

# Influence of auxins on somatic embryogenesis and alkaloid accumulation in *Leucojum aestivum* callus

Research Article

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**Abstract:** *In vitro* cultures of *Leucojum aestivum* are considered as an alternative for the production of galanthamine, which is used for the symptomatic treatment of Alzheimer's disease. We studied the effects of auxins 2,4-dichlorophenoxyacetic acid (2,4-D), 4-amino-3,5,6-trichloropicolinic acid (picloram), 3,6-dichloro-*o*-anisic acid (dicamba) at concentrations of 25 and 50  $\mu$ M on the induction of embryogenic callus and its capacity to induce somatic embryogenesis and alkaloid accumulation. The embryogenic response of the explants was from 30% for 25  $\mu$ M of dicamba to 100% for picloram (for both 25 and 50  $\mu$ M). 2,4-D (50  $\mu$ M) stimulated greater callus proliferation and somatic embryo induction as compared to the other auxins. Polyethylene glycol (PEG) stimulated somatic embryo maturation. Callus grown on media containing 50  $\mu$ M of auxins produced fewer phenolic compounds as compared with callus grown on media containing 25  $\mu$ M of auxins. GC-MS analyses showed seven alkaloids in the *in vivo* bulbs and two to four in callus culture. Galanthamine was detected in callus cultivated with 2,4-D (25, 50  $\mu$ M), picloram (25  $\mu$ M), and dicamba (50  $\mu$ M). Other alkaloids, trisphaeridine, tazettine, and 11-hydroxyvittatine were accumulated only in callus growing on medium with picloram (50  $\mu$ M).

**Keywords:** Somatic embryos • 2,4-D • Dicamba • Picloram • Alkaloids • Phenolic compounds

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## Abbreviations:

ABA	- abscisic acid;
BA	- benzyladenine;
2,4-D	- 2,4-dichlorophenoxyacetic acid;
FW	- fresh weight;
MS	- Murashige and Skoog;
NAA	- naphthalene acetic acid;
PEG	- polyethylene glycol;
SEM	- scanning electron microscopy.

## 1. Introduction

*Leucojum aestivum* L. is an important medicinal bulbous plant belonging to the family Amaryllidaceae.

Amaryllidaceae-type alkaloids possess antitumor and antiviral properties, as well as anticholinesterase activity [1]. Galanthamine is the most important alkaloid used in the symptomatic treatment of Alzheimer's disease [2]. A second alkaloid, lycorine, showed anti-tumor activity in cancer cells and growth-inhibiting effects in higher plants, and has been also studied for its antimalarial and antiviral effects [3].

Galanthamine is produced on a large scale from Bulgarian *Leucojum aestivum* [4] and also by chemical synthesis [5-7]. However, the synthesis is complicated and time consuming because galanthamine has three asymmetric carbons requiring stereochemically controlled synthesis. The increasing demands of the pharmaceutical market as a result of an ageing population led to the importance of the supply of this alkaloid [4].

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