Plant Cell Culture & Micropropagation

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Influence of 'atlantic' potato plant density under different micropropagation methods

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ABSTRACT

Potato (*Solanum tuberosum L.*) is among the most produced crop in Brazil. Since its propagation is vegetative, the plant presents problems due to its susceptibility to diseases. Thus, an *in vitro* propagation has been used to enable the production of quality plantlets with excellent sanitary conditioning in a short period of time. Aiming at optimizing micropropagation, bioreactors that allow the use of a liquid medium without the use of gelling agents were developed. The objective of this research was to study the effect of explant density for mass production of potato plantlets using a temporary immersion system (TIS) in comparison to conventional micropropagation. For potato culture, the conventional method using semi-solid medium presented better results for most of the variables analyzed when compared to TIS. The treatment using 60 plants was superior to 90 plants for explant fresh mass and the variables callus diameter and number of leaves in TIS, did not present significant results at the 5% probability level by the F test.

Index terms: Bioreactor; *in vitro*; *Solanum tuberosum* L.*;* TIS.

INTRODUCTION

Potato (*Solanum tuberosum L.*) is the third most produced vegetable crop in Brazil, with 3 688 029 tons of production in an area of 118 297 hectares (FAO, 2019). The main factor influencing plant yield is the seed tuber utilized. Since the conventional method of vegetative propagation of potatoes is very inclined to infection by pathogens such as fungi, bacteria and viruses, *in vitro* tissue culture becomes very advantageous, because originates plants with high sanitary quality (Altindal; Karadogan, 2010; Wang; Hu, 1982).

This biotechnological tool offers advantages in the propagation of various plant species and includes various cultivation techniques in a nutrient medium for plant cells, tissues or organs, under aseptic conditions and controlled photon flux density, photoperiod and temperature (Carvalho et al., 2011). It is based on the introduction of already disinfected mother plant tissue, denominated explant, in a sterile and species-specific culture medium, generating a plant genetically identical to its mother plant (García-Gonzáles et al., 2010; Singh, 2018). For potato culture, the most suitable nutritive medium is MS (Murashige; Skoog, 1962; Santiago et al., 2015). Badr; Angers; Desjardins (2011) observed that the addition of 30 g L^{-1} sucrose to this medium resulted in higher production of root biomass, smaller leaves and smaller length of internodes, in addition to favoring the accumulation of various substances such as sucrose, glucose and fructose, which play an important role during *ex vitro* acclimatization of potato plants.

In vitro cultivation in a conventional way uses nutrient media of gelatinous consistency (semi-solid) for better fixation of the explants. This consistency is acquired through the addition of gelling agents, usually based on agar, which is a hydrocolloid extracted from red seaweed and composed of two polysaccharides, agarose and agaropectin. This type of medium has disadvantages that increase the cost of production, such as a large need for labor, use of large amounts of bottles, need for costly components and low multiplication rate and biomass (Carvalho et al., 2011; Lemos, 2013; Teixeira, 2011).

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Aiming at optimizing this method, bioreactors were developed allowing the use of liquid media that provide greater contact with the plant and thus, a better absorption of nutrients (Lemos, 2013). Among the forms of immersion used in bioreactors, the temporary immersion system (TIS) is the most suitable cause reduces the prolonged contact of the explant with the medium, keeping it in motion, increasing the airing rate of the explants and increasing gas exchange (Teisson; Alvard, 1999) avoiding physiological disorders such as hyperhydricity, very common in permanent immersion bioreactors (Teixeira; Cid, 2014). Hyperhydricity is the physiological state in which the plant presents an abnormal accumulation of water inside cells and tissues, generally resulting in a characteristic translucent appearance (Fernadez-Garcia; Garma; Olmos, 2010). Peréz-Alonso et al. (2007) in their research state that the explant density and the immersion time should be optimized for production and quality increase, and in their study, the best results were obtained using 60 explants per bottle.

The aim of the research was to evaluate the effect of explant density on potato plant development using a temporary immersion bioreactor (TIS) and compare with conventional micropropagation.

MATERIAL AND METHODS

The potato (*Solanum tuberosum* L.) seeds cultivar Atlantic used to obtain the explants were cultivated in a semi-hydroponic system and were supplied by Castrolanda Cooperativa Agroindustrial Ltda, located in Colonia Castrolanda - Castro / PR, Brazil. These potatoes were kept in the dark at room temperature until the sprouts emerged.

Nodal segments with an average size of 2.0 cm, obtained from plants established *in vitro*, were placed in ½ MS liquid and semi-solid medium, supplemented with myo-inositol (100 mg L^{-1}) and sucrose (30 g L^{-1}) with the addition of agar (6 g L^{-1}) to the semi-solid medium. The pH of the nutrient medium was adjusted to 5.8.

The TIS works as described by Ayub et al. (2019). In the present experiment, were used two lines composed of three double flasks with a capacity of 600 mL each, programmed with two minutes immersion period and six hours interval. The explants were introduced in liquid medium and semi-solid medium in proportional densities, according to the defined treatments.

The experimental design is completely randomized, in a 2x2 factorial scheme, consisting of 2 explant densities (60 and 90 plants) and 2 micropropagation methods (TIS and conventional system) (Figure 1), totaling 4 treatments, with 3 replications. The flasks were kept in a growth room, with a temperature of 25 ± 2 °C, 16 hours photoperiod and a photon flux density of 27 µmol $m^{-2}s^{-1}$ for the light period. After 60 days, explant length (cm), number and fresh mass of shoots (g), root length (cm), number of hyperhydric plants and number of leaves were evaluated. Data were analyzed by the statistical program R Studio (RStudio Team, 2016) and the significant variables compared by Tukey's test at 5% significance.

RESULTS AND DISCUSSION

There was no interaction between plant density and cultivation method in any of the analyzed variables.

For the variable fresh mass, the density of 60 plants presented statistically superior results to density of 90 plants, with averages of 0.244g and 0.156g, respectively (Figure 2). According to Pérez-Alonso et al. (2007) the use of low densities can cause underutilization of culture flasks and growth rooms capacity, on the other hand, high densities lead to poor phenotypic formation of plants, reducing their quality, probably because of low oxygen availability inside the container due to large amount of biomass production. Corroborating our findings, Rahman et al. (2015), investigating potato micropropagation in nutrient spray bioreactor systems founded that 60 explants on each 250 mL of liquid medium was the best density, against 90 with the worst results.

Even though the media composition is the same, except for the agar addition in the conventional method, explant length, roots and number of shoots were statistically superior in the conventional system (Figure 3 A, B and C). This may have occurred because the composition of this medium is not the most suitable for potato cultivation in TIS, as according to Zhu, Li and Welander (2005) the sensitivity of cells and tissues can vary significantly between this system and the conventional one. This result may also be related to the immersion time applied, since the variable hyperhydricity was statistically superior

in TIS when compared to the semi-solid medium (Figure 2 D). According to Ziv (2005), there is an inversely relationship between the agar concentration in the nutrient medium and the degree of tissue hyperhydricity, since the culture in TIS showed hyperhydricity more often because the immersed tissues are under greater oxidative stress, with high concentrations of some reactive oxygen species. Tis is an alternative to control this disorder as it allows aeration of the liquid medium (Vasconcelos et al., 2012), but the immersion time must be optimized to ensure production efficiency (Pérez-Alonso et al., 2007). Another possibility to reduce hyperhydricity in TIS is to use higher concentrations of sucrose. Ayub et al. (2019) found a reduction in the number of vitrified blackberry plants grown in bioreactor when the amount of sucrose was high.

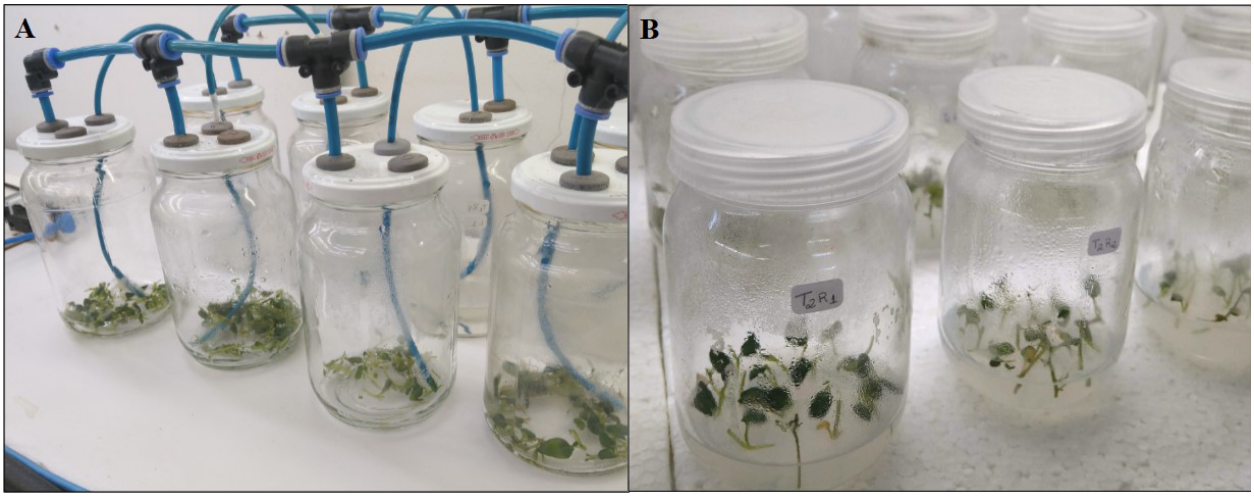

Figure 1: Micropropagation methods. TIS (A) and conventional (B) system.

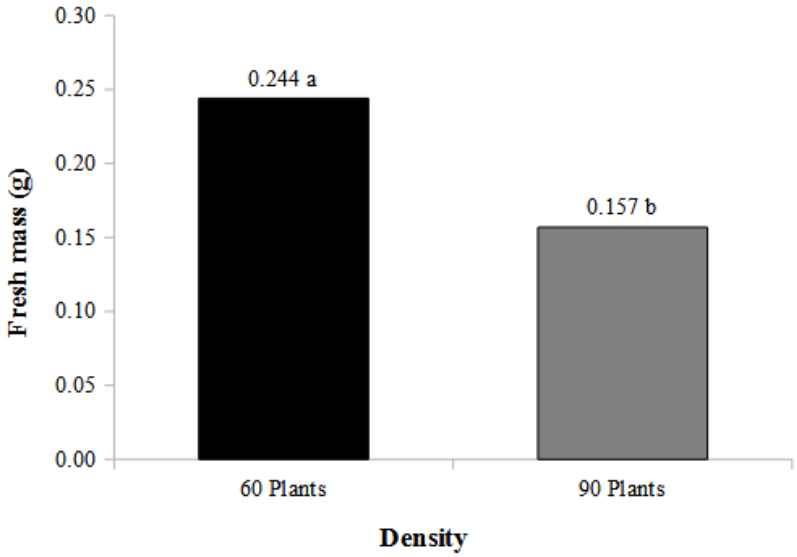

Figure 2: Fresh mass of potato cv. Atlantic explants in relation of plant density grown in TIS and semi-solid medium. Same letters are not different from each other according to Tukey's test at the 5% probability level.

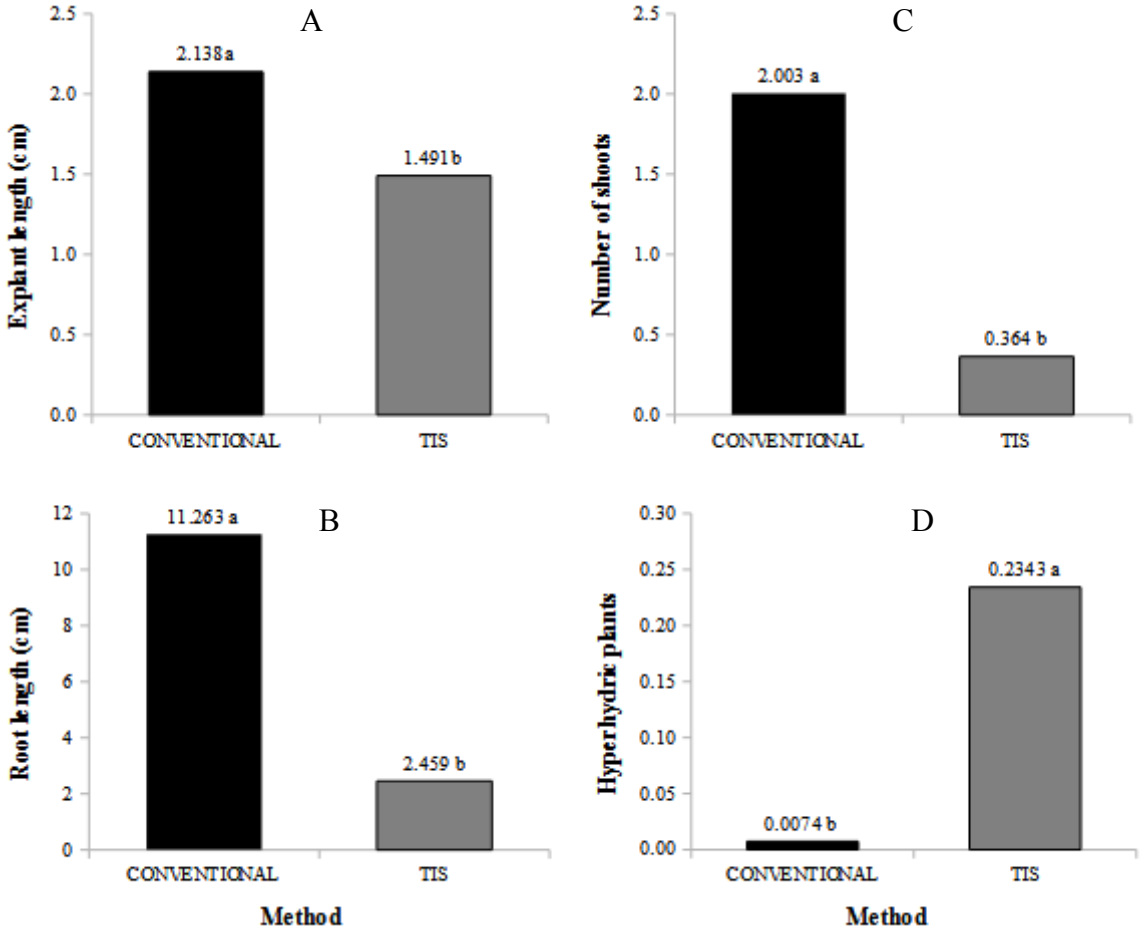

Figure 3: Explant length (A), root length (B), number of shoots (C) and number of hyperhydric plants (D) of potato cv. Atlantic in relation to methods of propagation (conventional micropropagation and TIS). Same letters are not different from each other according to Tukey's test at the 5% probability level.

The variables callus diameter ($\bar{X}$ 0.6246 mm) and number of leaves ($\bar{X}$ 1.007) did not present different means according the F test.

CONCLUSIONS

For potato culture the conventional method, using semi-solid medium, showed better results for the variables number of shoots, plant length and root length in comparison to TIS. To use TIS in this culture, the concentrations of the culture medium components, immersion time and plant density require further studies. The treatment with 60 plants was superior to 90 plants for fresh mass. It was not observed interaction between plant density and cultivation method in any of the analyzed variables.

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