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Efeito da concentração de ágar no meio de cultura no desenvolvimento in vitro de plantas de batata

Effect of agar concentration in culture medium on in vitro development of potato plants

Efecto de la concentración de agar em medio de cultivo sobre el desarrollo in vitro de plantas de papa

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Resumo

A técnica de propagação *in vitro* e cultivo de tecidos é alternativa para obter sementes sadias de batata. Entretanto, não há concordância sobre a consistência ideal do meio de cultura a ser utilizado *in vitro*. O objetivo neste estudo foi verificar a adaptação de cultivares de batata em meios de cultura com diferentes concentrações de ágar. O delineamento experimental foi inteiramente casualizado em esquema fatorial. O experimento testou o efeito de 3

concentrações de ágar do meio de cultura (sólido, semissólido e líquido) em 6 cultivares (Agata, Bel, Camila, Catucha, Clara e Eliza), com 5 repetições. Das plântulas foi quantificada a massa fresca e seca de raízes e da parte aérea, o número de nós, a capacidade de multiplicação da plântula e seu vigor. O estudo mostrou que as cultivares de batata respondem significativamente a diferentes concentrações de ágar, sendo que sua melhor resposta foi no meio semissólido.

Palavras-chave: Consistência; Micropropagação; Plântula; Semente; *Solanum tuberosum* L.

Abstract

In vitro propagation and tissue culture technique is an alternative for obtaining healthy potato seeds. However, data found in the literature differ on the optimal consistency of the culture medium to be used *in vitro*. The aim in this research was to look at the adaptation of potato cultivars to medium with different agar concentrations. The experimental design was entirely randomized in a factorial scheme. The experiment tested the effect of 3 agar medium concentrations (solid, semisolid and liquid) on 6 cultivars (Agata, Bel, Camila, Catucha, Clara and Eliza), with 5 repetitions. We quantified from the plantlets, fresh and dry mass of roots and shoot, the number of nodes, the multiplication capacity and vigor. The study showed that potato cultivars respond significantly to different concentrations of agar and their best response was in the semisolid medium.

Keywords: Consistency; Micropropagation; Plantlet; Seed; *Solanum tuberosum* L.

Resumen

La técnica de propagación *in vitro* y cultivo de tejidos es una alternativa para obtener semillas de papa saludables. Sin embargo, los datos encontrados en la literatura difieren en la consistencia ideal del medio de cultivo que se utilizará *in vitro*. El objecto en este estudio, es verificar la adaptación de los cultivares de papa a los medios de cultivo con diferentes concentraciones de agar. El diseño experimental fue completamente al azar en un esquema factorial. El experimento probó el efecto de 3 concentraciones de agar del medio de cultivo (sólido, semisólido y líquido) en 6 cultivares (Agata, Bel, Camila, Catucha, Clara y Eliza), con 5 repeticiones. A partir de las plántulas, se cuantificó la masa fresca y seca de las raíces y la parte aérea, el número de nodos, la capacidad de multiplicación de las plántulas y su vigor. El estudio mostró que los cultivares de papa responden significativamente a diferentes concentraciones de agar, y su mejor respuesta fue en el medio semisólido.

Palabras clave: Consistencia; Micropropagación; Plántula; Semilla; *Solanum tuberosum* L.

1. Introduction

Potatoes (*Solanum tuberosum* L.) have high social and economic importance, corresponding to the fourth most produced food in the world, surpassed only by wheat, corn, and rice (FAOSTAT, 2020). Its consumption is highly recommended for its energy properties derived from carbohydrates (Fernandes et al., 2015). There was a production of about 368 million tons of potato on 17.5 million hectares in the world in 2018 (FAOSTAT, 2020), of which around 3.7 million tons were produced in Brazil on 118 thousand hectares (IBGE, 2020).

Commercial potato cultivation is characterized by vegetative propagation through tubers. Therefore, the propagating material needs periodic renewal, as it may suffer degeneration, i.e., cumulative contamination from viruses, fungal and bacterial diseases (Al-Hussaini et al., 2015; Pereira & Fortes, 2003). Tissue culture is an alternative to obtaining high health seeds, the technique consists of aseptically cultivating cells, tissues or organ fragments of a given plant in artificial culture medium under a controlled environment, aiming at the development of new plants (Morais et al., 2018). This cultivation method is characterized by speed and a high rate of multiplication in a short time (Mohapatra & Batra, 2017).

In in vitro multiplication, the use of an appropriate culture medium for each stage of cultivation is an essential requirement; these mediums provide the nutrients necessary for the metabolism of plant cells in cultivation for growth and tissue differentiation (Kozai et al., 1997). Most *in vitro* potato micropropagation studies use the culture medium in the semisolid state, as they provide satisfactory multiplication rates of selected clones (Dutra et al., 2010). However, data found in the literature differ as to the appropriate consistency for potato. The use of liquid culture medium has provided equal or even greater efficiency for the multiplication rate of plant material (Pereira & Fortes, 2004). Therefore, it is necessary to investigate what is the best consistency of the culture medium for the development of *in vitro* potato plantlets.

The objective of this research is to provide data on the development of potato cultivars under different concentrations of the solidifying agent agar in the culture medium. Also, we want to obtain *in vitro* potato plantlets of phytosanitary quality, high vigor, and high propagation rate.

2. Material and Methods

This study used laboratory researches, and the nature of the word was quantitative (Pereira et al., 2018).

2.1 Experiment location

The experiment was conducted at the Tissue Culture Laboratory of the Center for Advanced Environmental Bioenergetic and Biotechnological Research (AMBIOTEC), Cedeteg campus, of the Midwestern Parana State University, UNICENTRO. The study was conducted in partnership with Biotech Seeds, a company currently incubated at the University.

2.2 Experimental design

The experimental design was entirely randomized in a factorial scheme. The experiment tested the effect of 3 concentrations of agar in the culture medium in 6 cultivars, with 5 repetitions.

The culture medium adopted as standard was that described by Murashige & Skoog (1962) (MS), with the adaptations indicated by Dutra et al. (2010). Three culture medium were tested according to the agar concentration: 1% agar (solid culture medium), 0.7% (semisolid culture medium) and 0% (liquid culture medium). The six cultivars used were: Agata, BRS Clara (Clara), BRS Eliza (Eliza), BRSIPR Bel (Bel), BRS F63 (Camila), and EPAGRI 361-Catucha (Catucha).

2.3 Study materials

Potato plantlets from micropropagation were acquired from Embrapa Temperate Agriculture. When they reached maximum growth *in vitro* (around 20 to 25 days to reach about 6 cm in height), they were sectioned, and each plantlet had one node/leaf. The explants were allocated to culture medium with different agar concentrations. The explants were directly allocated to solid and semisolid culture medium, with 10 explants per bottle. In liquid culture medium, germitest paper was used as support for the plantlet cuttings (Figure 1a). The plantlets remained for approximately 24 days in MS culture medium with different concentrations of agar.

2.4 Growth conditions

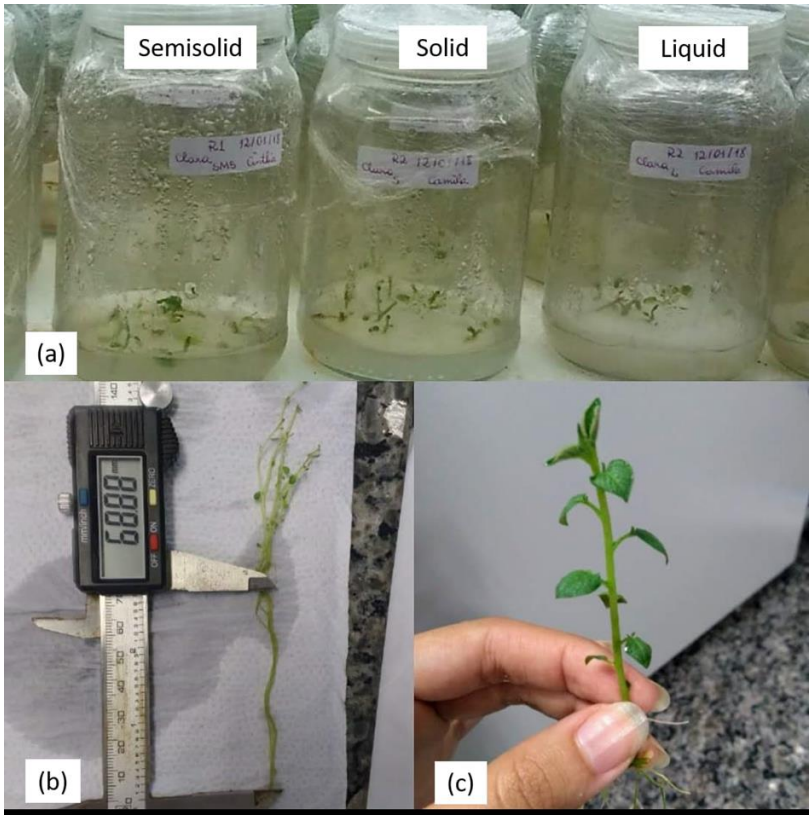
A glass bottle 13 cm high and 7.5 cm in diameter with a polypropylene cap was used (Figure 1a). The average temperature of the growth room was 24 ºC. The lamps used were Osram tubular fluorescent lamps, the amount of light was 90 μmol m^{-2} s^{-1}, and the duration of the photophase was 16 hours, being 8 hours the duration of the scotophase.

2.5 Measurements and statistics

From the plantlets originating from the explants (Figure 1c), the total fresh and dry weight (roots + shoot), plant height (Figure 1b), and the number of nodes were quantified. The vigor of the plantlets was determined by the ratio of total dry weight to height. The dry weight of the plants was determined after drying in an oven at 65 °C to constant weight.

The results obtained in each variable were submitted to analysis of variance, and when a significant effect was found ($p<0.05$), the mean comparative test of Tukey's ($p<0.05$) was performed.

Figure 1. *In vitro* grown potato plantlets under different agar concentration in the culture medium (a), measurement of the plant height (b) and vigorous potato plantlet obtained in *in vitro* cultivation (c).



Source: The authors.

3. Results and Discussion

In the present study, the objective was to obtain data that could clarify the divergences found in the literature regarding the adequate consistency of the artificial culture medium for the development of potato explants. As presented in the introduction, some studies describe the semisolid culture medium as ideal. However, other studies contest this information and demonstrate significant results for a higher rate of multiplication with the use of liquid medium.

In this study, there was no significant interaction between the cultivars and the agar concentrations used; that is, the cultivars respond in a similar way to variations in agar concentration. Thus, the tested cultivars can be managed similarly about the agar concentration in the MS medium.

The different agar concentrations resulted in significant differences for the variables: total fresh weight, total dry weight, and vigor (Table 1).

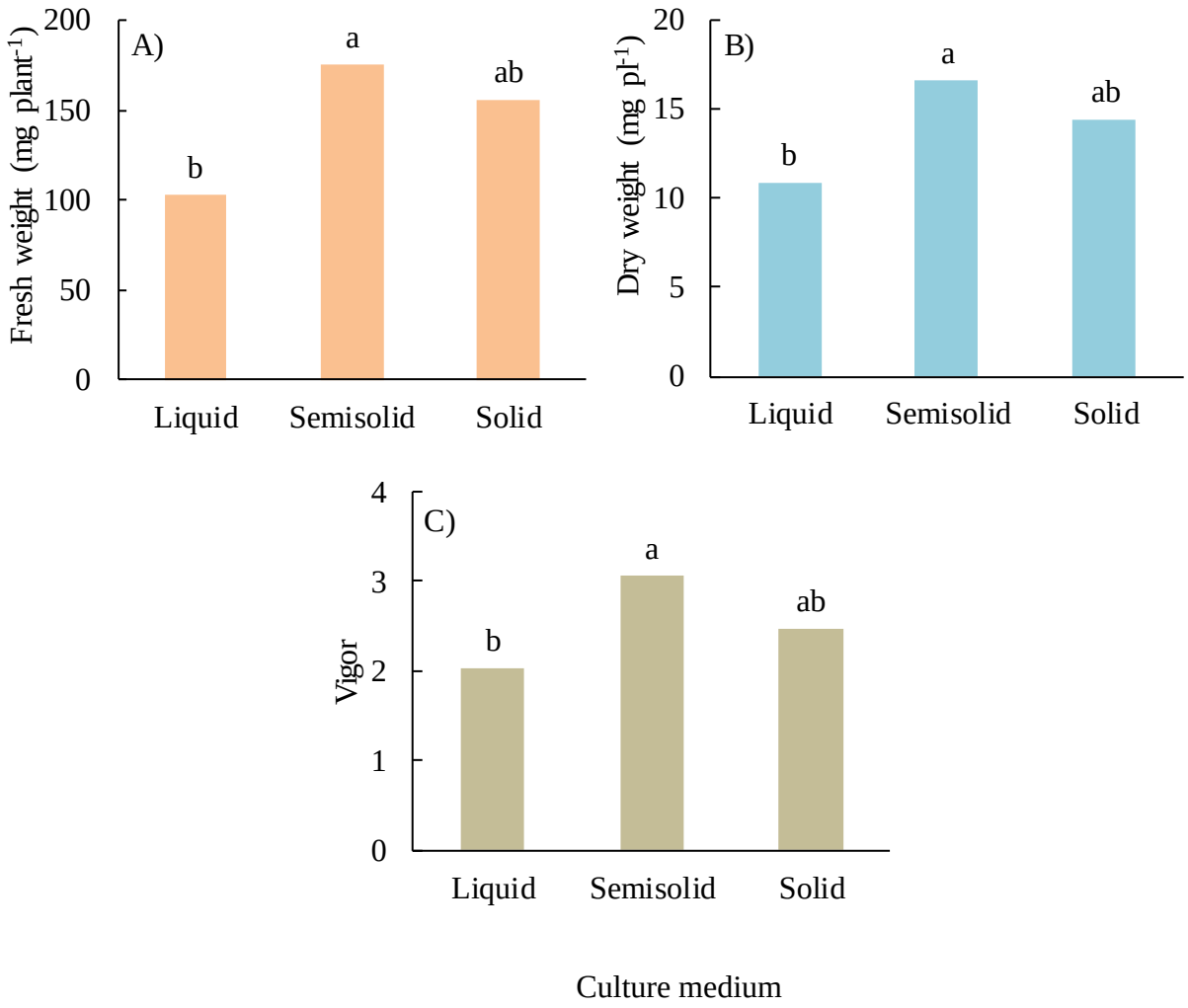
Table 1. Result of analysis of variance of different cultivars and agar concentration in the culture medium on morphological variables and quality of *in vitro* grown potato plantlets.

Variables	Plant height (cm)	Number of nodes	Fresh weight (mg)	Dry weight (mg)	Plant vigor
Agar concentration (AC)	ns	ns	**	**	*
Cultivar (C)	***	***	***	***	***
AC x C	ns	ns	ns	ns	ns
General mean	5.4	9.5	144.8	13.9	2.5

***, ** and *: significant statistical difference at 0.01; 0.1 and 0.5% probability, respectively. ns: non-significant statistical difference. Source: the authors.

The semisolid medium presented the best results for the measured variables (Figure 2), corroborating with the information described by Dutra et al. (2010) that the semisolid medium present satisfactory rates of multiplication.

Figure 2. Effect of agar concentration in the culture medium on total fresh weight (a), total dry weight (b), and vigor (c) of *in vitro* grown potato plantlets.



Means followed by the same letter do not differ significantly by Tukey's 5% probability test. Source: the authors.

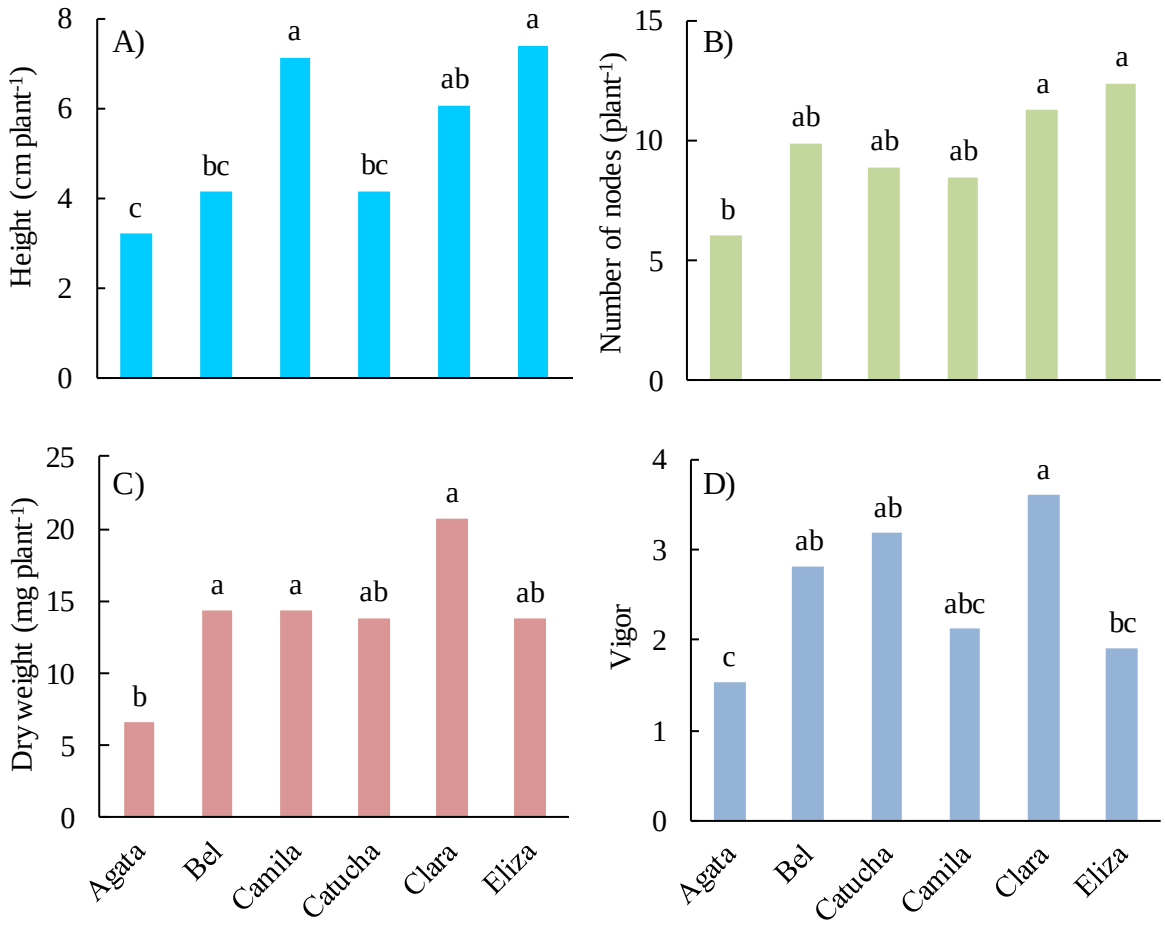
Unlike what occurred in the present study, in the experiment performed by Pereira & Fortes (2003), it was found that the fresh and dry weight of plants were significantly higher when cultivated in a liquid medium. Better results were obtained in the liquid medium compared to solid medium because high concentrations of agar, or very consistent medium, end up limiting the diffusion of nutrients until the explant (Andrade et al., 2011; Siqueira et al., 2013). Since the use of liquid culture medium has advantages over semisolid medium, the liquid medium may not be appropriate for some species, as it may induce hyperhydricity (i.e., vitrification) of sprouting (Mengarda et al., 2009).

In this study, we had a limitation because we did not have agitator equipment for the liquid medium, which could have limited the efficiency of nutrient arrival to the roots, thus being able to diminish the response of the plants to the liquid medium.

From the data obtained, we also found significant differences between cultivars for all variables: plant height, number of nodes, total fresh and dry weight, and vigor (Table 1).

The plant height is associated with competition for environmental factors, especially for light around the plants (Stürmer et al., 2014). As for height, the cultivars Eliza, Camila, and Clara were the ones that achieved the highest growth, and cultivar Agata had the lowest plant height (Figure 3a). Between the highest and lowest height, there was a variation of 57% difference.

Figure 3. Effect of cultivar on plant height (a), number of nodes (b), total dry weight (c), and vigor (d) of *in vitro* grown potato plantlets.



Cultivars Means followed by the same letter do not differ significantly by Tukey's 5% probability test. Source: the authors.

The variable that can define the potential of each cultivar for the formation of new plantlets in each time interval is the propagation rate, and this rate is expressed by the number of nodal segments (Flores et al., 2015). In this question, the number of nodal segments of the cultivars Eliza and Clara was higher than in the cultivar Agata (Figure 3b).

The total dry weight of the cultivars Clara, Camila, and Bel differed significantly from the cultivar Agata (Figure 3c). This result corroborates another study that also observed that potato cultivars had different growth under *in vitro* cultivation (Rocha et al., 2015; Salem & Hassanein, 2017). Dry weight accumulation and its distribution in the plant are essential processes for determining the yield of a crop (Fernandes et al., 2010). The potato crop yield depends on the efficiency of the plant in synthesizing carbohydrates in the leaves and mobilizing them to the developing tubers (Kim & Lee, 2019).

The cultivar with the highest vigor value was Clara, but it did not differ statistically from the cultivars Catucha, Bel, and Camila (Figure 3d). Cultivar Agata had the lowest vigor value. Vigor is understood by the potential - strength, and robustness - of development and growth of a given plant.

The data obtained in this study show that cultivars have higher total dry and fresh weight production and are also more vigorous when grown in semisolid culture medium. These results are relevant in practice as these variables are essential in potato farming as they are linked to higher photosynthesis and, consequently, higher yield.

4. Final Considerations

Data found in the literature differ on the optimal consistency of the culture medium to be used *in vitro* for the growth of potato plantlets. In this study, we looked at the adaptation of potato cultivars to medium with different agar concentrations.

The objective of the article was completely achieved. The study shows that potato cultivars respond significantly to different concentrations of agar, and their best response was in the semisolid medium. These results provide further insight into the ideal consistency of the culture medium for this crop.

New studies using agitators for liquid medium are welcome to shed light on the improvement of *in vitro* cultivation of potato plantlets.

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