Evaluación del perfil metabólico inducido por ácido salicílico en microtubérculos in vitro de papa *(Solanum tuberosum L.)* bajo estrés por heladas

Evaluation of the metabolic profile induced by salicylic acid in in vitro potato (Solanum tuberosum L.) microtubers under frost stress

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Abstract

Potato (*Solanum tuberosum L.*) is an economically and nutritionally relevant crop in the Andean region of Ecuador, whose yield has been affected by the frequency and intensity of frosts that damage tissues and reduce productivity. In this work, the role of salicylic acid (SA) as a biostimulant in *in vitro* potato microtubers of the cultivar Diacol Capiro grown under cold conditions was evaluated. Microtubers were established in supplemented MS medium and subjected to four contrasting treatments of temperature and SA. Preliminary results showed successful *in vitro* culture establishment as well as successful microtuber formation. On the other hand, SA is expected to promote protective physiological responses against frost, and metabolomic analysis will complement these findings by identifying differential compounds associated with cold tolerance.

Keywords: Biostimulants. Frost. Potato. Salicylic acid.

Resumen

La papa (*Solanum tuberosum* L.) es un cultivo de gran relevancia económica y nutricional en la región andina del Ecuador, cuyo rendimiento se ha visto afectado por la frecuencia e intensidad de las heladas, las cuales ocasionan daños en los tejidos vegetales y reducen la productividad. En este trabajo se evaluó el papel del ácido salicílico (AS) como bioestimulante en microtubérculos de papa de la variedad Diacol Capiro cultivados *in vitro* bajo condiciones

de frío. Los microtubérculos fueron establecidos en medio de cultivo MS suplementado y sometidos a cuatro tratamientos contrastantes de temperatura y ácido salicílico. Los resultados preliminares evidenciaron el establecimiento exitoso del cultivo *in vitro*, así como una adecuada formación de microtubérculos. Por otra parte, se espera que el AS promueva respuestas fisiológicas protectoras frente al estrés por heladas, mientras que el análisis metabolómico complementará estos hallazgos mediante la identificación de compuestos diferenciales asociados con la tolerancia al frío.

Palabras clave: Bioestimulantes; heladas; papa; ácido salicílico.

Introduction

Potato (*Solanum tuberosum L.*) is the fourth most important food crop worldwide and plays a central role in food security and rural economy in the Andean region. In Ecuador, potato is cultivated on more than 80,000 hectares and represents the main source of income for small farmers, who are especially vulnerable to adverse climatic factors (Basantes, 2020). Among these, frost is one of the main constraints, causing leaf area reduction, tissue necrosis, and yield losses of up to 60%. Climate change has increased the frequency and intensity of this phenomenon, making it urgent to develop alternative and sustainable mitigation strategies (Basantes, 2020).

Salicylic acid (SA) is a phytohormone involved in growth, development, and stress responses, reported as an inducer of cold tolerance through activation of antioxidant pathways, hormonal regulation, and accumulation of phenolic and osmoprotective compounds (Hayat et al., 2010). In this context, the present research hypothesized that SA application increases cold tolerance in potato microtubers, which was evaluated through an *in vitro* system combined with metabolomic analysis (Khan et al., 2015).

Methodology

The study was conducted with the cultivar Diacol Capiro. Explants were established from axillary buds in MS medium supplemented with 20 g/L sucrose, 5.5 g/L agar, and 1 g/L activated charcoal. The obtained plantlets were multiplied and then transferred to a microtuberization medium enriched with 8% sucrose and 0.5 mg/L BAP, under controlled photoperiod conditions (16/8 h light/dark, 25 °C). Subsequently, four treatments were applied: T1 (4 °C + SA at 0.5 mM), T2 (25 °C + SA), T3 (4 °C without SA), and T4 (25 °C without SA).

SA was applied by direct addition to the culture medium during the microtuberization stage. Morphological variables such as number of microtubers, average size, fresh weight, and degree of tissue oxidation were evaluated. The collected microtubers were stored in liquid nitrogen for metabolomic analysis, which will be carried out using UHPLC-QTOF-MS in positive and negative modes. Statistical analysis includes ANOVA with multiple comparison tests and multivariate analysis: PCA, heatmaps.

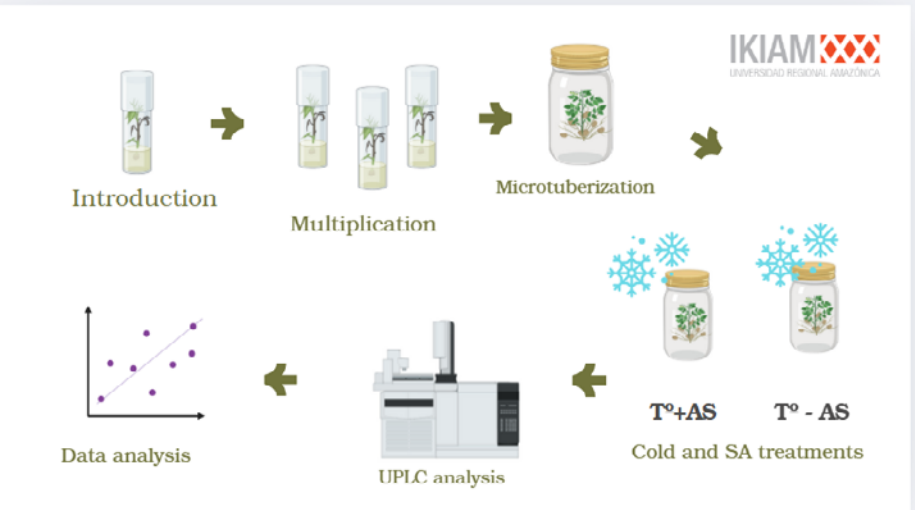

Figure 1. Methodology used

Results and discussion

In the initial phase of the experiment, an *in vitro* culture system was successfully established for the cultivar Diacol Capiro. With the established disinfection protocol, the 80% of contamination-free plantlets were obtained from axillary bud explants, with proper stem and leaf development. Later, plantlets were multiplied obtaining around 18 plantlets per bud explant and then they were transferred to microtuberization medium, where positive responses to the induction process were observed.

The obtained microtubers showed variations in size and number depending on the incubation time, displaying progressive growth throughout the weeks of culture. Likewise, the addition of high sucrose concentrations to the medium, together with the use of BAP, favored the induction of tuberous structures, which is consistent with previous reports in the literature (Rivas & Plasencia, 2011).

In the next phase of the study, SA treatments will be applied under controlled cold conditions and at room temperature, allowing evaluation of its isolated and combined effects on potato microtubers. Subsequently, the tissues will be analyzed using UHPLC-QTOF-MS to obtain

the differential metabolic profile between treatments, which will confirm these patterns by identifying differential metabolites related to soluble sugar pathways, amino acids, and antioxidant compounds (Zhang, 2025). This will strengthen the understanding of the biochemical mechanisms activated by SA .

Conclusions

The *in vitro* phase allowed the establishment of a reliable protocol for potato micropropagation and microtuberization, ensuring uniform material for the next stages of the study. This preliminary result represents an essential step for the implementation of salicylic acid treatments and subsequent metabolomic characterization, which will provide deeper insights into the biochemical mechanisms involved in potato tolerance to frost stress.

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