



"In vitro" maintenance of *Stevia rebaudiana* genotypes.

Gabriel de S. Leite*, Marcos N. Alves.

Resumo

Stevia rebaudiana Bertoni (Asteraceae) is an herb capable of producing non-caloric sweeteners sweeter than cane sugar. These sweeteners have not only pharmaceutical but socio-environmental importance¹. The industrial and economical interest opens up an opportunity for selection of genotypes and chemical profiles increasingly suited to such important demands, and the basis of this process can be achieved through the "in vitro" maintenance of these different genotypes through vegetative micropropagation. In this work, we developed an efficient propagation protocol of this species, and 10 genotypes of *S. rebaudiana* were established in our Germplasm Bank (GAB) at the Pluridisciplinary Center for Chemical, Biological and Agricultural Research - CPQBA/UNICAMP.

Palavras-chave:

Stevia, tissue culture, sweeteners.

Introduction

Stevia rebaudiana Bertoni (Asteraceae) is a native herbaceous from South America capable of producing sweetening diterpenes, which in addition to non-caloric, are 300 times sweeter than cane sugar and is therefore popularly known as sweet leaf. Other benefits of these sweeteners (such as stevioside and rebaudioside) include: antihyperglycemia, antidiabetes and even anticavities¹. This work aimed to improve the *in vitro* micropropagation process of *Stevia* varieties established in the CPQBA / UNICAMP Collection of Medicinal and Aromatic Plants (CPMA), thus maintaining a GAB (Germplasm Bank) with different potential genotypes to be used in improvement and selection programs, favoring pharmacological and economic evaluations and decisions.

Results e Discussions

From the general methodology of laboratory practice² and the methodology of explant collection and surface sterilization³, small changes were made in the sterilization method to test the improvement in the success rate of explant establishment. These, after sterilization, were inoculated into test tubes containing MS medium without addition of phytoestrogens in order to establish them aseptically. Sterilization assays were performed using different concentrations of sodium hypochlorite (3 and 5%) and Tween 20, and different treatment times (5 'and 10'). The material was maintained in growth room at 25 °C, photosynthetic photon flux density 40 mmol m⁻² s⁻¹ and photoperiod 14 hours light. After 10 days, the percentage of contamination of each treatment were analyzed. The best results were obtained using 6 drops of Tween 20 for each 100ml of 4% sodium hypochlorite for 7 minutes, with a 43% drop (from 64% to 21%) in losses due to fungal and /or bacterial contamination within periods of 10 days. For multiplication, the sterile explants were inoculated in MS medium containing auxins and cytokinins in diallelic combinations and after 30 days, rates of multiplication and growth were determined.

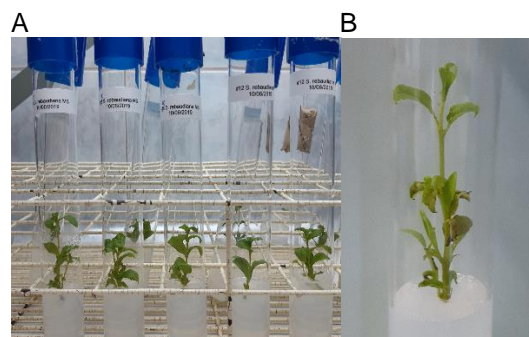


Figura 1. *S. rebaudiana* individuals successfully inoculated in culture medium (Murashige & Skoog) without addition of phytoestrogens (A) and the beginning of lateral sprout formation in MS medium plus auxins and cytokinins (B).

Conclusions

It is concluded that the relevance of care in asepsis and sterilization methodology are essential for the success of establishing the culture. The results have demonstrated that vegetative micropropagation is a tool that can serve as a method of preservation and maintenance of genotypes, serve as efficient method of clonal multiplication of elite genotypes, possessor of high levels of principles of interest. Qualitative and quantitative analyzes of steviosides and rebaudiosides present in each genotype have been carried out in order to select the most promising genotypes

Acknowledgments

I thank Dr. Marcos Alves and CPQBA / Unicamp for the opportunity to learn and grow professionally.

¹ LIMA FILHO, Oscar Fontão de; VALOIS, Afonso Celso Candeira; LUCAS, Zander Martinez (Ed.). **Sistemas de Produção 5: Estêvia**. 2004. Embrapa. Disponível em: <<https://www.infoteca.cnptia.embrapa.br/bitstream/doc/247888/1/SP52004.pdf>>. Acesso em: 01 jul. 2019.

² CARVALHO, Julita Maria Frota Chagas. **Técnicas de micropropagação**. 1999. Embrapa Algodão. Disponível em: <https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/271622?locale=pt_BR>. Acesso em: 04 jul. 2019.

³ NOORDIN, Norazlina; IBRAHIM, Rusli; SAJAHAN, Nur Hidayah. **MICROPROPAGATION OF *Stevia rebaudiana* Bertoni THROUGH TEMPORARY IMMERSION BIOREACTOR SYSTEM**. Agrotechnology and Biosciences Division, Malaysian Nuclear Agency. Disponível em: <https://inis.iaea.org/collection/NCLCollectionStore/_Public/44/096/44096871.pdf>. Acesso em: 05 jul. 2019.