



Collaboration for
Environmental
Evidence

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SYSTEMATIC REVIEW No. 70

**WORKING TITLE: THE USE OF MICRO-PROPAGATION TECHNIQUES TO
INCREASE THE REPRODUCTIVE RATE OF ORCHIDS**

DRAFT REVIEW PROTOCOL

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COVER SHEET

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1. BACKGROUND

The family Orchidaceae encompasses a great number of species of considerable interest and demand due to their ornamental value, which has favored a demanding market for plants and for cut flowers, where considerable amounts of money are spent. These species make up one of the most vulnerable families, due to habitat destruction and to the considerable amount of extraction to which they have been subjected to. The factors affecting the above problem and categorisation of many species into threat and endangered categories are: conservation difficulties, threats caused by habitat reduction, complex germination and long growth periods. The existence of wild orchid reproduction centers would help reduce the destruction of their natural habitats.

Consequently, the decision making that encompasses the sustainable management of orchids cannot be postponed. The use of *in vitro* culture techniques has its advantages: a rapid reproduction of materials in controlled and aseptic environments, which are disease-free, that is except for temperature fluctuations, climate changes, pathogen attacks and other problems that are constantly affecting field sample collections. Likewise, this permits to save on space and costs for the production of specific genotypes, for the mass and uniform production and for the conservation of germplasm which is particularly important to recalcitrant species that do not propagate easily. Aside from this propagation method, the obtained seedlings must be acclimatised. At this point considerable plant losses can occur due to the failure of the seedlings to adapt to *ex vitro* environmental conditions.

The establishment of micropropagation systems to mass reproduce orchids from seeds or vegetative tissues and its acclimatisation in greenhouses, can maintain a continuous production of specific or endangered species so that the extraction of the orchids from natural habitats may be reduced (Lee and Lee 1991). According to Arditti and Krikorian (1996), orchids were the first plants to be propagated *in vitro* from the sowing of sexual seeds, symbiotically by Noel Bernar, c. 1900, asymbiotically by Lewis Knudson in 1921, as well as clonally by introducing meristem culture techniques for vegetative or asexual propagation (Capellades *et al.* 1991).

Following the production of vitroplants, it is necessary to acclimatise them; this step refers to the final phase of most micropropagation procedures, where the plants need to adapt to new environmental conditions of relative humidity, lighting and temperature, among other variables. The intrinsic quality of *in vitro* seedlings is considered to be one of the most important factors that determine the success during the transition to *ex vitro* conditions. The main factors to overcome are those associated to the excessive loss of water by transpiration, and to their poorly developed photosynthetic system. Generally, most of the *in vitro* plant losses occur when they are moved to new environmental conditions (Roca 1991). Consequently their *in vitro* propagation success lies in being able to acclimatise the plants to new environmental conditions. During this phase, there occurs a gradual return to the autotrophic functioning of the plantlets, as well as the recovery of the species' typical

morphological and physiological characteristics. Likewise, in this phase the seedlings often suffer from a type of stress that is provoked by changes in humidity, temperature or lighting conditions which permit the plants to adapt to of their natural habitats conditions.

In this review, we will search for alternative solutions that will permit the mass production of plants, based on the use of artificial reproduction techniques which may be carried out efficiently, quickly and safely through the *in vitro* propagation and posterior acclimatisation of the plantlets which were obtained under greenhouse conditions.

The systematic review will be carried out on the family Orchidaceae, on those studies related to *in vitro* propagation, as well as with the acclimatisation of vitroplants.

2. OBJECTIVE

Examine the effectiveness of *in vitro* propagation and posterior acclimatisation in Orchids.

Primary question

Is *in vitro* propagation and posterior vitroplants acclimatisation an effective method to increase orchid reproductive rate?

3. METHODS

Search strategy

An Internet search will be performed using the following search engines:

- Google (<http://www.google.com>)
- Google Scholar (www.scholar.google.com)
- Intute (www.intute.ac.uk).
- www.alltheweb.com
- www.dogpile.com

Data bases

The following data bases will be consulted:

- Pre and postgrad theses in digital format
- Book editorials (Elsevier, CAB International, etc.)
- Technical reports
- Libraries in Venezuela (IVIC, FAGRO, etc.)
- Interview experts and members of the country's orchidology societies
- Abstracts from national and international meetings
- Ebsco (<http://web.ebscohost.com>)
- Directory of open access journals (<http://www.doaj.org>)
- Copac (<http://copac.ac.uk>)
- Scientific electronic library online (<http://www.scielo.org>)

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- Scirus for scientific information only (<http://www.scirus.com>)
- Index to theses online (<http://www.theses.com>)
- Agricola (<http://www.agricola.nal.usda.gov>)
- Jstor (<http://www.jstor.org>)
- Science. Gov (<http://www.science.gov>)

Other pertinent data bases and catalogues according to the experts will also be consulted:

- Scielo
- Biological abstracts
- CAB abstracts (<http://www.cabi.org>)
- Cambridge Scientific Abstracts
- USDA National Agricultural Library
- W3tropicos: nomenclatural and specimen database of the Missouri botanical gardens
- Epic: electronic plant information centre of royal botanical gardens, Kew
- Science direct
- ISI web of knowledge, Web of Science
- Science Citation Index (SCI), Biosis Previews
- Latindex Catalogue
- Visits to orchid growers (geographical distribution, phonological variables, morphology, etc.).

The following combination of terms will be searched both in Spanish and English:

Terms in English	Terms in Spanish
Orchids and propagation	Orquídeas y propagación
Orchids and micropropagation	Orquídeas y micropropagación
Orchids and <i>in vitro</i> culture	Orquídeas y cultivo <i>in vitro</i>
Orchids and <i>in vitro</i> propagation	Orquídeas y propagación <i>in vitro</i>
Orchids and acclimatisation	Orquídeas y aclimatación

A broad systematic review will be carried out: orchids-micropropagation-acclimatisation. In this way searches will be carried out on the acclimatisation of seedlings that have been obtained under *in vitro* conditions. The key words found in the studies found will also be included in the review. In each of the searches Word or PDF documents will be examined if found pertinent to the review. Experts in orchids will also be consulted.

Study inclusion criteria

- *Relevant subject*: Orchids on which micropropagation and *in vitro* propagation studies have been carried out
- *Types of intervention*: sexual and asexual micropropagation, acclimatisation of seedlings obtained under *in vitro* conditions.

- *Types of comparators:* Conventional propagation, types of acclimatisation. Mass plant production in small spaces. Acclimatized plants in greenhouses.
- *Types of studies:* Descriptive and quantitative. Studies that present relevant information on the review's relevant subject, intervention and proposed indicator variables will be considered.
- *Potential reasons of heterogeneity:* Variations in the collection times can entail variations in the *in vitro* response. The possible heterogeneity of the *in vitro* seedlings may present variations in the acclimatization process, interactions with local populations, environmental changes and potential plant plagues.

The inclusion criteria will be used in three phases with the objective of filtering out the most relevant studies. The three phases will be defined from the evaluation of the studies from:

1. *Title and keywords:* those studies whose title and keywords are associated to the objective of the review will be included.
2. *Summary:* After the first filter phase, the abstracts of the studies will be revised to select those that are more relevant to the review
3. *Whole article:* Those more relevant studies will be revised in order to select those that are most adequate for the analysis

3.3. Study quality assessment

Complete articles will be considered and subjected to a quality analysis using assessment tables. Special attention will be paid to those elements that have arisen as a result of bias. If the studies are found appropriate and if sufficient are available, a meta-analysis will be performed. The studies will be classified according to their importance based on the general objective. A validation analysis will be carried out, based on the reviewed protocols of *in vitro* propagation and acclimatisation and considering the relevant subject of the review. After the classification, a second reviewer will evaluate a sample of 25% of the studies to verify whether the criteria are clearly defined and have correctly been used to classify the studies.

3.4. Data extraction strategy

For the analysis of the selected studies for the review, repeatability in the extraction process will be guaranteed with an independent reviewer. Tables will be created for the qualitative and quantitative data of the previously defined variables of the intervention. Studies that measure the efficiency of the different propagation types will be considered in the review to establish comparisons and to determine the criteria of the quality of the data. A sample of 25% of the studies will be evaluated by a second reviewer to verify whether the extraction criteria of the data and efficiency of the tables is adequate. The bibliographical information will include the complete reference as well as the abstract (if available) of the studies, and this will be organised with a specific program (See appendix).

During the data extraction, the following characteristics of each study will be recorded: type of study (experimental, theoretical), species, location, date, research level, sources of heterogeneity, quality of the study, among others.

3.5. Data synthesis

If possible a meta-analysis will be included in the review that will describe the effects of the proposed intervention. If the quality of the data is not adequate, a descriptive review will be carried out. Summary tables will be created to compile information on the authors, organisation, year of study, source and others. For each of the analysed studies, summary tables and records will be created that will encompass the following variables: characteristics and quality of the study, sources of heterogeneity, important results (qualitative and quantitative), etc. If not sufficient and important data is available, tables will be created to compare and analyse the information in an organised manner.

4. POTENTIAL CONFLICTS OF INTEREST AND SOURCES OF SUPPORT

None expected. The project is financed by the NGO PROVITA and the Universidad Central de Venezuela. Facultad de Agronomía - CIBA.

5. REFERENCES

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APPENDIX

Reference	Relevant subject	Intervention	Response variable	Summary of main results	Methodology used
	Orchids	In Vitro propagation and acclimatisation of vitroplants	<ul style="list-style-type: none">▪ Reproduction rate▪ No. of acclimatised plants		

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