



Effect of biotic and abiotic elicitors on *Teucrium polium* L. callus in vitro

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Abstract

Plant tissue culture is a modern biotechnology technique that enables the detailed study of physiological processes and the regulation of cell growth under controlled conditions. In this study, the effects of two types of elicitors, biotic (chitosan) and abiotic (silver nitrate), on induction and growth parameters of callus of the medicinal plant *Teucrium polium* L. were investigated. Leaf explants were cultured on MS medium supplemented with various concentrations of the hormones BAP (6-Benzylaminopurine) and NAA (1-Naphthaleneacetic Acid), followed by treatments with different elicitors concentrations. The results showed that the main effects of hormones and elicitors, and their interactions, on the fresh and dry weight of callus were significant ($p \leq 0.01$). The highest fresh weight of callus was observed in the combined treatment of BAP (1.5 mg L⁻¹) + NAA (0.5 mg L⁻¹) with chitosan at 150 mg L⁻¹. In addition, silver nitrate at 12 mg L⁻¹ also positively affected callus growth. Findings suggest that the application of biotic elicitor chitosan and abiotic elicitor silver nitrate can improve cell growth and division in *T. polium* tissue culture by stimulating physiological pathways and hormonal regulation.

Introduction

The medicinal plant *Teucrium polium* belongs to the mint family (Lamiaceae). This plant is valued in traditional medicine and pharmacy due to its medicinal properties (Bahramikia *et al.*, 2022). Previous studies have shown that the hormones BAP and NAA significantly affect the efficiency of callus formation in *T. polium* (Tabarifard *et al.*, 2021). In tissue culture, the induction of defense and metabolic pathways can be enhanced by applying biotic and abiotic elicitors. Chitosan is a biotic elicitor that, in recent studies, has been shown to promote plant growth and stimulate the biosynthesis of secondary metabolites under in vitro condition. This biopolymer, chitosan, mimics pathogen attack in tissue culture and triggers the production of active metabolites (Cañizares *et al.*, 2024). Various studies have demonstrated that silver nitrate application can stimulate the biosynthesis of specialized metabolites associated with stress responses. For instance, research on *Arabidopsis thaliana* has shown that silver nitrate application leads to the accumulation of various specialized metabolites (Roy *et al.*, 2025). This study aimed to investigate and compare the combined effects of plant hormones, the biotic elicitor chitosan, and the abiotic elicitor silver nitrate on the fresh and dry weight of callus. Given the economic and medicinal value of *T. polium*, the findings of this study can be applied to advance in vitro cultivation strategies for practical mass propagation of the plant and for enhancing the production of its bioactive metabolites.

Materials and methods

This research was conducted as a factorial experiment in a completely randomized design (CRD) with three replications. *T. polium* plant were obtained from healthy specimens cultivated in a hydroponic system in the research greenhouse of Ferdowsi University of Mashhad. Young leaves were selected as explants and, after washing with distilled water, were surface-sterilized with 70% (v/v) ethanol solution (for 30 seconds) and then 5% (w/v) sodium hypochlorite (for 10 min). The MS basal medium was prepared by adding 30 g L⁻¹ sucrose and 7 g L⁻¹ agar. To induce callus, various combinations (BAP 1 mg L⁻¹, BAP 1.5 mg L⁻¹, and BAP 1.5 mg L⁻¹ + NAA 0.5 mg L⁻¹) of hormones were added to the medium. The cultures were kept in total darkness at 25±2°C for 4 weeks until the initial callus formed. Biotic elicitor (chitosan) at concentrations of 0, 50, 100, and 150 mg L⁻¹ and abiotic elicitor (silver nitrate) at concentrations of 0, 4, 8, and 12 mg L⁻¹ were added to MS medium. After 10 days of treatment, the calli were transferred to light condition (16 hours of light and 8 hours of darkness). Callus fresh and dry weights were measured, and the data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple-range test at the 1% significance level ($p \leq 0.01$).

Results and discussion

The present study demonstrated that the optimal combination of BAP and NAA hormones, together with biotic and abiotic elicitors, plays a key role in enhancing callus induction and cell growth in the medicinal plant *T. polium*. Chitosan at 150 mg L⁻¹ had the most significant positive effect on callus fresh and dry weight, while silver nitrate at 12 mg L⁻¹ also showed a significant effect. Accordingly, the use of biotic elicitors such as chitosan is a practical approach for optimizing tissue culture conditions in medicinal plants. Continued research, especially examining the effects of these compounds on physiological and molecular pathways of growth, can lead to a deeper understanding of the regulatory mechanisms of cell division under in vitro conditions.

References

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