



**European Cooperation
in the Field of Scientific
and Technical Research
- COST -**

Secretariat

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COST 288/99

Subject : Draft Memorandum of Understanding for the implementation of a European
Concerted Research Action designated as COST Action 843 "Quality
enhancement of plant production through tissue culture"

DRAFT
Memorandum of Understanding
for the implementation of a European Concerted Research
Action designated as
COST Action 843
Quality enhancement of plant production
through tissue culture

The Signatories to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the Technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 400/94 "Rules and Procedures for Implementing COST Actions", the contents of which are fully known to the Signatories.
2. The main objectives of the Action are (1) innovation of plant propagation methods that permit and enhance sustainable and competitive agriculture and forestry in Europe and (2) initiation and extension of networks amongst European scientists in the field of plant tissue culture that facilitate knowledge exchange and expertise transfer.

3. The overall cost of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 15 million at 1999 prices.
4. The Memorandum of Understanding will take effect on being signed by at least five Signatories.
5. The Memorandum of Understanding will remain in force for a period of five years, unless the duration of the Action is modified according to the provisions of Chapter 6 of the document referred to in Point 1.

COST ACTION 843

**Quality enhancement of plant production
through tissue culture**

A. BACKGROUND

The key role of plant tissue culture

Plant breeding and crop production, both by traditional and biotechnological methods, increasingly rely on plant tissue culture (in-vitro culture) as a mainstream tool that provides key opportunities for plant quality enhancement and subsequent economic sustainability. For example, the development of pest- and disease-resistant plants through biotechnology depends on a tissue-culture growth stage; as a result, these resistances enable growers to reduce or eliminate the application of crop-protection chemicals. By propagation in vitro, new and/or elite plants can be mass-propagated with far greater speed than through traditional methods. Additionally, micropropagation produces high-quality plants that may be free from viral and bacterial diseases and that have an increased cropping capacity.

Thus, in vitro culture is an essential cornerstone for a sustainable crop-based agricultural industry across the EU.

Bottlenecks

The high costs of labour in the EU, needed for the skilled manual processes inherent in the current processes of micropropagation, present a major economic obstacle if in vitro culture is to be fully exploited. Thus, in the EU the competitiveness of these plant-based industries is compromised. Furthermore, the benefits that may be achieved through tissue culture are being applied successfully only to a limited number of crops, because many crops are unresponsive to tissue culture.

European perspective

The EU member states benefit directly and indirectly from the proposed action: pan-EU scientific co-operation is required to provide the critical scientific mass and transfer of expertise that will lead to widely applicable advances in this field. The proposed action focuses on two strategies to increase competitiveness of the European in-vitro plant production industry:

- the development of high-tech micropropagation methods which reduce labour input and
- the production of plants of superior quality compared with the plants that are usually produced in tissue culture.

Research

It is obvious that the bottlenecks described above, require extensive research inputs. Therefore, a new action is proposed that deals with research aimed at innovation of the in-vitro technology and at plant quality enhancement. This is a logical successor to previous COST actions in the field of plant tissue culture that focused on protocol development for specific crops, 'mass propagation' and understanding the underlying mechanisms of processes occurring in vitro. Many of the aims of the previous COST actions have been achieved.

B. OBJECTIVES AND BENEFITS

The main objectives of the action are (1) innovation of plant propagation methods that permit and enhance sustainable and competitive agriculture and forestry in Europe and (2) initiation and extension of networks amongst European scientists in the field of plant tissue culture that facilitate knowledge exchange and expertise transfer.

Objectives

Through co-operative research in this action, tissue culture methods will become available that are required for agriculture and forestry of the 21st century. Future agriculture and forestry are sustainable, they use little or no crop-protection chemicals, have a low energy input, have a high yield and produce plants of highest quality (e.g. better post-harvest storage or enhanced flavour).

Biotechnological breeding is an essential tool to achieve these goals, and, as noted before, tissue culture is an integral part of plant breeding through biotechnology. In addition, propagation in vitro is essential. The introduction of "improved" plants on the market usually takes several years, since propagation is slow. For example, it may take a tulip breeder 20-25 years to produce sufficient bulbs of a newly bred cultivar before they can be marketed. Propagation in vitro can considerably speed up this process so that only ten years are needed. However, commercial tissue-culture is reliant, mainly, on "conventional" tissue-culture techniques that are still relatively slow (namely, auxillary propagation on gelled media). The proposed action will target the development of potentially faster techniques such as somatic embryogenesis, propagation in liquid medium or automated culture preparation/handling systems.

Process innovation is not only necessary for faster production of improved crops but also to reduce costs. Currently, labour accounts for 60-70% of the costs of a plant produced in vitro. Since labour costs are relatively high in the EU, development of faster - and therefore cheaper - propagation methods is necessary to maintain and increase competitiveness and to exploit the advantages of tissue culture for a broad range of crops.

The techniques of tissue-culture itself also offers many possibilities for production of plants of high quality but up to now, this potential has been little exploited. During growth in vitro, plants can be "prepared" for optimal growth after transfer to ex-vitro conditions. Potentially, following such manipulations, tissue-cultured plants out-perform conventionally propagated plants. Research focused on this aspect will receive much attention in the proposed action.

Thus, for a sustainable and competitive agriculture and forestry in Europe, in-vitro culture is essential: it is a prerequisite for the successful application of plant breeding by biotechnological methods, for the rapid introduction of improved plants in the market and it offers unique possibilities for the production of plants of superior quality.

Benefits

Because of the increasing importance of plant tissue culture and the rapid developments in the field of biotechnology, co-ordination of research efforts is essential to ensure that the EU will remain one of the world leaders in this vital area.

This action will result in extensive networks of European scientists. This will lead to swift exchange of information, synergy and cooperative research. The success of previous COST actions in the field of plant tissue culture demonstrates the effectiveness of the COST-approach.

C. SCIENTIFIC PROGRAMME

Focus of the action will be on 1. innovation of plant propagation methods and 2. plant quality enhancement.

The research carried out will deal with the following subjects:

Advanced propagation techniques

Currently, most commercial propagation *in vitro* is done by axillary bud propagation on gelled media. During growth of plants *in vitro*, new shoots are formed by activation of buds in the axils of leaves or branches. In this way, from every bud, a cluster of shoots is formed within a few weeks. Then, the newly formed shoots are excised and subcultured to allow outgrowth of their axillary buds in the next subculture cycle. This method closely resembles the way many plants are propagated *in vivo*, namely, by making cuttings.

Culture on gelled nutrient media requires frequent transfer of plantlets to fresh media, which is exceptionally labour-intensive. Two potentially faster methods are hardly being used commercially: axillary propagation in liquid medium and somatic embryogenesis. By using liquid medium instead of gelled medium, propagation is accelerated, culture transfer frequencies may be decreased and labour is less intensive. However, liquid media provide a substantially different environment for the plantlets, and widespread use is hampered by several problems including hyperhydricity of the tissue, rapid spread of contaminants (e.g. bacteria and fungi), plantlet asphyxiation and changed direction of growth with respect to gravity. Even faster propagation can be obtained using somatic embryogenesis. In this method, single cells or cell clusters, not buds, are propagated. When required, cells are stimulated to form embryos which then develop into new plants. Potential propagation rates are extremely high (millions of cells can be grown in a litre of medium) and labour input is very low. However, at the moment, large-scale somatic embryogenesis is feasible only in a few crops including conifers. Process bottlenecks are technical (bioreactor technology) or physiological (e.g. establishment of stable embryogenic cell suspensions, embryo maturation and conversion into whole plants).

Labour input can also be reduced by automation of the propagation system (robotisation); e.g. robotised handling of plantlets grown on gelled media, automation of bioreactor systems, automated selection of embryos from a suspension and transfer to maturation media. Up to now, automation has been problematic. Drawbacks are related to the heterogeneity of plant material which makes image analysis (e.g. determination of the site at which a shoot cluster should be machine-cut) difficult to realise, high costs for developing an automated system and impossibility of propagating different crops by the same system.

A prerequisite for developing new propagation techniques is fundamental knowledge of regeneration. This research will have highly significant spin-off: the knowledge generated about the mechanisms operating in adventitious regeneration will be exploited both in biotechnological breeding techniques and in conventional propagation techniques (e.g. rooting of conventional cuttings).

Plant quality enhancement

The quality of plants is improved by in vitro culture, giving rise to plants that are free of most, or even all, endogenous pathogens. Therefore, tissue-cultured plants show a far better performance after transfer to soil than plants obtained by conventional propagation techniques.

There are, though, more and often still non-explored aspects about quality of tissue-cultured plants. Plant quality can be influenced by many different factors: by the manipulation of the physiological, nutritional and physical culture environment; by rooting treatments; through the induction of culture photosynthesis; by the application of endophytic and epiphytic organisms. Thus, during growth in vitro, plants can be prepared for optimal growth after their transfer to ex vitro conditions. This means that the in vitro system may also be used to increase the quality of the plants.

It should be noted that because of the in vitro environment, the performance of the plant may suffer instead of benefit. Plant growth regulators used during tissue culture may have unwanted after-effects. Furthermore, because of high humidity and low light intensity during the tissue-culture stage, following transfer to soil, the plants need to adjust to their new environment. Optimal performance after transfer to ex vitro conditions is determined by different plant characteristics such as the capacity to withstand "hardening" (preferably, plants should be conditioned in such a way that no hardening treatment is necessary), the capacity to form a well developed root- and leaf-system and genetic stability. Research in this part of the action will focus on the influence of in-vitro conditions on ex-vitro performance.

D. ORGANISATION AND TIMETABLE

D.1. ORGANISATION

The new action will consist of 5 working groups (WGs) with distinct and well defined missions. Working groups will deal with the problems outlined above and will be defined according to disciplines. Interactions between WGs and with other COST-actions are proposed.

In detail, the WGs deal with:

INNOVATION

1. Developmental biology of regeneration

A prerequisite for developing new propagation technologies is fundamental knowledge of regeneration. This WG deals with molecular, biochemical, physiological and histological studies on adventitious regeneration of roots, shoots and embryos. A major point is pre-existing and acquired developmental competence.

2. Advanced propagation techniques

This WG deals with development of innovative propagation technologies for mass propagation such as somatic embryogenesis, propagation in liquid medium and automated systems. Its topics include establishment of embryogenic cell suspensions, propagation in liquid medium, bioreactor technology and somatic embryo conversion to plants.

QUALITY ENHANCEMENT

3. Physiology related to the micro-environment

In this WG, non-plant growth regulator factors affecting plant quality in vitro are studied: nutritional factors, uptake of nutrients, physical (stress) conditions, photosynthesis, culture vessel headspace, hyperhydricity.

4. Biochemistry and physiology related to plant growth regulators

This WG focuses on the effects and after-effects of plant growth regulators ("traditional hormones" and new substances).

5. "Stage 4"-technology

This WG deals with preparation for growth ex vitro (hardening, rooting) and performance ex vitro (genetic stability, screening for genetic variation, field trials).

WORKSHOPS

Because some aspects are dealt with in various working groups, albeit from another perspective, easy interactions between the working groups in specially organised workshops are guaranteed and will result in a high level of synergy. These workshops have an essential role in the proposed action. Also, in workshops, topics can be treated that are adjacent to the mission of the new COST action, and interactions with other COST-actions can be pursued. Topics may include:

Genetic modification

Many problems in genetic engineering are related to tissue culture, in particular to regeneration. Other items include: isolation of desirable genes, making genetic constructs, making gene banks, gene transfer techniques, expression of introduced genes, stability of genes, introduction of genes for virus resistance, testing for virus resistance, bio-safety, field performance of transformed plants, disturbance of performance by Agrobacterium.

Micro-biological aspects

Contamination can be a serious problem in commercial plant propagation. However, except for use of antibiotics or heat-treatments, there are no possibilities to eradicate contaminants. Another problem is spreading of insects or mites in culture rooms. This workshop can be organised in co-operation with COST Action 823 (Early detection of pathogens). Use of beneficial micro-organisms in tissue culture might be discussed in a joined workshop with COST Action 821 (Mycorrhizae).

In vitro protocols

There is a need for exchange of detailed information on technical aspects of in-vitro protocols. This information is often not given in sufficient detail which might hinder replication of experimental results and commercial protocols in other laboratories. This subject is probably of interest for people in different working groups. It may include (in various small workshops): gene transfer techniques, propagation protocols of different plant species, sterilisation methods and HACCP-norms (Hazard Analysis Critical Control Points) for commercial tissue culture laboratories.

Crop specific workshops

For example, regeneration in monocotyledons: the topic regeneration is dealt with in various WGs from different perspectives. This workshop will provide state-of-the-art presentations from the various perspectives. It may be organised together with COST 824.

Publications

To ensure efficient transfer of knowledge to the field of application, knowledge gained in the course of this COST-action must become available throughout Europe. This will be achieved by articles in scientific journals, special "COST" issues' of journals, by publications of the EU and via the Internet.

There will be collaboration with working groups of the International Society of Horticultural Science (ISHS) and with the International Association of Plant Tissue Culture and Biotechnology (IAPTC&B).

D2. TIMETABLE

Every working group will organise one meeting per year in the course of the action. In the first year, every working group will make a time schedule on the scientific targets to be achieved by the working group. There will be a number of workshops during the action to increase the communication between groups and to invite external experts.

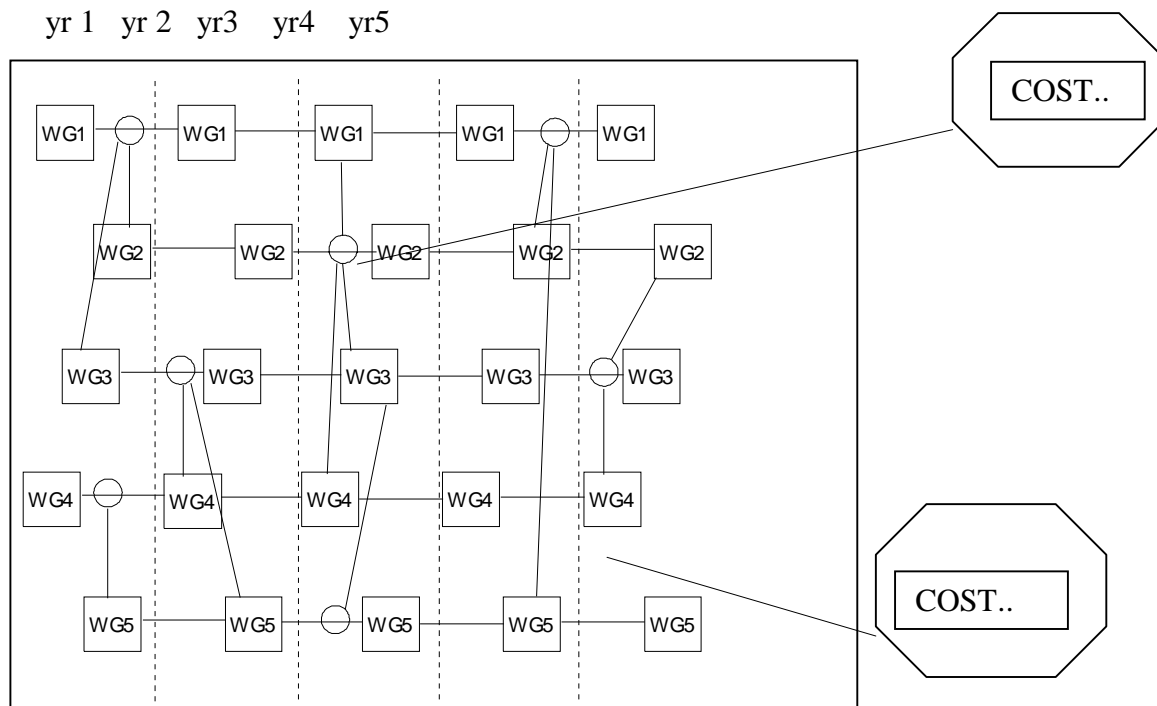


Diagram showing the organisation of the proposed action. Squares represent WG meetings and circles represent examples of workshops. Also interactions with some other COST actions are shown.

E. ECONOMIC DIMENSION

In 1996 about 505 tissue culture laboratories were found in Europe with an estimated production of 180 million plants per annum. In these laboratories about 3330 people are employed.

Furthermore, already some 30 scientists have expressed strong interest in participating in the proposed action. This corresponds with an estimated EUR 15 million of research costs contributed by the participating countries.
