

In vitro plants: How to open a jar containing bioactive phytochemicals

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Abstract

Due to the increasing demand for phytochemicals, plant *in vitro* cultures are a noteworthy, environmentally sustainable method, enabling the biotechnological production of bioactive compounds. Medical writers can find writing scientific articles in this field quite tricky due to the knowledge required at the intersection of botany, chemistry, pharmacy, and biotechnology. This article aims to provide helpful tips for writing about plant biotechnology to minimise the risk of article rejection by scientific journals due to inaccurate description of methods or inadequate data analysis.

Introduction

Plant biotechnology is defined as the use of tissue culture and genetic engineering techniques to produce genetically modified plants that exhibit new or improved desirable characteristics.¹ Plant biotechnology is used to obtain genetically modified crops but also to preserve human health. In human health green (agricultural) and red (pharmaceutical) plant biotechnology cross over each other. Plant biotechnology harnesses modern techniques to use plants as bioreactors to produce bioactive phytochemicals with potential applications in the pharmaceutical industry.

Phytochemicals are divided into primary and secondary metabolites based on



their function in plant metabolism. Primary metabolites are directly involved in plant growth and development (carbohydrates, proteins, and lipids). Secondary metabolites are those that plants biosynthesise in a defense response to threats and stresses in the natural environment (alkaloids, terpenes, flavonoids, and steroids). These compounds are valuable for human use in medicines, flavours, dyes, repellents, and cosmetic ingredients. The market demand for secondary metabolites is still growing, which may endanger some medicinal plants due to over-harvesting.² Yet, chemical synthesis of organic compounds with complex structures is economically unfeasible. Nevertheless, for the last 60 years, plant cell, tissue, and organ cultures have provided assistance, enabling *in vitro* production of bioactive secondary metabolites.

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inner bark of yew trees (*Taxus* sp.) is ecologically

unsustainable as the content of the compound is too low. To treat one patient, six 100-year-old slowly growing trees are required to produce 2 g of pure Taxol[®].^{3,4} *Taxus* spp. cell suspension cultures proved to be an alternative production route.³⁻⁶ The cell suspension culture of *Taxus chinensis* elicited with methyl jasmonate resulted in one of the highest yields of paclitaxel (565 mg L⁻¹, or 29.3 mg g⁻¹ dry weight) so far.⁷ Currently, the production system, developed by Phyton Biotech, which is based on *T. chinensis* cultivation in 75,000-L bioreactors, is the largest commercially viable plant cell culture application.⁵

Among many other medicinally important compounds biosynthesised in plant *in vitro* cultures, it is worth mentioning antitumour indole alkaloids (*Catharanthus roseus*), anticholinergic tropane alkaloids (*Datura stramonium*, *Atropa belladonna*), or immunomodulating ginsenosides (*Panax ginseng*).^{6,7} Although research on *in vitro* cultures has been conducted since the mid-1960s, broad commercialisation of plant *in vitro* systems has not yet occurred.⁷ Efforts are still being made to overcome limitations in scaling up secondary metabolite production, such as insufficient knowledge of biosynthetic pathways and slow *in vitro* cultures growth.⁸

After entering the search term “*in vitro* plant cultures”, PubMed displays over 1000 publications per year for the recent decade, which indicates the potential for medical and scientific writers to show off their skills in this field. Since the world of plants has its own rules (even if they

grow in a jar), it is helpful to know which ones are important when writing a manuscript about plant *in vitro* cultures.

Botanical nomenclature

Scientific plant names, according to the International Code of Nomenclature for algae, fungi, and plants (ICN),⁹ are required when publishing research about plants. Using this nomenclature ensures scientific accuracy and avoids citation errors. Writing “ginseng *in vitro* cultures” is insufficient because various species of *Panax* (ginseng) genus can be used to establish *in vitro* cultures and produce ginsenosides. The most popular species is *Panax ginseng*, but *P. quinquefolius* or *P. sikkimensis* are also used. Importantly, each of these species cultured *in vitro* produces different ginsenoside yield.⁷ Therefore, the precise botanical name of the explant source must be provided in the manuscript (Fig. 1A). To do this, it is preferable to report the genetic identification of the mother plant species and the location where the preserved and archived herbarium specimen of mother plant (representative voucher specimen)

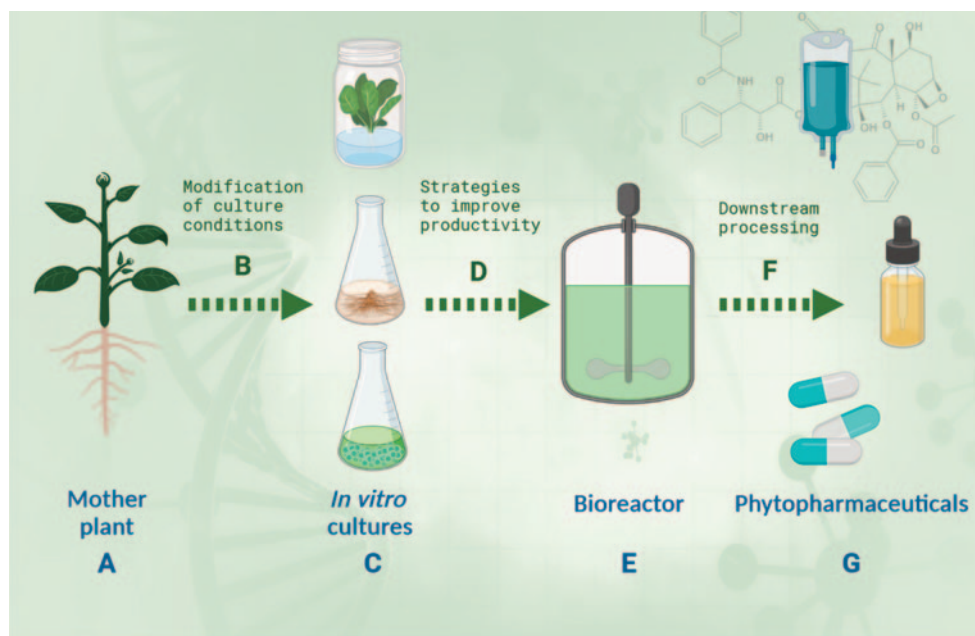
is stored (e.g. university department or horticultural library).¹⁰

The standard Latin binomial nomenclature system, first developed by Carl Linnaeus and still used today, has its own spelling rules. The full plant name is written in italics. The first name identifies the genus and is capitalised, while the second name represents the species and is written in lowercase. The name of the author who named the plant using scientific nomenclature, usually given when the plant is first mentioned in a manuscript, is not italicised (*Mentha piperita* L. – L. is used to indicate Linnaeus). Once the full botanical name has been used in the manuscript, the genus name can be abbreviated to the first initial if there is no possibility of confusion (*M. piperita*), or the common name (mint) can be used, neither italicised nor capitalised. The names of higher taxa, such as family or order, should be written in standard lettering (Lamiaceae, formerly called Labiatae; the common name: the mint family). Placing “sp.” after a genus refers to the genus in general, while “spp.” means “several species of that genus.” Be aware of the synonyms for the same plant resulting from taxonomic

changes (e.g. *Rhododendron tomentosum* Harmaja, previously *Ledum palustre* L.). Using only outdated names may lead to the failure of bibliographic searches to find the article.¹¹ However, the outdated plant name may still be referenced as a synonym (syn.), especially where there is no consensus among taxonomists or the older name is widely known.

Culture conditions

Plant *in vitro* cultures are incubated in controlled aseptic conditions in closed flasks or jars spaced evenly apart in a growth room. Because external factors may affect photosynthesis and other biochemical processes in plants and induce anatomical and physiological changes, data on light (intensity, spectrum, photoperiod), humidity, and temperature should be provided (Fig. 1B). If basic nutrient culture media were applied, indicate references to the original formulations without specifying their exact composition but explain any modifications that were made, e.g. the changes to medium acidity. Abbreviations such as MS (Murashige & Skoog) or SH (Schenk & Hildebrandt) are commonly used to describe



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Figure 1. The production of bioactive secondary metabolites in plant *in vitro* culture

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| <p>A. Initiation of <i>in vitro</i> cultures from the mother plant</p> <p>B. Multiplication of primary cultures and optimisation of culture conditions</p> <p>C. Selection of high-yielding lines</p> <p>D. <i>In vitro</i> strategies to improve productivity</p> | <p>E. The bioreactor system for large scale production of secondary metabolites</p> <p>F. Extraction and phytochemical analysis of bioactive compounds</p> <p>G. Production of phytochemicals as potential drugs</p> |
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plant culture media. Similarly, the names of regulators of plant growth and development (plant growth regulators, phytohormones) are usually abbreviated (BAP – 6-Benzylaminopurine, TDZ – Thidiazuron, etc.). The amount of cytokinins, auxins, gibberellins, or other substances added to the medium should be expressed in μM . Precise reporting of the combinations of plant growth regulators used and their accurate concentrations is essential because of their high impact on shoot regeneration, rhizogenesis, or callus formation. Another necessary piece of information is whether the culture medium was liquid (for shaken and stationary cultures), solid (for stationary cultures), or semi-solid (with support for the developing plants, e.g. during micropropagation), which influences the growth of biomass.

Type of *in vitro* culture

Depending on the explant and the culture medium, cell cultures (cell suspensions, protoplasts, or gametic cells), undifferentiated biomass (callus), differentiated tissues (meristems), or organ cultures (shoots, roots, or zygotic embryos) can be established (Fig. 1C).^{6,7} Cell suspensions seem to be a promising source of phytochemicals due to their fast growth, facilitating scale-up to bioreactor systems. However, a certain degree of differentiation is indispensable for producing some secondary metabolites, e.g. essential oils, since several biosynthetic pathways may be fully developed only in specific plant organs.⁷ In the manuscript, explant sources (any meristematic tissues of the mother plant, consisting of continuously dividing cells, that serve as the starting material for *in vitro* cultures) and culture initiation should be described in detail, just as the individual stages of micropropagation (if applicable). If root cultures are to be mentioned in the article, make sure their character is described as adventitious or hairy. Hairy root cultures are formed as a result of an infection with different *Rhizobium rhizogenes* strains (formerly *Agrobacterium rhizogenes*), a soil-borne Gram-negative bacterium, which leads to the integration of the root-inducing plasmid into the plant genome. Transgenic hairy roots, unlike non-transformed adventitious roots developed from stems or leaves, grow rapidly in

culture media without an exogenous application of auxins. They are genetically stable and provide high secondary metabolite production.¹

Strategies for improving productivity

A medical writer may come across specific names of biotechnological approaches employed to improve biomass and secondary metabolite production after high-yielding plant cultures have been selected and culture conditions optimised (Fig. 1D). These approaches should be described in the Materials and Methods section in sufficient detail, with technical specifications, experimental design, and data collection, to enable repetition of the study and verification of the findings.¹² Techniques that typically need to be described are elicitation (the use of biotic or abiotic compounds or factors to trigger the plant's defense response), permeabilisation (the release of product from biomass into culture media), immobilisation (fixing of plant biomass in or on a supporting material or matrix), precursor feeding (the addition of exogenous or endogenous compounds that are converted by plant cultures into secondary metabolites through biosynthetic pathways), and metabolic engineering (introduction of genes encoding specific metabolic enzymes into the plant).⁸

Type of bioreactor

The bioreactor system is a fundamental tool for scaling up the *in vitro* production of secondary metabolites (Fig. 1E). This system allows for the bulk transfer of nutrients and gases and provides constant micro-environmental conditions, promoting rapid growth of biomass. The automated cultivation processes save time and reduce labor costs.¹ The bioreactor design for plant cultures differs from that applied in microbial cultures because not only organs but also plant cells, which are larger than bacterial cells (10-100 μm vs 0.5-5 μm long), may experience mechanical damage in stirred tank bioreactors and foam formation in bubble-aerated bioreactors.¹³ The selection of each bioreactor is unique and

adapted to the type of plant culture, considering efficient oxygen and nutrients supply, low shear stress, proper mixing, and a suitable support system.¹⁴ Because bioreactors show numerous modifications, such as the addition of mist spray, temporary immersion, different shapes of columns, having mesh or basket, using various agitation options, etc., an accurate description is essential, preferably combined with a schematic drawing. Providing references is sufficient if the bioreactor is commercially available, e.g. RITA® systems, and no modifications have been made.

Downstream processing

Downstream processing is often described in manuscripts on secondary metabolite production in plant cultures as an integral part of research. The extraction of bioactive compounds from the plant material¹ and qualitative and quantitative analysis of phytochemicals^{15,16} are essential for screening *in vitro* cultures as well as assessing the effectiveness of the developed large-scale system (Fig. 1F). Without going into details of plant analysis methods, some basic advice can be given to medical writers. The product yield is usually expressed in the following units: mg g^{-1} DW (dry weight), less frequently in mg g^{-1} FW (fresh weight), or mg L^{-1} (in case of cell suspension culture). Bioreactor productivity can also be reported as $\text{g L}^{-1} \text{day}^{-1}$. The spelling of the names of the obtained secondary metabolites should be carefully checked to avoid mistakes, especially when long names based on the chemical structure are concerned. If a compound is not widely known, including its chemical

formula may be considered. To discuss the potential biological activity of the final products (Fig. 1G), assigning them to the appropriate phytochemical group (e.g. essential oils, flavonoids, alkaloids) is helpful.¹

Publishing in the field of plant biotechnology

The instructions for authors and style guide in the target journal will clarify many points not covered in this article. Interestingly, some submission guidelines do not insist on writing common Latin terms such as *in vitro* and *in vivo* in italics. The difficulty in publishing in plant biotechnology is the relatively long time necessary to develop a high-yielding plant *in vitro* system. In this situation, the challenge for the scientist is to meet the expectations for writing a

Precise reporting of the combinations of plant growth regulators used and their accurate concentrations is essential because of their high impact on shoot regeneration, rhizogenesis, or callus formation.

The bioreactor system is a fundamental tool for scaling up the *in vitro* production of secondary metabolites.

scientific article every year, which requires creativity to propose new solutions and innovative strategies.¹² According to a recent study, the main reasons for manuscript rejection by scientific journals in agriculture and plant biology were the lack of novelty, flaws in methods or data interpretation, inadequate data analysis, and poor critical scientific thinking.¹⁷ Therefore, a vital task for a medical writer is to clearly emphasise the novelty in the research and transparently put the complexity of *in vitro* secondary metabolite production on paper.

Conclusions

Although research articles on plant biotechnology do not differ significantly from other papers with an IMRAD (Introduction, Methods, Results, and Discussion) structure, there are a lot of nuances specific to describing plant *in vitro* cultures. Starting from botanical nomenclature, through *in vitro* culture establishment and a bioreactor system development for secondary metabolite production, to the extraction and analysis of bioactive compounds – all these issues may seem complicated to a medical writer outside the field. However, with a good approach to the subject, no jar with plants will be too difficult to open.

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The opinions expressed in this article are the author's own and not necessarily shared by EMWA.

Disclosures and conflicts of interest

The author declares no conflicts of interest.

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