

Introduction

Pharmaceutical companies are tackling the great demand in medicinal compounds by extracting them from plants (Ventola, 2011). However, the harvesting methods are both detrimental to the plant and environment. In-vitro culture systems can be an ideal strategy in producing the essential metabolites while decreasing environmental impact (Bakhtiar et al., 2015). *Atropa belladonna*, is an important plant in obtaining atropine used in medication against Parkinson's disease and as an antipsychotic (Hodgson, 2012). This study on *Atropa belladonna* will explore the optimal conditions in obtaining atropine through callus formation and the highest quantity to be obtained through additional elicitors such as methyl jasmonate.



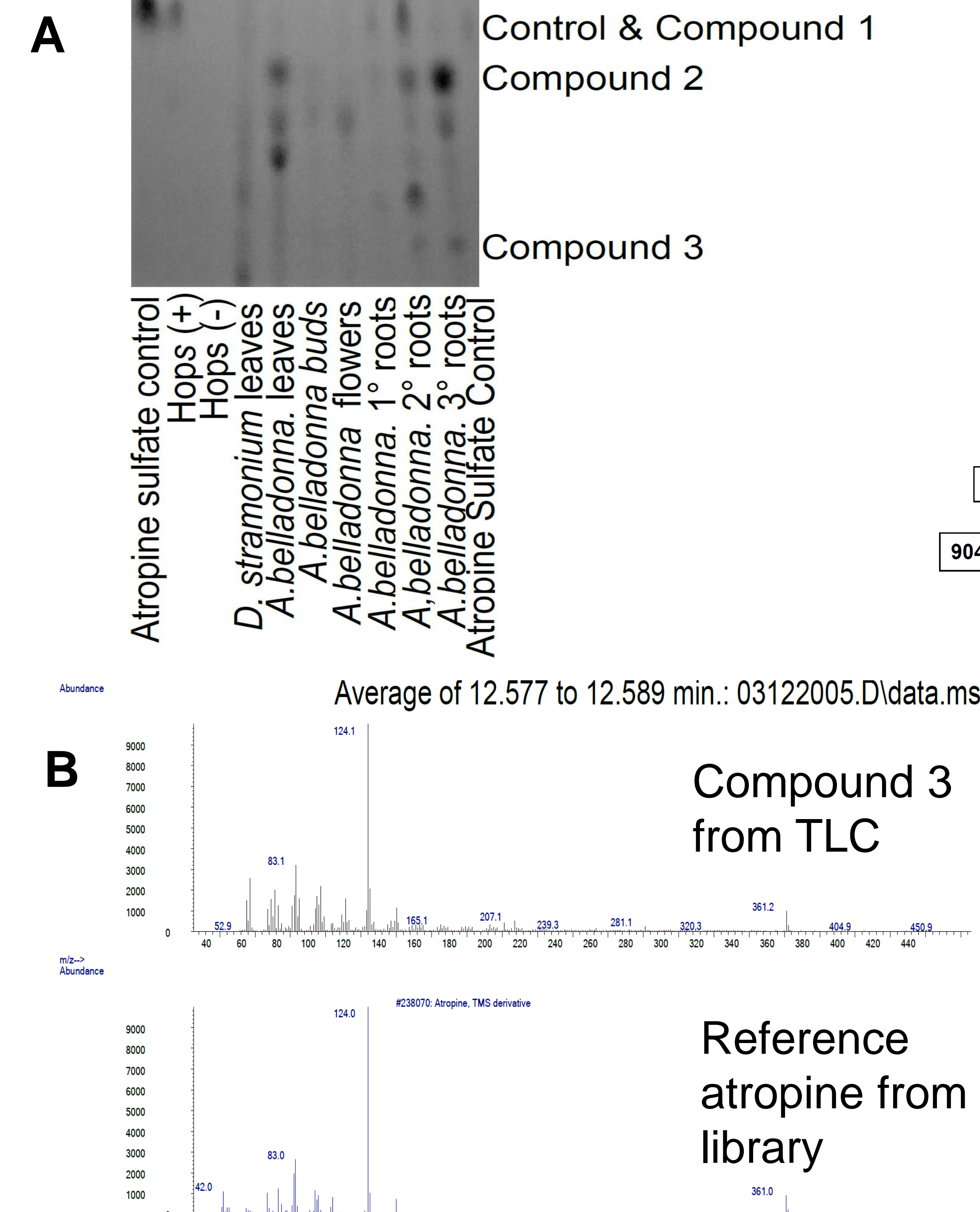
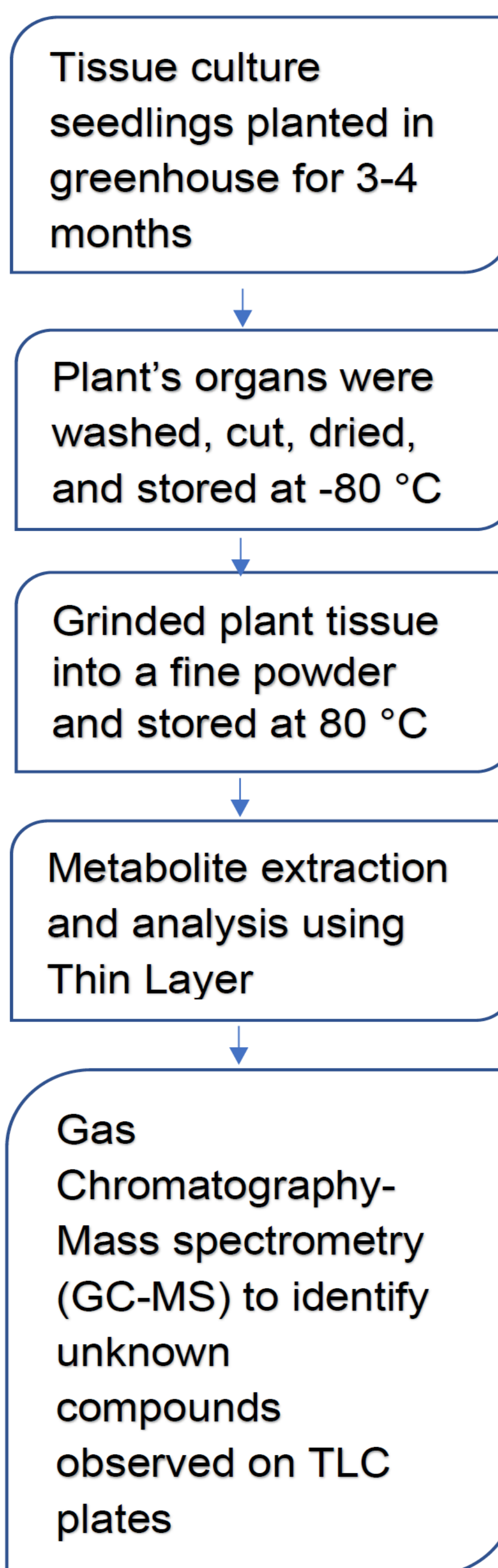
Adapted from Encyclopaedia Britannica (2017)

Objectives

- 1) What are the **optimal conditions** needed for in-vitro culture systems for callus growth?
- 2) What tissues have **higher alkaloid content** in greenhouse-grown plants?
- 3) Can this study find the **right tissue and hormone concentrations** to induce the formation of callus *in vitro*?
- 4) Can the accumulation of alkaloids be increased by **applying the stress hormone**, methyl jasmonate (MeJA)?

Experiment I

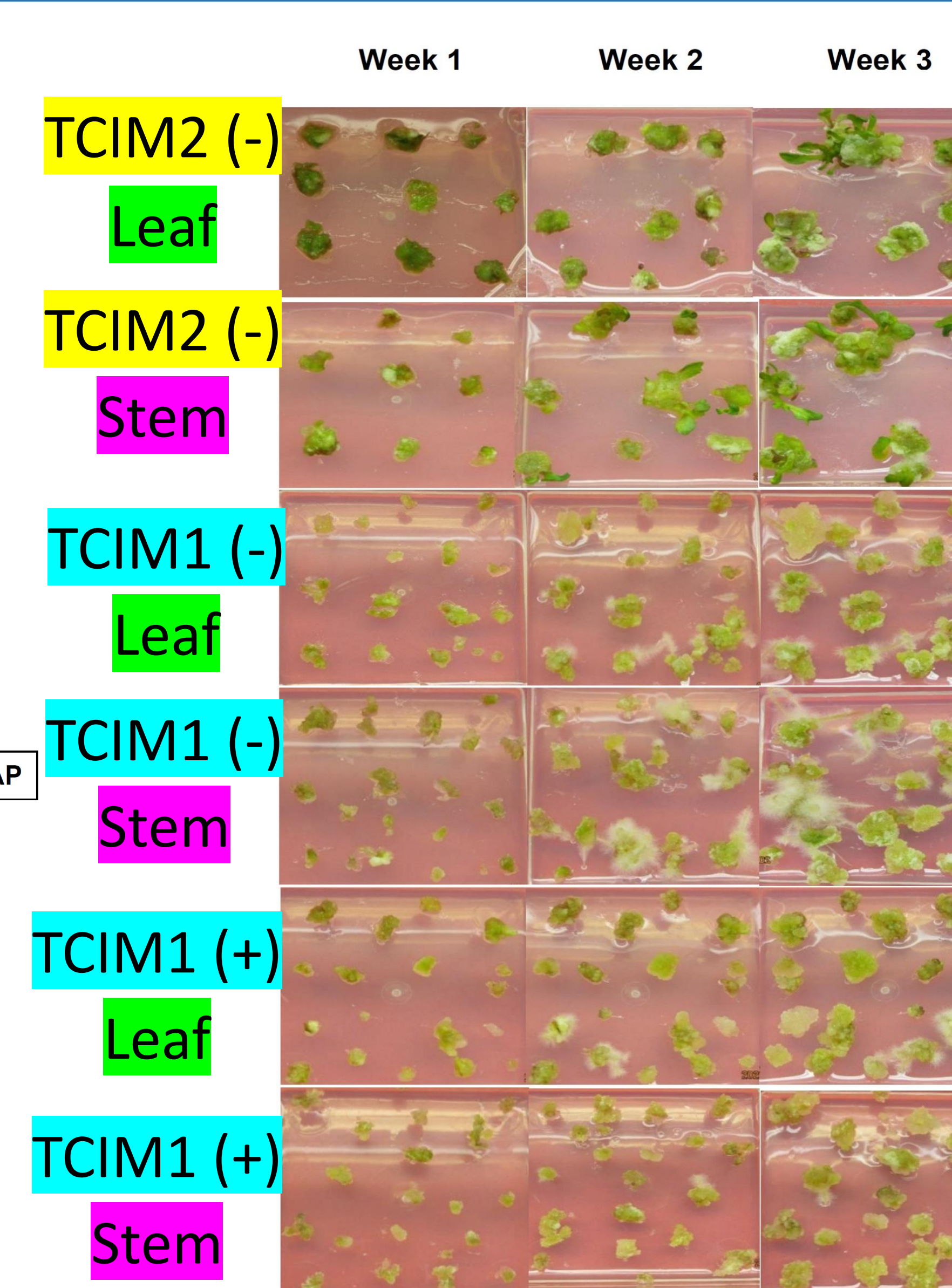
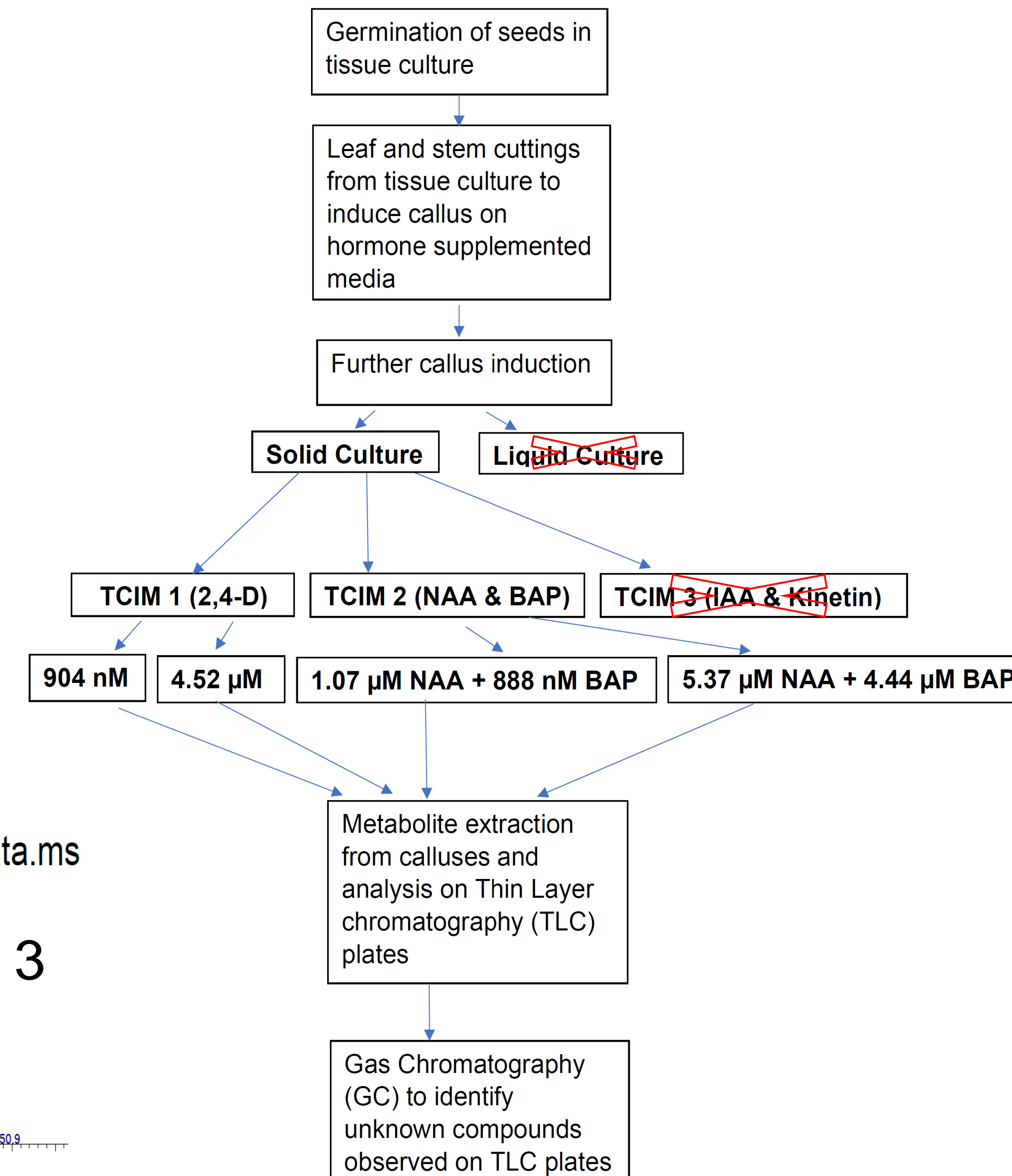
Methods



- A) Thin layer chromatography (TLC) displays three compounds in *Atropa belladonna* greenhouse-grown tissue.** Chromatography was performed with an ethyl acetate, methanol, & ammonium hydroxide solvent system (75:20:5) and visualized using an iodine solution to detect atropine in greenhouse-grown tissue.
- B) GC-MS confirms the presence of atropine in greenhouse-grown *Atropa belladonna*.** Compounds taken from the TLC plates were derivatized using BSTFA, pyridine, and methanol and analyzed using the GC-MS (Gas chromatography-Mass spectrometry). The peaks associated with atropine (361.0 and 124.0) were observed only in compound 3 that was identified in comparison to a library of compounds (bottom graph).

Experiment II

Methods

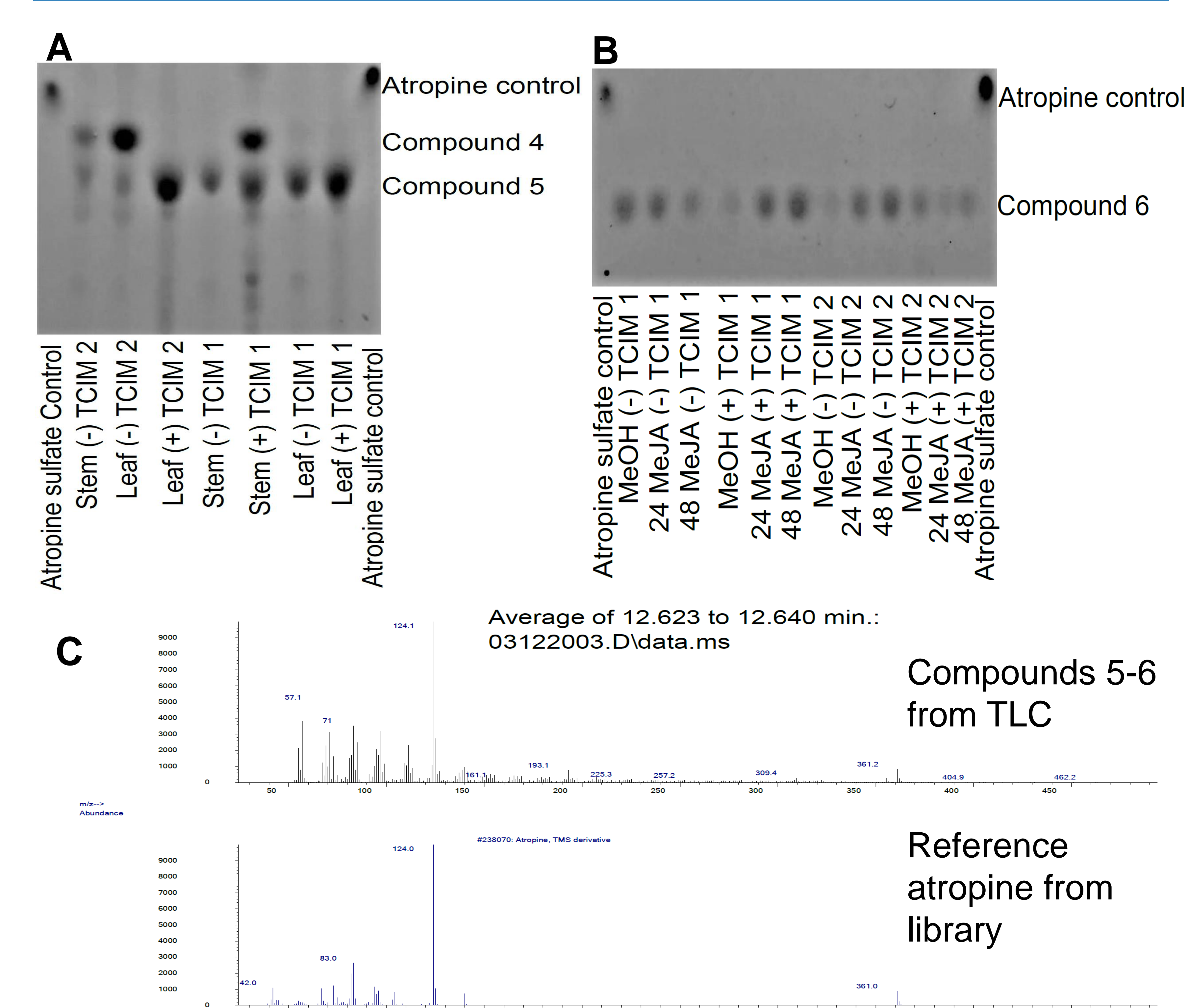


Optimal conditions for callus growth. Callus formation was explored under solid and liquid media conditions and with four different treatments in Tissue Culture Induction Media (TCIM). (-) represents the lower concentration tested and (+) represents the higher concentration. TCIM1 is of 2,4-dichlorophenoxyacetic acid (2,4-D) and TCIM2 consists of Naphthaleneacetic acid (NAA) + 6-Benzylaminopurine (BAP). Growth was best on solid agar media with plant preservative mixture (PPM), using leaf explant tissue, and 5.37 μ M of NAA + 4.44 μ M of BAP (TCIM 2).

Conclusions

This study identified TCIM 2 as the best hormone for inducing callus formation. This study demonstrated that the highest concentration of alkaloids is present in both TCIM1 and TCIM2 calluses. Plate A calluses were induced for 1 month and plate B had calluses induced for 2 months, resulting in a difference for exponential callus growth and atropine accumulation for different hormone treatments (Jamil et al., 2018). Moreover, callus culture systems subjected to stress hormones such as methyl jasmonate, can be used to generate atropine. Further research must be conducted and focused toward the use of tissue culture systems on a diverse range of herbal plants as pharmaceutical corporations struggle to meet the growing demands of drugs.

Experiment III



- A) TLC Chromatography of callus samples from different explant tissues, and under different concentrations and combinations of regulators in *Atropa belladonna*.** Compound 5 on the TLC had the greatest quantity in the leaf calluses of TCIM2.
- B) TLC tests of callus cultures treated with the stress hormone methyl jasmonate (MeJA), a mock treatment of methanol (MeOH), or no treatment in *Atropa belladonna*.** Compound 6 was detected in this TLC in all the calluses, with the greatest quantity found in 4.52 μ M of 2,4-D of TCIM1.
- C) GC-MS identifies atropine in *Atropa belladonna* callus.** Atropine was detected in compounds 5-6. All calluses accumulated atropine, however the amount in each callus differed in quantity.