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Tissue Culture on Stevia Plant

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Stevia *(Stevia rebaudiana Bertoni)*, a natural sweetener, has gained significant attention due to its non-caloric sweetness and potential health benefits. Traditional methods of propagation, such as seed or cutting propagation, have limitations in terms of speed, uniformity, and disease transmission. Tissue culture techniques have emerged as a promising alternative for the mass propagation of Stevia plants. This review aims to summarize the recent advances in Stevia tissue culture, including its applications, advantages, challenges, and future perspectives.

Introduction

Stevia rebaudiana Bertoni, a plant native to Paraguay and Brazil, is renowned for its sweet compounds, primarily steviol glycosides, which are 50–300 times sweeter than sucrose (Yadav et al., 2010). Due to its growing demand in the food, pharmaceutical, and cosmetic industries, efficient propagation of Stevia has become essential. While conventional propagation methods like seeds and cuttings are commonly used, they face challenges such as slow growth, genetic variation, and susceptibility to diseases (Nogueira et al., 2015). In this context, tissue culture techniques provide a controlled and reproducible approach to the propagation of Stevia plants.

Tissue Culture Techniques in Stevia Propagation

1. Callus Induction and Organogenesis: Tissue culture of Stevia typically begins with the establishment of an aseptic culture from explants like leaf, stem, or nodal segments. Callus induction, which involves the formation of undifferentiated plant tissue, is the first step in most protocols. Optimal callus formation requires specific plant growth regulators (PGRs), such as auxins (e.g., 2,4-Dichlorophenoxyacetic acid or NAA) combined with cytokinins (e.g., BAP or Kinetin) **(Rani et al., 2020)**.

Following callus formation, organogenesis is induced to differentiate shoots and roots. The concentration and type of PGRs influence this differentiation process. For shoot induction, combinations of cytokinins like BAP or TDZ (Thidiazuron) with lower concentrations of auxins are often used, while rooting is typically achieved using auxins like IAA or IBA (Indole-3-acetic acid and Indole-3-butyric acid) (Maffei et al., 2003).

2. Micropropagation: Micropropagation, which refers to the mass production of genetically identical plants through tissue culture, has proven effective for Stevia. Using shoot-tip or nodal explants, micropropagation ensures the production of uniform, disease-free plants. The process involves the steps of initiation, multiplication, rooting, and acclimatization. Key parameters, including light intensity, temperature, and nutrient composition of the medium, are crucial for maximizing shoot multiplication (Munir et al., 2018).

3. Somatic Embryogenesis: Somatic embryogenesis, the formation of embryos from somatic cells, has been reported in Stevia (Ali et al., 2019). This technique is advantageous for large-

scale production of plants, particularly in cases where seed propagation is inefficient. Somatic embryos can be cultured into complete plants under appropriate conditions, offering a method for generating genetically uniform and high-quality Stevia plants.

4. Secondary Metabolite Production: One of the most compelling reasons to use tissue culture for Stevia is the potential to enhance the production of bioactive compounds, such as steviol glycosides, which contribute to Stevia's sweetness. Studies have shown that tissue culture can be used to manipulate the production of these secondary metabolites by adjusting culture conditions, such as PGR concentration, light intensity, and stress conditions (Chaudhary et al., 2021). Additionally, the use of bioreactor systems for large-scale in vitro culture has been explored as a way to increase the yield of steviol glycosides.

Challenges in Stevia Tissue Culture

Despite its potential, tissue culture of Stevia faces several challenges. These include:

Contamination: Like many other plant species, Stevia explants are prone to bacterial and fungal contamination during culture initiation.

Low Regeneration Efficiency: Some Stevia cultivars exhibit low response to regeneration protocols, requiring optimization of various factors such as PGR combinations and medium composition.

Genetic Stability: While tissue culture generally produces genetically uniform plants, there is a risk of somaclonal variation, which can result in undesirable traits such as altered steviol glycoside content (Yadav et al., 2010).

Acclimatization: Transferring tissue-cultured Stevia plants to soil or greenhouse conditions can be a challenging process. Plants often suffer from wilting or poor survival due to inadequate hardening protocols (Tiwari et al., 2016).

Applications of Tissue Culture in Stevia Improvement

Genetic Improvement: Tissue culture techniques are essential tools for genetic modification of Stevia, facilitating the introduction of new traits, such as improved disease resistance or enhanced steviol glycoside production (Soni et al., 2020).

Mass Propagation: Tissue culture allows the rapid and disease-free multiplication of Stevia plants, which is beneficial for commercial scale-up. This is particularly important for high-demand crops like Stevia, which are often cultivated in regions with challenging environmental conditions (Rao et al., 2014).

Conservation of Germplasm: In vitro conservation through cryopreservation or slow-growth culture offers a reliable method for the long-term storage of Stevia germplasm, helping to preserve valuable genetic resources and access superior genotypes (Kong et al., 2019).

Future Perspectives

The future of Stevia tissue culture lies in overcoming existing limitations through continued optimization of protocols, enhanced understanding of genetic factors influencing regeneration, and integration with biotechnological advances like gene editing. Innovations in bioreactor technology could also scale up the production of high-value compounds. Furthermore, precision breeding techniques, such as CRISPR/Cas9, could be employed to enhance the production of steviol glycosides or improve stress tolerance in Stevia plants (Soni et al., 2020).

Conclusion

Tissue culture offers a powerful and versatile tool for the mass propagation, genetic improvement, and conservation of *Stevia rebaudiana*. Despite challenges such as low regeneration efficiency and contamination, advances in culture media, growth regulators, and biotechnological applications promise to improve the efficacy of Stevia tissue culture. With

continued research and development, tissue culture could play a pivotal role in meeting the global demand for Stevia-based sweeteners.

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