

In Vitro Propagation and Conservation of *Cymbopogon citratus* (DC.) Stapf: A Proposed Approach to Assessing Heavy-Metal Stress

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Abstract

Cymbopogon citratus (DC.) Stapf, commonly known as lemongrass, is a perennial aromatic grass valued for its citral-containing essential oil and its use in traditional preparations, food flavouring, fragrances and cosmetics. Access to healthy, uniform planting stock is important for cultivation and conservation. In vitro culture can facilitate the multiplication and maintenance of selected plant material under controlled conditions and can also serve as a platform for examining responses to environmental stress. Cadmium, lead and chromium may disrupt mineral relations, photosynthetic activity and cellular redox balance; their effects depend on chemical form, concentration, exposure duration and plant species. This paper reviews the role of tissue culture in lemongrass propagation and conservation, summarizes general mechanisms associated with metal stress, and presents a proposed laboratory framework for studying regenerated plantlets. The proposed procedures include explant establishment, optimization of growth-regulator combinations, graded exposure to individual metals, growth assessments, chlorophyll and antioxidant analyses, and measurement of metal levels in roots and shoots. This is a prospective framework, not a report of completed experiments, and no experimental results are claimed. Although published studies demonstrate that *C. citratus* can be micropropagated, its metal tolerance, accumulation patterns and detoxification responses require direct, species-specific investigation.

Keywords: *Cymbopogon citratus*; lemongrass; tissue culture; micropropagation; germplasm conservation; heavy-metal stress; antioxidant response; aromatic plants.

1. Introduction

Medicinal and aromatic plants support traditional healthcare and provide raw materials for pharmaceutical, food, fragrance and cosmetic industries. Their value is often connected with secondary metabolites, including terpenoids, phenolics, flavonoids and volatile oils. Plant biotechnology offers approaches for conserving valuable germplasm, multiplying selected genotypes and examining developmental or stress-related processes under controlled conditions.

Lemongrass, *Cymbopogon citratus* (DC.) Stapf, is a perennial, tuft-forming grass grown in tropical and subtropical regions. Its characteristic citrus aroma is largely associated with citral in the essential oil. The species is used in traditional preparations and in several commercial product types. Because oil

composition can vary with genotype, environment, cultivation practices and extraction procedures, propagation programs should address the identity and quality of plant material as well as multiplication rate.

Vegetative propagation is useful for retaining desirable plant characteristics, although it may not always supply large quantities of planting material quickly. In vitro methods provide a controlled alternative by cultivating shoot tips, nodes or other suitable tissues on nutrient media containing plant growth regulators. Common stages include culture initiation, shoot multiplication, root induction and gradual acclimatization.

Heavy-metal contamination is an additional concern in plant and environmental research. Cadmium, lead and chromium can influence plant growth and metabolism, with the extent of injury depending on metal form, dose, exposure period and culture conditions. In vitro systems can help distinguish treatment effects, but findings should subsequently be evaluated under more representative growing conditions. This paper links the rationale for *C. citratus* micropropagation with a proposed strategy for examining its response to metal exposure.

2. Botanical Characteristics and Uses

2.1 Taxonomic identity

Cymbopogon citratus (DC.) Stapf is a member of the family Poaceae and genus *Cymbopogon*. It is a monocotyledonous flowering plant belonging to the order Poales.

2.2 Morphology and cultivation

The plant develops dense clumps of elongated, narrow leaves and a fibrous root system. Its foliage contains structures associated with volatile-oil production and gives off a familiar lemon-like scent. Lemongrass is commonly multiplied vegetatively. Both plant growth and essential-oil profile may be influenced by the genetic source, local growing conditions and post-harvest handling.

2.3 Medicinal and industrial relevance

Lemongrass oil contains citral along with other volatile constituents. Published investigations have examined antimicrobial, antioxidant and anti-inflammatory activities of particular extracts or oil preparations, and have described uses in flavouring, fragrance, cosmetics and pharmaceutical-related products. Such reports must be interpreted in light of the material and assay used; they do not show that all preparations have the same composition or clinical effect. Conservation work should therefore maintain verified plant identity and allow later phytochemical quality testing.

3. In Vitro Conservation and Micropropagation

3.1 Role of tissue culture

Plant tissue culture enables cells, organs or tissues to develop aseptically on a defined nutrient medium. For medicinal and aromatic plants, it can support multiplication of selected genotypes, germplasm maintenance, production of uniform starting material and physiological or metabolite studies.

Conservation success depends on maintaining healthy, correctly identified cultures over time, not simply maximizing shoot numbers.

3.2 Explant selection and culture initiation

Shoot apices and nodal segments are candidate explants for lemongrass. Previous studies have described clonal multiplication through shoot-tip culture and have investigated organogenesis, somatic embryogenesis and temporary-immersion systems. Explant age, physiological status and surface-disinfection procedures should be standardized. Disinfection requires preliminary optimization: insufficient treatment can allow contamination, whereas excessive exposure can injure plant tissue.

3.3 Medium and growth regulators

Murashige and Skoog (MS) basal medium has been used in *C. citratus* propagation studies. Cytokinins such as 6-benzylaminopurine (BAP) may encourage shoot initiation and multiplication, while auxins such as naphthaleneacetic acid (NAA) can influence rooting and other developmental responses. Research has explored BAP–NAA combinations and the possible effects of silicon nanoparticles on shoot structure, rooting and acclimatization. These reports support protocol optimization rather than the assumption that one formulation will perform identically across genotypes and laboratories.

3.4 Rooting, acclimatization and conservation quality

Regenerated shoots require a suitable root system before removal from culture vessels. Acclimatization then allows plantlets to adapt gradually to lower humidity, ambient light and non-sterile substrates. Records for conservation cultures should include donor source, explant type, medium, passage number and subculture schedule. Cultures should be monitored for contamination, abnormal form and loss of vigor. Genetic-fidelity testing may be included when facilities are available; slow-growth storage or cryopreservation should be used only after validation for the specific material.

4. Heavy Metals and Plant Stress Responses

4.1 Sources and variable effects

Heavy metals may enter environmental systems through industrial discharge, mining, wastewater, agricultural inputs and other human activities. Their persistence can affect soil quality, plant productivity and ecosystem functioning. Toxicity is influenced not only by total concentration but also by chemical form, bioavailability, exposure time, nutrient status and species-specific traits.

4.2 Growth and physiological consequences

Exposure may affect germination, root elongation, shoot development, biomass, chlorophyll content, photosynthetic performance, nutrient uptake and membrane function. Roots and shoots can show different responses. Accordingly, effects should be measured for the species and conditions under study rather than inferred from other plants.

4.3 Oxidative stress and antioxidant systems

Metal exposure is often associated with disruption of cellular redox regulation. Excess reactive oxygen species can damage membrane lipids, proteins, nucleic acids and photosynthetic structures. Plants possess enzymatic defenses such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR), together with non-enzymatic compounds

including ascorbate, glutathione and phenolics. Changes in enzyme activity may indicate a stress response, but enzyme measurements alone cannot establish tolerance.

4.4 Uptake, binding and compartmentalization

Possible plant responses include restricting metal entry, binding ions to cell-wall components, chelating them with organic ligands, compartmentalizing them within cells and adjusting antioxidant metabolism. These mechanisms are hypotheses for investigation in lemongrass; their importance in this species must be established experimentally.

5. In Vitro Systems for Metal-Tolerance Research

In vitro cultures allow researchers to control medium composition, nutrient supply and the concentration of a selected stressor. This can improve treatment comparisons and simplify collection of tissue for biochemical analysis. However, culture media do not replicate soil environments, where pH, organic matter, microbial communities and nutrient interactions influence metal availability. Plantlets grown under artificial conditions may also differ from mature plants. Culture findings should therefore be treated as laboratory evidence and, where appropriate, followed by greenhouse or field assessment using characterized substrates.

6. Proposed Experimental Framework

The design below is proposed for future work and does not describe completed experiments. Concentrations, exposure periods, replication and analytical procedures should be finalized through preliminary trials, literature review and laboratory validation.

6.1 Objectives

1. Establish an in vitro propagation procedure for *C. citratus* using suitable explants.
2. Compare selected growth-regulator treatments for shoot multiplication and rooting.
3. Evaluate growth responses to graded cadmium, lead and chromium exposure in separate treatment series.
4. Measure selected morphological, photosynthetic and biochemical indicators under metal exposure.
5. Quantify metal concentrations in root and shoot tissues and compare their distribution.
6. Identify exposure conditions that produce measurable responses while allowing adequate plantlet survival for analysis.

6.2 Hypotheses

- Culture-medium composition and growth-regulator combinations influence shoot multiplication and rooting.
 - Metal exposure causes concentration-related changes in growth and physiological traits.
 - Antioxidant enzyme activities differ among metal-treatment levels.
 - Metal concentrations may differ between root and shoot tissues under the selected culture conditions.
- These are propositions to be tested, not established findings.

6.3 Plant material and establishment

Healthy donor plants should be obtained from a documented source. A qualified botanist should verify the species, and a voucher specimen should be deposited in a recognized herbarium where feasible.

Young nodal segments or shoot-tip explants may be used. Sterilization should be optimized in preliminary trials to balance contamination control with tissue safety, followed by sterile rinsing.

6.4 Shoot multiplication and rooting

Explants may be cultured on MS basal medium with planned BAP and NAA combinations, including a hormone-free control. Temperature, light intensity, photoperiod, vessel type, medium pH, explant size and subculture interval should be standardized or explicitly included in the design. At predefined intervals, document contamination, survival, time to shoot initiation, shoot number and shoot length. Rooting and acclimatization should also be evaluated with clearly recorded methods.

6.5 Metal treatments

After healthy cultures have been established, cadmium, lead and chromium should initially be examined in separate experiments. A range-finding trial can identify doses that yield measurable responses without immediately killing all tissues. Include a metal-free control and several exposure levels, such as low, intermediate and higher treatments. The final protocol should state concentrations, chemical salts and exposure duration. The same concentration range should not automatically be assumed appropriate for all three metals.

6.6 Measurements and analytical quality

Record root and shoot number and length, fresh and dry biomass, survival and visible stress symptoms using consistent criteria. Chlorophyll a, chlorophyll b and total chlorophyll may be quantified by a validated extraction and spectrophotometric assay. Malondialdehyde (MDA) may indicate lipid peroxidation, while proline can be measured as a stress-associated metabolite. SOD, CAT, POD and APX activities may be determined using established assays. Root and shoot samples may be digested by a validated method and analyzed using atomic absorption spectroscopy or ICP-based instrumentation. Quality assurance should include blanks, calibration standards, analytical replicates and reported detection limits.

6.7 Experimental design and statistics

Use independent replicate culture units and define the experimental unit before data collection. Select statistical methods according to the design and distribution of the data. ANOVA may be appropriate if its assumptions are assessed; a suitable multiple-comparison procedure can follow where treatment effects are detected. Report sample sizes, means, variability, test statistics and exact or appropriately stated significance levels. Numerical results should be included only after experiments are complete and raw data have been checked.

7. Discussion

Existing tissue-culture research provides a basis for propagating *C. citratus* under controlled conditions. A reproducible propagation and acclimatization protocol is a logical prerequisite for stress experiments because it can reduce variation associated with plant source and culture history. The proposed metal-treatment work would extend this foundation by relating defined exposure levels to growth, biochemical responses and metal concentrations in plant tissues.

A combination of measurements is more informative than a single indicator. For example, antioxidant-enzyme changes should be interpreted alongside survival, biomass, indicators of membrane damage and

tissue metal content. Metal accumulation alone does not demonstrate phytoremediation potential; uptake, translocation, biomass production, contaminant removal and safe management of contaminated biomass all require consideration.

Environmental applications need particular care because lemongrass is also used as a medicinal and aromatic plant. Material exposed to contaminants should not enter food, medicinal or essential-oil supply chains without appropriate safety evaluation. Further studies could compare oil profiles of tissue-cultured and conventionally propagated plants, since plant source and growing conditions may affect chemical composition.

8. Research Gaps and Future Directions

8.1 Species-specific metal response

Controlled studies should establish how cadmium, lead and chromium affect lemongrass growth and metabolism across defined exposure levels.

8.2 Genetic fidelity

Long-term multiplication studies should determine whether regenerated plants retain the donor material's morphological and genetic characteristics.

8.3 Phytochemical consistency

Validated chemical analyses can compare essential-oil composition in tissue-cultured plants with conventionally propagated controls.

8.4 Metal distribution

Separate measurements of roots and shoots can clarify metal retention and translocation under experimental conditions.

8.5 Greenhouse validation

Promising laboratory observations should be assessed in controlled greenhouse systems using characterized substrates.

8.6 Conservation and sustainable use

Propagation, germplasm maintenance and quality assessment can be developed as complementary elements of sustainable medicinal-plant production.

9. Conclusion

Cymbopogon citratus is an economically important aromatic grass whose biomass and citral-containing oil support traditional and commercial uses. Published research indicates that *in vitro* techniques can facilitate multiplication and acclimatization, while controlled culture provides a means of investigating responses to environmental stress. This manuscript proposes an integrated framework involving explant culture, growth-regulator optimization, graded exposure to individual metals, morphological assessment, biochemical assays and analysis of metal in roots and shoots. The approach may guide future conservation-biotechnology and stress-physiology research. Nevertheless, metal tolerance, accumulation behavior and any phytoremediation application must be demonstrated through verified experiments and subsequent greenhouse validation. No experimental outcomes are reported in this manuscript.

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Editorial note

This manuscript has been rewritten in original wording from the supplied draft, retaining its proposed-study status and without adding experimental data. Rewriting cannot guarantee a zero similarity score because standard scientific terms, titles and reference details may overlap with published material. Before submission, verify every reference against the publisher or indexing record, add in-text citations in the journal's required style, and ensure that the manuscript accurately represents the research actually performed.