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Agronomic performances of temporary immersion bioreactor-derived potato microtubers in a Peruvian low input cropping agriculture system

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In Peru, potato cultivation represents 25% of the agricultural gross domestic product yet only 0.2% of the agamic seed used is from certified sources. The use of temporary immersion bioreactors (TIB) has improved the quality of microtubers micropropagated along with savings in costs of production. The current study investigated the agronomic performances of Peruvian Canchan potato microtubers derived from TIB (basic agamic seed 1 and 2) under the low-input agro-technology in the coastal zone of Peru. Following 75 days 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 as the plants from TIB derived-basic agamic seed 1 and 2 produced the highest numbers of tubers per plant. 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.

Key words: Agronomic traits, micropropagation, potato microtuber, *Solanum tuberosum* L., temporary immersion bioreactors.

INTRODUCTION

Feeding an ever increasing global population is placing unprecedented pressure on agricultural systems and finite natural resources. This is exacerbated by the impacts of climate change, regional conflicts, migration,

the COVID-19 pandemic, etc. The aforementioned factors have highlighted the importance ensuring a stable, sustainable supply of food. 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). The crop is cultivated in temperate regions of the northern hemisphere, highlands of the Andes and Africa, in the Rift valley, volcanic mountains of West Africa and South East Asia. In the sub-tropics, production occurs in the Mediterranean areas, North India and Southern China (Devaux et al., 2020). Currently, global production is estimated to be approximately of 370 million tons on 17 million hectares of land. Peru is regarded as the major center of origin of potato (Devaux et al., 2020) and farmers of this country cultivate four species of potatoes in the highlands and on the coast.

The cultivation of potato faces several challenges including pests, diseases, increasing soil salinity, incidences of drought and susceptibility to high temperatures (Gastelo et al., 2014). A major difficulty faced by producers worldwide is access to high quality planting material, that is, seed tubers (Sharma-Thomas et al., 2015). In many developing countries, seed tubers are not regularly renewed. This leads to the accumulation of endophytic pathogens which ultimately causes gradual degeneration in both quality and yield (Wasilewska-Nasciemento et al., 2020). In Peru, only a dramatically limited proportion of the agamic seeds used by farmers are certified (Corrêa et al., 2009).

Over the years alternative propagation technologies have been investigated in efforts to promote production efficiency. One of such 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 and with efficient multiplication rates. In this regard, virus-free plants have been propagated through meristem cultures, successfully multiplied and minitubers produced *in vitro* (Al-Shareefi et al., 2020; Mamiya et al., 2020; Rojas et al., 2020; Yagiz et al., 2020; Belguendouz et al., 2021). *In vitro* plants can be planted in the field but they generally require a fairly technical acclimatization intermediate stage to outdoor conditions prior to field transfer and since these plants are delicate, they are also difficult to handle and transport (Wróbel, 2015). Microtubers are more robust than *in vitro* plants and provide an easier alternative to *in vitro* plants for potato propagation. Microtubers produced from in vitro plants provide ideal propagules for direct field planting. This strategy can be planned with consideration of planting seasons to ensure that sufficient in vitro plants are produced for subsequent generation of appropriate amounts of minitubers when required for planting (Igarza et al., 2011; Igarza et al., 2012, 2014; Rokka et al., 2014). An added advantage of microtubers is that they can be planted directly into the field without the need for acclimatization, provided that microtubers are large enough unlike *in vitro* plants (Jiménez et al., 1999).

There also exist the possibility of reducing production costs of microtubers through semi-automation in liquid media to produce basic potato agamic seed (Jiménez et al., 1999). A range of bioreactors have been developed over the years (e.g. temporary immersion systems) (Jiménez et al., 1999; Valdiani et al., 2019; Vidal and Sánchez, 2019). Temporary immersion systems have been successfully used to produce microtubers in potato (Jiménez et al., 1999; Higgins et al., 2017; Tapia et al., 2018) and yam (Jova et al., 2011; Balogun et al., 2014). However, as with any new technology, it is imperative that the agronomic performance of bioreactor-derived microtubers be investigated under local conditions before the micropropagation system can be scaled-up and applied on a large scale for roll out in potato agamic seed production schemes. Therefore, the current study investigated the agronomic characteristics of the Peruvian Canchan potato, propagated from microtubers regenerated in TIBs, in on farm field trials in the coastal zone of Peru and under the low-input agro-technology.

MATERIALS AND METHODS

Plant and culture conditions to obtain microtubers

Tubers were sourced from the germplasm bank of the International Potato Center (IPC, Lima, Peru) and *in vitro* plants were obtained via meristem culture. The elite plant tubers received were planted in greenhouses under controlled conditions allowing the material to keep free of pest and diseases. After 60 days of growth, apical cuttings were harvested and subsequently disinfected with 2.5% (v/v) sodium hypochlorite for 15 min. Meristems (0.1-0.3 mm) from apical and lateral buds were excised from plants using a dissecting microscope. The excised meristems were placed onto culture medium in a growth chamber at 25°C with a Photosynthetic Photon Flux Density (PPFD) of 50 µmol m^{-2} s^{-1} with a photoperiod of 16 h light/8 h dark. The meristems were transferred weekly onto fresh medium. After 6 to 8 weeks of growth, seedlings were obtained, which were micropropagated for indexing. The plants were evaluated at the IPC for any persistent virus infection. The following tests were carried out: nucleic acid spot hybridization (NASH) for the detection of potato spindle tuber viroid (PSTVd) and serological enzyme linked immunosorbent assay (ELISA) tests of indicator plants (Lizarraga et al., 1980). Pathogen-free plants generated from meristems with a height of approximately 15 cm and diameter of 1.5 mm were sectioned into nodal segments and placed in culture

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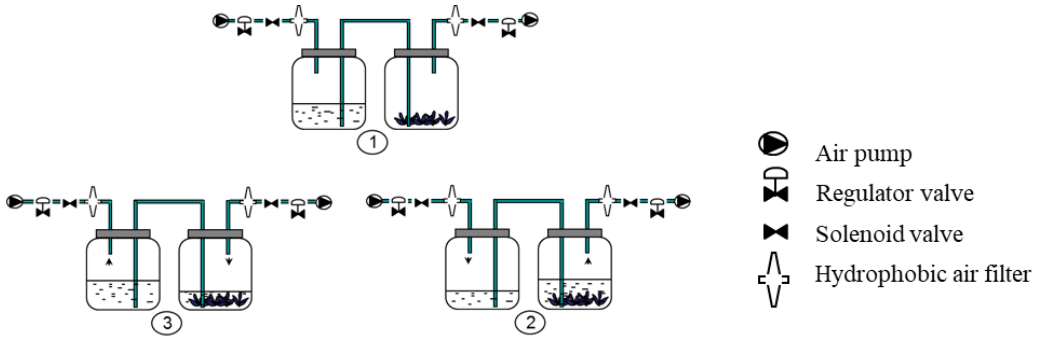

Figure 1. Operating cycle of a TIB. (1) Non-immersed stage, plant materials were free-standing on the bottom of the culture vessel. (2) Beginning of the immersed stage; an overpressure were applied and the medium was pushed up into the plant container immersing the plant material for 4 min. (3) End of the immersed stage, a second solenoid valve was opened and the culture medium was removed into the reservoir. These steps were performed every 3 h. The air pump and electric valves were under control of a timer.
Source: Lorenzo et al. (1998) and Escalona et al. (1999).

flasks with semi-solid medium for further multiplication.

The microtubers (pre-basic agamic seed) were obtained in 4 L-TIB (Lorenzo et al., 1998; Escalona et al., 1999) (Figure 1) in two stages, the first targeted for growth and multiplication of the nodal segments and the second was for microtuberization. The inoculation density was 50 explants/TIB (segments with 2 nodes) and 15 mL medium per segment in the bioreactors. Plants were immersed in liquid medium for a frequency of 4 min every 3 h over a period of 28 d under a photoperiod 16 h light/8 h dark. The microtuberization was induced in the same bioreactor by replacing the nutritive medium with the same medium enriched in 50 g/L sucrose to reach 80 g/L as final concentration. In such conditions, microtubers were induced after 60 day in the dark at 22°C.

All culture media were composed of Murashige and Skoog (1962) salts and vitamins modified as follows: 1750 mg/L ammonium nitrate, 2 g/L potassium nitrate, 450 mg/L calcium chloride, 175 mg/L phosphate, 0.4 mg/L thiamine, 2 mg/L glycine, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 1.0 mg/L calcium pantothenate, 1 mg/L folic acid and 4 mg/L arginine. The medium was supplemented with 100 mg/L myo-inositol. Sucrose was supplied at 30 g/L except for the microtuberization step which contained 80 g/L. The pH of all culture media was adjusted to 5.8 before autoclaving by steam sterilization at 121°C and 1.2 kg/cm^2.

In vitro culture procedure to obtain basic 1 and 2 agamic seeds

In order to obtain basic agamic seed 1, microtubers were rinsed and placed in trays exposed to ambient environmental conditions (natural light and 22 ± 2°C) for a month until they started budding. Before planting, they were disinfected with a 1 g/L (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 the same as described earlier.

Assessment of agronomic performances

The performances of Peruvian Canchan potato plants derived from different propagation methods were compared in May to August, 2018. The following planting materials were used (about 50 g/seed tuber; planting density: 0.3 m × 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 days of field growth, agricultural traits of plants were evaluated (Egúsquiza, 2014). Plants grew on sandy soil, without any fertilizer or pesticide, at 200 m above sea level, with superficial irrigation, and at 15 to 27°C. Average rainfall was 0.12 mm and temperature 16.7°C.

Statistical analysis

The field experiment was composed of 4 independent blocks used as replicates. Each block was divided in three lines of 15 plants, each line representing one of the 3 planting/propagation material tested. In total, 60 plants for each type of propagation material were characterized agronomically. All data were statistically evaluated using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform one-way analysis of variance (ANOVA) and Tukey post-hoc tests (p=0.05).

RESULTS AND DISCUSSION

Potato agamic seed production remains a challenge in many developing countries. TIBs have been used in attempts to improve the efficiency of microtuber production for this purpose. However, it is necessary to first evaluate the field performance of propagules produced in this manner before this technology can be rolled out. This is particularly important when local cultivars are used under resource constrained conditions, as is the case in potato production in Peru. Hence, the current study investigated the field performance of Canchan potato microtubers produced in TIBs cultivated under typical low input systems characteristic of this country. The performances of this new planting material

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 days	Percentage of sprouting*	80.0 ± 7.3^{c}	90.0 ± 8.7^{a}	85.0 ± 7.6^{b}
After 75 days	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 days	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 $y' = 2 \arcsin(y/100)^{0.5}$. Vertical bars represent ±SE of original data.

were compared to those of conventional tubers propagated by farmers themselves in non-controlled conditions.

The agronomic performances of microtubers derived from cultures in TIBs are summarized in Table 1 and Figure 2. Sprouting of tubers was recorded following 30 days of field growth and other agronomic parameters after 75 and 150 days. 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 (that is at 75 days), 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 the aforementioned 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 tubers/plant). No significant difference was observed between the three planting materials for the tuber FW per plant and the diameter and length of tubers.

The field trial was harvested 150 days after planting and final agronomic performances of plants depending on the sowed tuber type were assessed. In contrast to the preliminary findings at 75 days, the plants derived from both types of microtubers derived from TIB-basic agamic seed 1 and 2 generated the significantly highest numbers of tubers per plant (12.6 and 10.9, respectively) while the plants grown from commercial tubers produced 8 tubers per plant (Table 1). However, these plants grown from commercial tubers still yielded the highest tuber FW/plant (143.9 g), compared with basic 1 and 2 agamic seeds (86.6 and 93.0 g, respectively). Furthermore, the 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 the 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 is 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 tuber propagation. The results showed that the significantly higher numbers of agamic seed tubers per plant were produced by plants generated by microtubers basic agamic seeds 1 (8.5) and 2 (6.7) in comparison with plants from commercial tubers yielding very low number (3.0) (Figure 2A). Then, an elevated

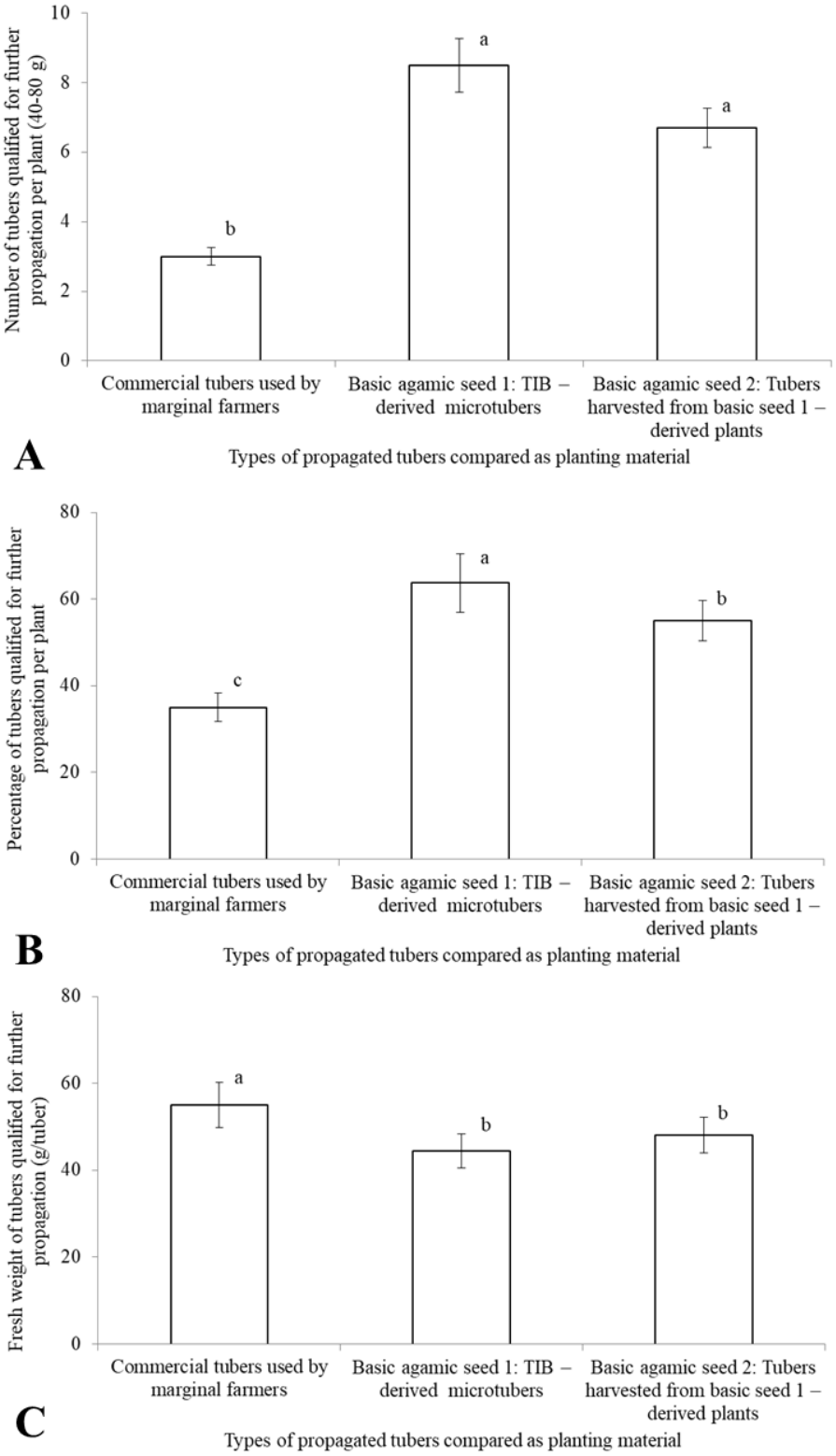

Figure 2. Quality of the materials obtained for further propagation after 150 days of planting. Results with the same letter are not statistically different (one-way ANOVA, Tukey, $p>0.05$). For the statistical analysis only, numbers of tubers were transformed according to $y" = y^{0.5}$, and the percentage as $y" = 2 \arcsin(y/100)^{0.5}$. Vertical bar represent ±SE of original data.

percentage of tubers harvested on plants generated from basic agamic seed 1 (63.7%) can be used for further new propagation cycle, higher than those obtained with plants from basic agamic seed 2 (55%) and much higher than the low percentage obtained with plants from commercial tubers (35%) (Figure 2B). In terms of the FW of tubers, selectable for subsequent propagation cycles, plants raised from commercial tubers produced bigger tubers (55 g) than plants from microtubers that produced similar tubers in a range of 44 to 48 g (Figure 2C). The highest emergence of sprouts observed in microtubers derived from bioreactors during the initial 30 days-vegetative growth stage must be related to the setting up of a series of particular biochemical, physiological and morphological processes leading to the induction of buds to ultimately give rise to new organs of the plant (Wattimena et al., 1983). This process is also influenced by environmental conditions and genotype (Dieme and Sy, 2013; Wróbel, 2015). It has been postulated that the size of tubers might influence the rate of emergence, however there are conflicting reports regarding this. Ranalli et al. (1994) reported that smaller tubers were prone to slower rates of emergence with reduced vigor of plants while Kawakami and Iwama (2012) found differences in emergence only with the smallest tubers tested (0.3 - 0.5 g) and not with other size classes. However, this observation was not consistent across years. Others authors argue that it is the nutrient status of tubers that determines sprouting. In this regard, Escalona et al. (2003) highlighted that the superiority of *in vitro* derived microtubers is as a consequence of the improved assimilation of nutrients that occurs under *in vitro* conditions leading to more vigorous tubers than from conventional sources. It has been shown that a substantial pool of reserves in tubers, particularly carbohydrates, allows for further more efficient plant development (Desire et al., 1995). In addition, the method of *in vitro* propagation has also been reported to affect nutrient accumulation in microtubers and subsequent plant emergence. For example, potato microtubers generated from *in vitro* culture on semi-solid nutritive media have been reported to display lower percentages of emergence (Lommen and Struik, 1994).

Despite the higher levels of sprouting observed in microtubers derived from bioreactors, these plants displayed the slowest relative growth rates within the first 75 days 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 on the contrary 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 basic agamic seed 2 originated from the same source, that is, culture in TIBs, the latter was able to produce more vigorous plants. Ultimately, the faster vegetative development of these plants derived did not translate into higher tuber yields as both plant types produced similar numbers of tubers with comparable morphological characteristics.

Wattimena et al. (1983) and Leclerc and Donnelly (1990) also found no differences in the FW of tubers per plant between basic agamic seed 1 and 2. Similar results were obtained by Kawakami and Iwama (2012) who observed that the differences found in the vegetative development of plants from microtubers and commercial tubers disappeared as plants developed, with no significant differences in the number of tubers produced at harvest. However, tubers produced from commercial sources were larger (with higher diameters and lengths) and heavier than those produced from basic agamic seed 1 and 2. Similarly, Kawakami et al. (2003), Wróbel (2015) and Higgins et al. (2017) also reported a larger fresh mass of tubers generated from commercial tubers. Hence, in the present study, it was observed that fewer, larger tubers were produced by plants raised from commercial tubers while many smaller tubers were produced by basic seed 1 and 2. A similar finding was reported by Wattimena et al. (1983) in potato and by Jova et al. (2011) in yam. The reason for this observation is not clear but a few suggestions have been proposed. For example, it has been highlighted that the temporary immersion system permits contact between liquid culture medium and all parts of the plant for prescribed amounts of time. This allows for the induction of tubers in a more uniform manner among axillary buds leading to the formation of more tubers (Jiménez et al., 1999). It has also been suggested that microtubers might have a greater number of eyes than tubers from other sources leading to more stems which in turn could generate more tubers (Radouani and Lauer, 2015). Regardless of the exact mechanism involved, 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, 2009b, a) leading to deterioration

(particularly in informal or non-certified schemes). These results suggest that the use of agamic seed from biotechnological methods (microtubers from temporary immersion: pre-basic) would be especially useful in countries where the production of quality agamic seed with phytosanitary conditions is challenging. In addition, the observation in the current study 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 evidences the possible use/potential 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.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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