for growth and multiplication of the nodal segments and the second for microtuberization. The inoculation density was 50 explants/TIB (segments with 2 nodes) and 15 mL medium per segment in bioreactors. Plants were immersed in liquid medium for 4 min every 3 h over a period of 28 d under a photoperiod 16 h light/8 h dark. Microtuberization was induced by replacing the medium with fresh medium of the same composition but with 80 g L^{-1} sucrose (60 d, dark, 22°C).

To obtain basic agamic seed 1, microtubers were rinsed and placed in trays exposed to ambient conditions (natural light, 22 ± 2°C, 1 mo.). Before planting, they were disinfected with a 1 g L^{-1} (w/v) Benomyl solution for 10 min. Only microtubers with a fresh weight greater than 0.5 g (Kawakami and Iwama 2012) were planted directly in sandy loam soil. Basic agamic seed 2 was obtained from the sowing of basic 1 agamic seed with 50 g of fresh weight. The procedure was as described above.

The performance of plants derived from different propagation methods were compared in May – August 2018. The following planting material were used (approximately 50 g per seed tuber, planting density: 0.3 m x 1.0 m): 1) first generation tubers obtained in TIBs (basic agamic seed 1), 2) second vegetative generation (basic agamic seed 2), and 3) a commercial agamic seed control used by the Peruvian farmers. After 30, 75 and 150 d of field growth, agricultural traits of plants were evaluated. Plants grew on sandy soil, without any fertilizer or pesticide, at 200 m above sea level, with superficial irrigation, and at 15 - 27°C. Average rainfall was 0.12 mm and temperature 16.7°C.

The field experiment was composed of four independent blocks used as replicates. Each block was divided into three lines of 15 plants, each line representing one of the three propagation material tested. In total, 60 plants for each type of propagation material were characterized. All data were statistically evaluated using SPSS (Version 8.0 for Windows, SPSS Inc., New York).

The agronomic performances are summarized in Table 1 and Fig. 1. Sprouting of tubers was recorded following 30 d of field growth and other agronomic parameters after 75 and 150 d. Good levels of sprouting were achieved from all propagation material with the highest values observed from bioreactor-derived microtubers (90%) followed by basic agamic seed 2 (85%) and commercial tubers (80%). At the mid-point of the trial (75 d), plants derived from commercial tubers were more vigorous showing the significantly highest number of leaves (187), leaf FW (692 g), stem FW (523 g) and root FW (67 g). For all of the abovementioned parameters, the significantly lowest indicators were observed in plants directly derived from bioreactors (basic agamic seed 1). At this intermediate stage, plants from commercial agamic seeds also produced the highest number of tubers per plant (53) compared with basic agamic seed 1 and basic agamic seed 2 that regenerated similar numbers of tubers (14). No significant difference was observed between the three planting materials for tuber FW, and diameter and length of tubers. The field trial was harvested 150 d after planting and the final agronomic performance of plants were assessed. In contrast to the preliminary findings at 75 d, plants derived from both types of microtubers derived from TIBs - basic agamic seed 1 and 2 - generated the significantly highest numbers of tubers per plant (12.6 and 10.9, respectively) while plants grown from commercial tubers produced 8 tubers per plant (Table 1). However, the plants grown from commercial tubers still yielded the highest tuber FW per plant (143.9 g), compared with basic 1 and basic 2 agamic seed (86.6 and 93.0 g, respectively). Furthermore, tuber size as expressed by both diameter and length was larger in plants from commercial tubers (59.1 and 63.3 cm, respectively) than in the plants grown from basic agamic seed sources. Plants propagated from commercially sourced tubers produced fewer but larger tubers than those regenerated by microtubers produced from bioreactors. It is noteworthy that tubers of a similar size were produced by plants grown from basic agamic seed 1 and 2 (Table 1). As the objective of the study was to develop methods for the production of more efficient planting material in potato, tuber morphological characteristics were also measured to determine the ability of plants from each of the three sources to generate tubers suitable for subsequent propagation. The results showed that significantly higher numbers of agamic seed tubers per plant were produced by plants generated by microtuber basic agamic seed 1 (8.5) and 2 (6.7) in comparison with plants from commercial tubers which yielded comparatively lower numbers (3.0) (Fig. 1A). In terms of tuber FW suitable for subsequent propagation cycles, plants raised from commercial tubers produced bigger tubers (55 g) than plants from microtubers which produced tubers of a similar size range (44 - 48 g, Fig. 1C). The highest emergence of sprouts was observed in microtubers derived from bioreactors during the initial 30 d-vegetative growth stage. Despite the higher levels of sprouting observed in microtubers derived from bioreactors, these plants displayed the slowest relative growth rates within the first 75 d in the field as evidenced by the low levels of biomass generated. While there are studies on the field performance of plants derived from microtubers and commercial tubers, there is still a scarcity of information on the field behavior of the basic agamic seed 1 and 2 microtubers obtained from *in vitro* culture compared with the conventional sowing of commercial tubers. Nevertheless, this lower plant development did not ultimately affect the yield of tubers obtained as plants grown from microtubers produced a higher number of tubers at harvest. Kawakami and Iwama (2012) also noted that the observation of initial low leaf area index in plants derived from microtubers was transient and disappeared after flowering. Plants from basic agamic

seed 2 microtubers also displayed consistently higher vegetative growth than those from basic agamic seed 1 microtubers. Although both basic agamic seed 1 and 2 originated from the same source, i.e. TIBs, the latter was able to produce more vigorous plants. Ultimately, the faster vegetative development of these plants did not translate into higher tuber yields as both plant types produced similar numbers of tubers with comparable morphological characteristics. Tubers produced from commercial sources were larger and heavier than those from basic agamic seed 1 and 2. The results indicate that microtubers and basic agamic seed 2 provide appropriate and optimized sources of material for potato propagation schemes. Furthermore, there are phytosanitary advantages in using microtubers as these are generated from virus-free *in vitro* meristem cultures, therefore the quality of these propagules are superior to commercial tubers in this regard. Indeed, this might have contributed to the observation of reduced sprouting in commercial tubers, which are known to accumulate pathogens over time (Badoni and Chauhan 2009) leading to deterioration (particularly in informal or non-certified schemes). The results suggest that the use of agamic seed from biotechnological methods (microtubers from TIB: pre-basic) would be especially useful in countries where the production of quality agamic seed is challenging. In addition, the observation that plants generated from basic agamic seed 1 and 2 can produce high levels of tubers for subsequent propagation, makes this method feasible for potato agamic seed schemes. The current work provides evidence for the potential use of TIBs to produce microtubers as a source of material for potato agamic seed schemes. The results showed that bioreactor-derived propagules initially displayed slower rates of vegetative growth than commercial tubers but ultimately produced a higher number of tubers per plant. Bioreactor-derived microtubers were smaller in mass but more abundant in number than conventional tubers with a higher percentage of tubers suitable for subsequent propagation.

Abbreviations

International Potato Center (IPC, Peru), Temporary immersion bioreactors (TIBs)

Declarations

Author contribution

MLTF, JFBT, EH, ME, HE and JCL designed the research, MLTF and JFBT conducted the experiment, MLTF, JFBT, EH, ME, HE and JCL analyzed the data and wrote the paper, and JCL had primary responsibility for the final content. All authors have read and approved the final manuscript.

Acknowledgements

This research was supported by the National Agricultural University La Molina (Peru), the Bioplant Centre, University of Ciego de Avila (Cuba), and the Agricultural Research Council (South Africa).

Compliance with Ethical Standards

Conflict of interest

The authors declare no conflicts of interest.

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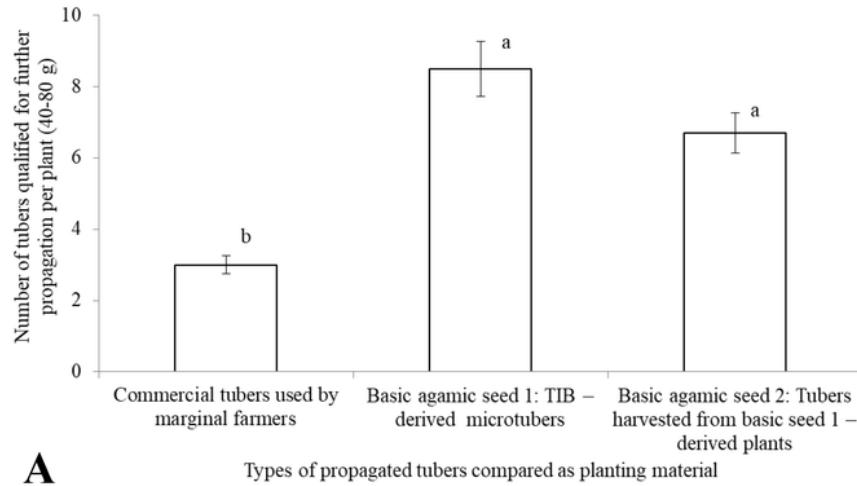
Tables

Table 1: Field performance of TIB – derived potato microtubers in under a low input cropping system in Peru

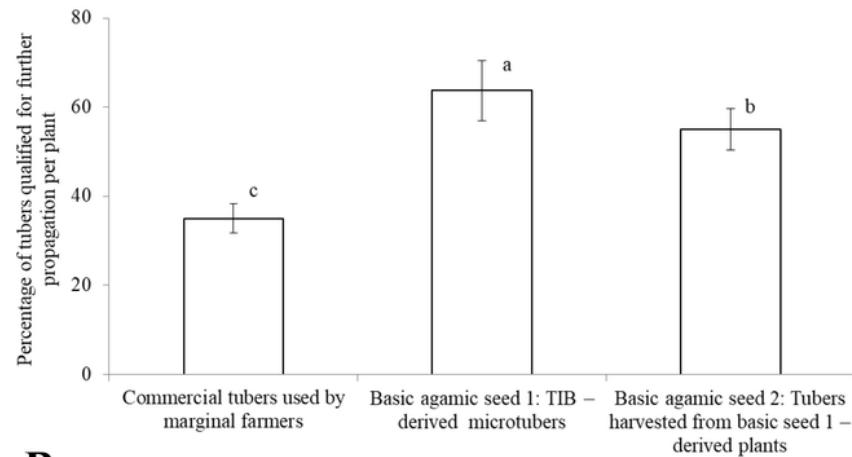
Agronomic trait observed after field planting		Types of propagated tubers compared as planting material		
		Commercial tubers used by marginal farmers	Basic agamic seed 1: TIB – derived microtubers	Basic agamic seed 2: Tubers harvested from basic seed 1 – derived plants
After 30 d	Percentage of sprouting *	80.0 ± 7.3 c	90.0 ± 8.7 a	85.0 ± 7.6 b
After 75 d	Number of leaves per plant *	187.2 ± 15.4 a	86.7 ± 7.5 c	142.7 ± 11.5 b
	Total leaf fresh weight per plant (g) *	692.8 ± 55.3 a	188.1 ± 13.3 c	413.3 ± 39.4 b
	Total stem fresh weight per plant (g) *	523.7 ± 45.1 a	101.7 ± 9.5 c	380.5 ± 35.2 b
	Total root fresh weight per plant (g) *	67.7 ± 4.7 a	5.4 ± 0.4 c	23.2 ± 2.3 b
	Number of tubers per plant *	53.0 ± 4.3 a	14.2 ± 1.2 b	14.0 ± 1.2 b
	Total tuber fresh weight per plant (g) *	23.5 ± 1.9 a	16.5 ± 1.5 a	25.7 ± 1.9 a
	Tuber diameter (cm) *	28.9 ± 2.4 a	24.9 ± 2.1 a	33.1 ± 3.0 a
	Tuber length (cm) *	32.4 ± 2.8 a	26.1 ± 1.8 a	32.4 ± 2.8 a
After 150 d	Total number of tubers per plant *	8.0 ± 0.7 b	12.6 ± 1.1 a	10.9 ± 1.1 a
	Total tuber fresh weight per plant (g) *	143.9 ± 12.5 a	86.6 ± 7.6 b	93.0 ± 8.8 b
	Tuber diameter (cm) *	59.1 ± 4.8 a	51.6 ± 4.9 b	53.3 ± 4.5 b
	Tuber length (cm) *	63.3 ± 5.6 a	53.1 ± 4.1 b	53.9 ± 5.2 b

* Results with the same *letter* are not statistically different (One-Way ANOVA, Tukey, $p>0.05$). For the statistical analysis only, numbers of leaves and tubers were transformed according to $y'=y^{0.5}$, and the percentage variables as $y'=2 \arcsine(y/100)^{0.5}$. Vertical bars represent $\pm SE$ of original data.

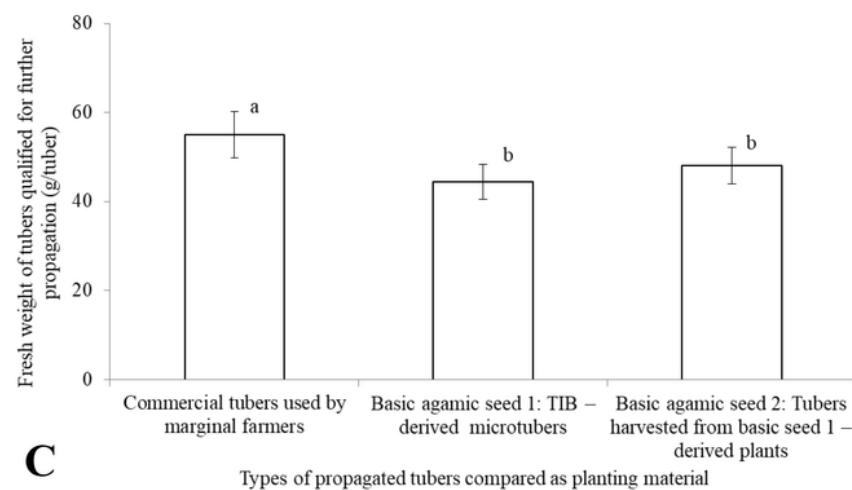
Figures



A Types of propagated tubers compared as planting material



B Types of propagated tubers compared as planting material



C Types of propagated tubers compared as planting material

Figure 1

Quality of the materials obtained for further propagation after 150 d of planting. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, $p>0.05$). For the statistical analysis only, numbers of tubers were transformed according to $y' = y^{0.5}$, and the percentage as $y' = 2 \arcsine(y/100)^{0.5}$. Vertical bars represent $\pm SE$ of original data.