CORPUS PUBLISHERS

Archives of Agriculture Research and Technology (AART)

ISSN: 2832-8639

Volume 3 Issue 3, 2022

Article Information

Received date : September 20, 2022

Published date: December 23, 2022

*Corresponding author

Ricardo Antonio Ayub, Departament of Plant Sciences, Ponta Grossa State University, Brazil

DOI: 10.54026/AART/1041

Keywords

Micropropagation, polysaccharides, *Solanum tuberosum L.*

Research Article

Gelling Agents and Partial Agar Replacement *In Vitro* for Production of Potato Plants

Leticia Manuela de Morais, Jessica Andressa Wosniack, Camila Audrey dos Reis, Luiz Gustavo Batista and Ferreira and Ricardo Antonio Ayub*

Departament of Plant Sciences, Ponta Grossa State University, Brazil

Abstract

The potato crop (*Solanum tuberosum L.*) is susceptible to pathogens that reduce its production. The micropropagation technique is used to reduce contaminants in seedlings, which requires gelling agents, with agar being the most used, which increases the cost of production. This study consists of two experiments: one with the cultivar Atlantic, under a completely randomized design, containing five treatments, with four replications, where three concentrations of Agar (6,5 and 4 gL^{-1}) were tested, one concentration of Phytagel (2.5 gL^{-1}) and the partial replacement of Agar adding Galactomannan (5+5 gL^{-1}) and another with the Atlantic and Markies cultivars, under a completely causal experimental design, in a 2x5 factorial analysis, with the two cultivars and the same five types of gelling agents, containing 10 treatments, with four repetitions; 30 days after multiplication, morphometric evaluations were performed. After 30 days of acclimation, ex vitro survival, length of root, shoots and number of leaves were evaluated. For the statistics analysis, were used the R program and the variables compared using the Tukey test at 5 % of probability. It was concluded the partial replacement of Agar by Galactoman is possible, lowering the concentration of Agar to $5gL^{-1}$, where great results are obtained in the growth of explants, reducing the production cost in micropropagation and acclimatization of the cultivars Atlantic and Markies. There were no differences between agar at lower concentrations and the use of phytagel in the production of seedlings of cv.s Atlantic and Markies.

Introduction

The potato crop *(Solanum tuberosum L.)* is the third most produced vegetable crop in Brazil, with 3,688,029 tons, with an area of 118,297 hectares [1]. For the food industry, it is necessary to use cultivars of adequate shape and size and lack of physiological disturbances besides a high content of soluble solids, dry mass and light coloring after frying. Cultivar 'Markies' meets these requirements, possessing culinary properties for cooking and frying [2,3]. Another potato cultivar widely planted for chip processing industries in Brazil is 'Atlantic' [4,5].

As a conventional method of vegetative propagation, the potato is extremely prone to infection by pathogens, such as fungi, bacteria and viruses, and tissue culture in vitro is able to produce plants with high microbiological quality [6,7]. The most used culture medium is MS, and its consistency can be established with the use of gelling agents [8,9], which, depending on the type and concentration, can affecting the growth of explants [10,11]. The main distinction among the solidifying agents which influences the in vitro growth characters is the water retention capacity of the gels and the availability of nutrients to the cultured tissue [12].

According to the literature, the key gelling agents used for that context is Agar and Phytagel [13-15]. Agar is a hydrocolloid extracted from red seaweed and composed of two polysaccharides, agarose and agaropectin [13]. The concentration generally used for potato cultivation is 6 g L^{-1} of Agar [16]. Phytagel, a compound from the fermentation of bacteria, is considered superior to agar, as it is highly pure and translucent, which improves the production of plant shoots and reduces the cost of production [15]. Galactomannans are seed polysaccharides and despite having high viscosity it is not considered a gelling agent, however, has the potential to be used in micropropagation because they are non-toxic, stabilizers, thickeners and adhesives, controlling the viscosity of the solution [14]. Studies have verified that its use has positive results in proliferation, growth of shoots, rhizogenesis and can partially replace gelling agents, reducing costs [17].

Thus, this study aimed to carry out the different gelling agents in potato micropropagation and its acclimatization effects.

Material and Methods

Two experiments were carried out for this study. In the first experiment, potato explants *(Solanum tuberosum L.)* from the Atlantic cultivar, with an average size of 1.5cm, obtained from in vitro plants, were grown in MS medium [18], supplemented with 30g L^{-1} sucrose, 0.1g L^{-1} myo-inositol and gelling agents according to each treatment being T1: 4g L^{-1} agar, T2: 5g L^{-1} agar, T3: 6g L^{-1} agar, t4: 5 g L^{-1} agar + 5g-1 Galactomannan and T5: 2.5g L^{-1} Phytagel. The experimental design was completely randomized, with five treatments and four replications containing 4 explants each.

The second experiment repeated the first with explants of Atlantic and Markies cultivars in a completely randomized design, in a 2x5 factorial arrangement, with two cultivars and the same five treatments. Each treatment contains four replicates and each replicate is represented by a bottle containing 4 explants. The flasks were placed in a growth room, at a temperature of 25±2°C, with a 16-hour photoperiod and a light-period photon flux density of 27µmol m-2s-1. After 30 days, the evaluation of the variables number of shoots, whole roots, broken roots, number of leaves, shoot length, largest shoot and largest root, fresh mass and broken root mass was carried out. After 30 days of acclimatization of plants in 100ml plastic cups with Carolina Soil® commercial substrate, ex vitro survival, length of the largest root, shoot and number of leaves were evaluated. Data were analyzed using the R statistical program and variables with significant differences were compared using the Tukey test at 5% of probability.

Results and Discussion

In the first experiment, in the evaluation 30 days after multiplication, there was no significant interaction by the F test for the amount of shoots, whole roots, broken roots, length of the larger shoots, fresh mass and broken roots mass. There was a significant difference between the concentrations of gelling agents only for the largest root length where treatment with Phytagel 2.5 g L^{-1} was statistically superior to treatment with Agar 4g L^{-1} (Figure 1), as well as the result found in the work of Rodrigues et al. [15], where the researcher showed greater root length for the gelling agent Phytagel, in the cultivation of manna cubiu *(Solanum sessiliflorum)*.

Different media types have different physical properties, and root behavior on those media is altered potentially using similar mechanisms as would be employed in natural soils with differing obstacles. Harder media, for example, would prevent the root tip from penetrating the surface, while softer media would allow for roots to sink into the surface [19-21].

Agar was softer than Phytagel when measured on the texture analyzer, but was qualitatively stickier than Phytagel. Perhaps this stickiness increased physical interactions and exaggerated the amount of root skewing on agar. Phytagel contains different cross-linking carbohydrates, which likely contributes to the difference in tackiness between this gelling agents [22].

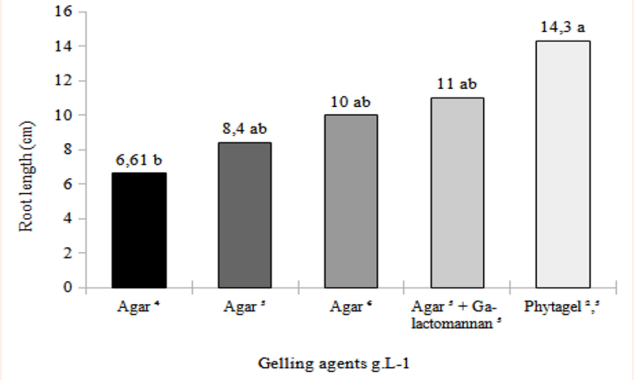

Figure 1: Gelling agents on the largest root length in potato micropropagation cv Atlantic. Lowercase equal letters are not differentiated from each other by Tukey test at the 5 % of probability.

The results obtained for the evaluations 30 days after the acclimatization of the plants did not show statistically significant differences between the treatments for the variables length of the largest root, length of the aerial part, number of leaves and survival.

In the second experiment, the interaction between cultivar factors and gelling agents was not significant for the variables number of shoots, number of leaves, number of roots, number of broken roots, shoot length, length of the largest shoot, length of the largest root, fresh mass and callus diameter, within 30 days after multiplication. At 30 days after the acclimatization, there was no significant interaction between the factors for the variables root length, shoot length, number of leaves and survival.

However, at 30 days after multiplication, there was a significant difference only for shoot length (Figure 2) and length of the largest shoot among the gelling agents, where 5g L^{-1} Agar was statistically superior to Phytagel (Figure 3), corroborating the result found by Kaur and Kumar [23], where the development of potato plants in vitro was superior using agar when compared to Phytagel. As for cultivars, the variable fresh mass and number of leaves showed a significant difference, with cv. Atlantic statistically superior to cv. Markies (Figures 4 & 5). At 30 days after acclimatization, only the survival variable showed a significant difference between the levels of gelling agents, with Agar 5g L^{-1} and Agar+Galactomannan being statistically superior to Phytagel (Figure 6).

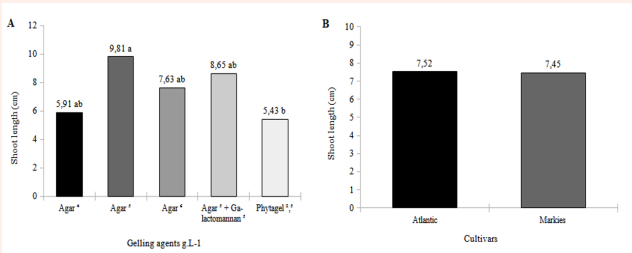

Figure 2: Gelling agents on shoot length in potato cv Atlantic and Markies (A) micropropagation and on shoot length cultivars in potato cv. Atlantic and Markies (B) Lowercase equal letters are not differentiated by Tukey test at the 5% probability level.

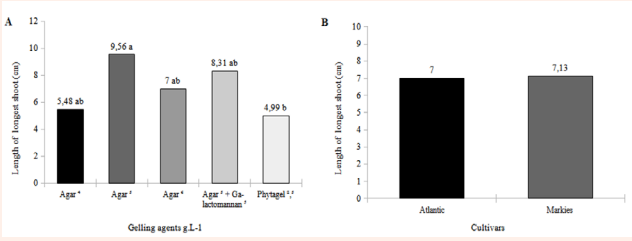

Figure 3: Gelling agents on the length of the largest shoot in the micropropagation of potato cv. Atlantic and Markies (A), and cultivars on the length of the largest shoot in the micropropagation of potato cv Atlantic and Markies. Lowercase equal letters are not differentiated from each other by Tukey test at the 5 % probability.

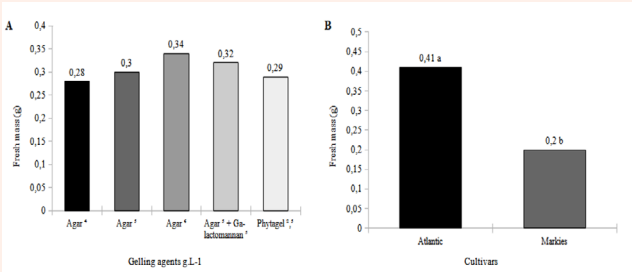

Figure 4: In A, gelling agents on fresh mass in potato cv. Atlantic and Markies. In B, cultivars on fresh mass in potato cv. Atlantic and Markies. Lowercase equal letters are not differentiated from each other by Tukey test at the 5 % probability.

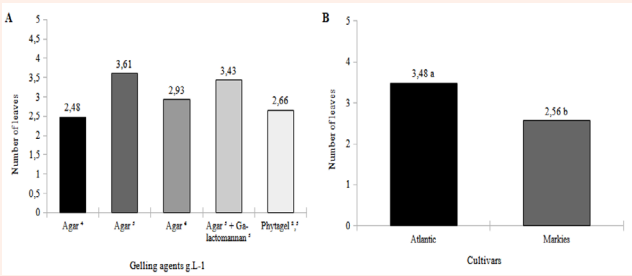

Figure 5: In A, gelling agents on the number of leaves in potato cv. Atlantic and Markies. In B, cultivars on the number of leaves in the micropropagation of potato cv. Atlantic and Markies. Lowercase equal letters are not differentiated from each other by Tukey test at the 5 %.

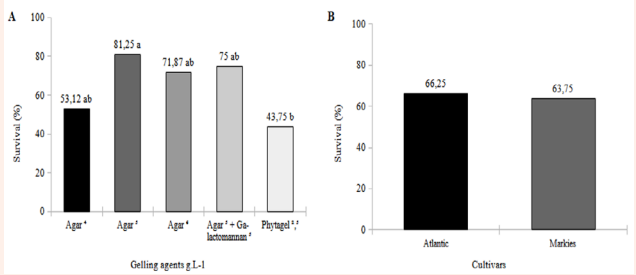

Figure 6: In A, Gelling agents on micropropagation survival of potato cv. Atlantic and Markies. In B, cultivars on micropropagation survival of potato cv. Atlantic and Markies. Lowercase equal letters are not differentiated from each other by Tukey test at the 5% probability level.

Thus, it can be stated that Agar can be partially replaced, as in the work of Gordo, Gonzalez and Pacheco [24], where they proved the effectiveness of partial replacement of Agar by Galactomannans in the in vitro propagation of strawberry.

Plants grown in medium gelled with Phytagel showed lower values for most variables. In contrast with our results, some researches found that Phytagel is better overall to potato micropropagation than agar [12,25,26]. More studies with known genotypes of Atlantic and Markies cultivars need to be done to confirm our findings [27].

Conclusion

Our results show that reduce the concentration of Agar used to 5g L^{-1} can be done with satisfactory results in explant growth, reducing production cost in micropropagation and acclimatization of Atlantic and Markies cultivars. Although Galactomannan does not interfere with plant development, the partial replacement of agar by it is not advantageous, as only the reduction of agar has already been shown to be satisfactory.

References

1. FAO (2020) Food and Agriculture Organization's Statistics.
2. Keijbets MJH (2008) Potato processing for the consumers: Developments and future challenges. Potato Research 51: 271-281.
3. Gonçalves DN, Pereira AM, Soares LG, Da Silva Guimarães ME, Petrucci KPDOS, et al. (2020) Post-Harvest Quality of cv. Markies Potatoes Submited to Mechancial Damages. Journal of Agricultural Science 12(2).
4. Evangelista RM, Nardin I, Fernandes AM, Soratto RP (2011) Nutritional quality and postharvest greening of tubers of potato cultivars. Brazilian Agricultural Research 46(8): 953-960.
5. Yagi R, Nazareno NRX, Soratto RP (2020) Agronomic and Economic Interactions between Sidedressed Nitrogen and Potassium Fertilizations on 'Atlantic' Potato. Brazilian Archives of Biology and Technology 63.
6. Wang P, Hu C (1982) *In vitro* mass tuberisation and virus free seed potato production in Taiwan. American Potato Journal, Orono-ME 59: 33-37.
7. Oseni OM, Pande V, Nailwal TK (2018) A review on plant tissue culture, a technique for propagation and conservation of endangered plant species. International journal of current microbiology and applied sciences 7(7): 3778-3786.
8. Mohamed MAH, Alsadon AA, AL Mohaidib MS (2010) Corn and potato starch as an agar alternative for Solanum tuberosum micropropagation. African Journal of Biotechnology 9(1).
9. Mohapatra PP, Batra VK (2017) Tissue culture of potato *(Solanum tuberosum L.)*: A review. International Journal of Current Microbiology and Applied Sciences 6(4): 489-495.
10. Shilpa S (2011) Influence of gelling agents and nodes on the growth of potato microplant. Potato Journal 38(1): 41-46.
11. Madege RR, Msemwa J (2015) Evaluation cassava, sweet potato and Irish potato starches as cheap alternative gelling agents for micropropagation of sweet potato (Ipomoea batatas L.). International Journal of Innovation and Applied Studies 12(2): 390.
12. Venkatasalam EP, Sood R, Pandey KK, Thakur V, Sharma AK, et al. (2013) Development of low cost technology for in vitro mass multiplication of potato *(Solanum tuberosum L.)*. African Journal of Agricultural Research 8(49): 6375-6382.
13. Carvalho ACPP, De Torres AC, Braga EJB, Lemos EEP, De Souza FVD, et al. (2016) Plant tissue culture glossary. Plant Cell Culture & Micropropagation, Recife 7(1): 30-60.
14. Soares PAG (2015) Development and characterization of a new hydrogel based on galactomannan and κ-carrageenan. Carbohydrate polymers 134: 673-679.
15. Rodrigues FA, Rezende RALS, Pasqual M, Lopes MTG (2017) Solidifying agents and activated carbon in the in vitro culture of Solanum sessiliflorum. Brazilian Agricultural Research, Manaus 52(11): 1123-1126.
16. Pereira JES, Fortes GRL (2003) Protocol for the production of potato propagation material in liquid medium. Brazilian Agricultural Research, Brasilia 38(9): 1035-1043.
17. Lucyszyn N, Quoirin M, Koehler HS, Reicher F, Sierakowski MR (2006) Agar/galactomannan blends for strawberry (Fragaria x ananassa Duchesne) cv. Pelican micropropagation. Scientia Horticulturae 107(4): 358-364.
18. Murashige T, Skoog FA (1962) Revised Medium for Rapid Growth and Bioassays with Tobaco Tissue Cultures. Physiologia Plantarum 15: 437-496.
19. Okada K, Shimura Y (1990) Reversible root tip rotation in Arabidopsis seedlings induced by obstacle-touching stimulus. Science 250(4978): 274-276.
20. Evans M (2003) Touch sensitivity in plants: be aware or beware. Trends Plant Sci 8(7): 312-314.
21. Bisgrove SR, Lee YR, Liu B, Peters NT, Kropf DL (2008) The microtubule plus-end binding protein EB1 functions in root responses to touch and gravity signals in Arabidopsis. Plant Cell 20(2): 396-410.
22. Schultz ER, Paul AL, Ferl RJ (2016) Root Growth Patterns and Morphometric Change Based on the Growth Media. Microgravity Sci. Technol 28: 621-631.
23. Kaur A, Kumar A (2020) The Effect of Gelling agente, médium pH and silver nitrate on adventitious shoot regeneration in Solanum tuberosum.
24. Gordo DAM, Gonzalez OC, Pacheco JC (2012) Substances used as gelling agent alternatives to agar in culture media for in vitro propagation. Agricultural and Environmental Research Magazine 3(2): 49-62.
25. Sharma S, Venkatasalam EP, Patial R, Latawa J, Singh S (2011) Influence of gelling agents and nodes on the growth of potato microplant. Potato Journal. 38(1): 41-46.
26. Al Shhab M, Shatnawi M, Abu RS, Almajdalawi M, Odat N (2021) Micropropagation and in vitro conservation of Ruta graveolens plants. Research on Crops 22(2): 398-409.
27. Peixoto PHP, Pasqual M (2015) Grapevine micropropagation: effects of pH and agar. Ceres, Judge from Outside 42: 242.