Brussels, 27 May 2022

COST 071/22

**DECISION**

Subject: Memorandum of Understanding for the implementation of the COST Action "European Network for Innovative Woody Plant Cloning" (COPYTREE) CA21157

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action European Network for Innovative Woody Plant Cloning approved by the Committee of Senior Officials through written procedure on 27 May 2022.

## MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

**COST Action CA21157**
**EUROPEAN NETWORK FOR INNOVATIVE WOODY PLANT CLONING (COPYTREE)**

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to address the major research challenges of in vitro cloning of woody plants, its public acceptance, risk assessment and promotion of commercial applications, in order to achieve a well connected and informed scientific community and better informed policy makers, stakeholders and market. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

## OVERVIEW

**Summary**
In vitro culture of woody plants is leaving the academic laboratories and is now being developed in a range of commercial applications in horticulture and forestry that respond to the challenges of climate change and changing global food and wood consumption habits. It is therefore urgent that the research challenges, public acceptance, risk assessment and commercial application are confronted now in order to establish a well informed scientific community, policy makers and market place. This proposal concerns the following challenges, whose solution will have a significant scientific, social and economic impact: How can we overcome recalcitrance in a lot of woody plants? What are the best tools for diagnosis, sanitation and storing clean stocks? How can the production of elite clones be scaled up at a acceptable price? What are the real risks of this technology and how can the public be informed so that they appreciate and accept the applications ? How can foresters and landowners be persuaded to invest in planting poly-clonal forests? Taking these aspects into account, it seems more than urgent to us to set up a European network to connect the researchers involved from various domains, so that they can share innovations and develop new research strategies, assess the risks of the technology and improve communication with stakeholders and the general public.

| Areas of Expertise Relevant for the Action | Keywords |
|---|---|
| ● Agricultural biotechnology: Biotechnology (non-medical)<br>● Agriculture, Forestry, and Fisheries: Agriculture related to crop production, soil biology and cultivation, applied plant biology, crop protection | ● Woody<br>● shrub<br>● biotechnology<br>● somatic<br>● micropropagation |

**Specific Objectives**
To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination
● Improving the understanding of all aspects of micropropagation of woody plants. The action aims to take this technology to the next level to address the challenges of 'Recalcitrance', 'Diagnosis and sanitation' and 'Scaling up and automation', as well as the challenges 'Technological risk and public acceptance' and 'Commercialisation'.
● Comparative performance evaluation of emerging technologies through the exchange of research tools, know-how and practical experience. This platform will benefit from the skills of the different groups to integrate in vitro protocols, diagnostic methods and production experience to address the above challenges.
● Encouraging open access publication of joined research results
● Vulgarisation of breakthroughs and their impact, tailored to stakeholders, including the general public, and providing input on future market application for investors
● Providing information on and raising awareness of the potential risks of the technology

Capacity Building
● Bridging in vitro technology with other disciplines to achieve breakthroughs that require an inter-disciplinary approach

● Promoting knowledge exchange and the development of joint projects and doctoral research themes around new or emerging research areas.
● Building a stakeholder platform for exchanging information and stimulating interaction
● Stimulating investments in cost effective and innovative business models for in vitro production of woody plants.

# TECHNICAL ANNEX

# 1. S&T EXCELLENCE

## 1.1. SOUNDNESS OF THE CHALLENGE

### 1.1.1. DESCRIPTION OF THE STATE OF THE ART

Trees and shrubs together are called woody plants. They differ from herbaceous plants because they form wood as a structural tissue. This action focuses on woody plants that produce timber wood, edible fruits, nuts, berries, saps, forage and plant-based medicines or have an ecological, ornamental or even cultural value. Although historically, there has been a focus on herbaceous ornamentals, nowadays micropropagation of trees and shrubs is gaining great importance. The past years have seen an ever-growing need for superior planting material for fruit and nut orchards, commodity and biomass plantations and timber forests due to changing food patterns and climate change. Sustainable re- and afforestation and the restoration of degraded forests can increase absorption of CO2 while improving the resilience of forests and promoting the circular bioeconomy. In Europe, researchers from universities and institutes are anticipating this evolution and trying to solve a number of challenges, which are listed below.

#### Challenge 1: Recalcitrance

In vitro recalcitrance is the inability of plant cells, tissues, and organs to respond to tissue culture manipulations. With respect to plant propagation and regeneration, recalcitrance is a major limiting factor for the biotechnological exploitation of economically important woody plant species, and it can also im- pair the wider application of in vitro conservation techniques.

*Gene regulation*

In vitro explants derived from mature woody plants usually exhibit low morphogenic ability. In contrast to juvenile seedlings, they often lack vigorous growth and root poorly. Only recently detailed studies are highlighting the physiological and molecular events involved in plant rejuvenation. DNA methylation, histone modifications, microRNAs and telomere length could play an important role. In the last decade, successes in the field of -omics have stimulated scientists to elucidate the molecular mechanism initiating and controlling in vitro growth and development. Nevertheless, insights obtained in the model plant Arabidopsis are not readily applicable to woody plants due to their large size, long life cycle and large genome size. To advance, there is a need to outline the major regulatory genes implicated in in vitro rooting, shoot organogenesis and somatic embryogenesis in woody crops as well as to understand the metabolic and signalling pathways involved.

*Proteomics*

The proteome refers to the set of proteins expressed by the genome of an organism at any given time or condition. Proteomics allows the quantitative and qualitative evaluation of differentially expressed proteins in different physiological events occurring in cells, tissues, and organs. It generated remarkable results that improved our understanding of, for example, somatic embryogenesis in trees. Recently, gel-free separation and tag-free quantitation considerably enhanced the sensitivity of protein identification and the recently introduced protein networks could help to find solutions to the irregular or even non-existent germination of somatic embryos of so many species.

*Epigenetics*

Plant developmental processes, as differentiation and proliferation of shoots, roots and somatic embryos are accompanied by chromatin remodelling and epigenetic reprogramming. Methylation constitutes a prominent epigenetic modification that can lock the DNA in a transcriptionally inaccessible con- formation. Analysing DNA methylation distribution patterns informs on the regulatory mechanisms of these processes, helping in the design of efficient protocols in different species. in situ localization approaches using modern bioimaging technology have become potent tools to analyse spatial and temporal patterns of methylation.

### *A new toolbox to solve old problems*

New in vitro chemicals are regularly developed, but their application to woody crops is delayed due to a lack of knowledge transfer. Examples are the so-called topolin cytokinins. Many research groups have shown that these compounds can make the difference between success or failure in the micro-propagation system of a plant species. Moreover, derivatives of phenylurea compounds have recently been developed that act as pure cytokinin oxidation inhibitors and hold promise as a new tool in the in vitro toolbox. Furthermore, the "chemical genetic approach" has become a popular method for identifying interesting plant growth regulators and compounds that interfere with signal formation. Using pipette robots and image recognition, chemical libraries are screened with literally tens of thousands of small molecules. Almost always, the model systems used are based on a particular developmental stage of young Arabidopsis seedlings in multiwell plates. Compounds have been identified that affect cell elongation (brassinosteroid signalling), cell division and lateral root induction (auxin signalling), shoot organogenesis (cytokinin signalling) and protoplast regeneration, apical hook opening (ethylene signalling). Together, these studies have resulted in an impressive collection of unique molecules. The application of these molecules in cloning woody plants is still a virtually unexplored area. Other tools include affordable LED lamps that allow morphology and development to be altered in vitro through manipulation of phytochromes and cryptochromes. New bioimaging technologies also offer great opportunities. Worth mentioning are the huge range of cameras and imaging software that can track bio- luminescent genetic reporters, estimate stress factors using epifluorescence and enable 3D micros-canning.

## Challenge 2: Diagnosis, sanitation and germplasm conservation

### *Virus/bacteria diagnosis by Next Generation Sequencing*

Vegetatively propagated woody plants can be systemically infected with one or more plant pathogens, not only in the field, but also in nurseries. Traditionally, selected viruses have been detected by ELISA and RT-PCR and bacteria have been characterized by using culture-based methods followed by further confirmation. These methods will not detect other potential viruses that are not included in the test with specific antiserum or primers and the bacteria that are not able to grow on standard artificial media. Biological indexing has been applied to screen potential pathogens in relative long indexing period with several indexing cycles. However, high-throughput sequencing (HTS), also called next-generation sequencing (NGS) or deep sequencing, are powerful tools for both targeted and non-targeted pathogen analysis for certification of nuclear stocks and propagation materials. HTS has the potential to re- place the traditional testing procedures, shortening the indexing cycles and increasing pace flow of novel varieties to the market. In addition, microbial profiling using HTS can be applied to identify the community of non-culturable microorganisms that often exist in in vitro mother plants. They often present a real burden for reliable micropropagation protocols because they produce plant hormones and toxins that influence growth and development. We are at a turning point in this regard because routine pathogen diagnosis and cleaning in woody plants will be needed to set new international standards, which are essential for research, production, and international trade.

### *Sanitation technology*

In addition to the development of sensitive techniques for detection, identification and characterization of viruses and bacteria, substantial progress must be realized in plant sanitation. For viruses, these methods are essentially based on meristem culture, preceded by thermotherapy, and followed by shoot tip culture. In order to improve the efficiency of those methods, additional tools such as chemotherapy, electrotherapy and cryotherapy have been developed. For many species, making an in vitro mother plant completely bacteria-free is an enormous challenge.

### *Medium and long-term storage*

Today, conservation of plant species has become a high priority to ensure sustainable use of biological resources and prevent further loss of plant diversity. Efficient conservation of plant germplasm is essential not only to prevent further loss of interesting genotypes, but also to preserve the certified stocks obtained with such great effort.

Cryopreservation, i.e. the storage of plant material in liquid nitrogen has become the only safe and cost-efficient option for the long-term conservation of various categories of plants, including non-ortho- dox seed species, vegetatively propagated plants and rare and endangered species. The utilization of new vitrification-based techniques including vitrification, encapsulation-dehydration, encapsulation-vitrification, droplet-vitrification, and V-cryo-plates has extended the applicability of cryopreservation to a broad range of plant species. However, the effectiveness of these protocols is highly genotype dependent and as the regeneration capacity of cryopreserved tissues is affected by the physiological stage of plant material, its tolerance to osmotic, chemical, desiccation and cold stress etc. Hence, additional research is needed and should focus on additional genotypes and species and validation of improved cryopreservation protocols in different laboratories.

## Challenge 3: Lack of scaling up and automation

### *Somatic embryogenesis*

Somatic embryogenesis (SE) is the developmental process by which somatic cells, under suitable in-duction conditions, undergo restructuring and go through a series of morphological and biochemical changes, resulting in the formation of a somatic embryo. The regulation of SE development, maturation and subsequent germination requires the concerted action of several signalling pathways that integrate genetic and epigenetic programs, as well as hormonal and metabolic signals. SE has been observed in a wide range of tissue culture systems but in Gymnosperms it is the only available micro- propagation method (Park 2013). For many commercial conifer species, many research efforts have already been made to render SE industrially applicable. However, despite these efforts, large scale industrial application has so far been problematic due to a limited number of species that react well. A lot of effort is needed, especially to improve the frequency of conversion of embryos into viable plants. Somatic embryogenesis has a lot of advantages in comparison with other propagation techniques, but the main advantage is that embryogenic tissue can be cryopreserved while field testing is still in progress. Once field tests show which genotypes perform best, the corresponding embryonic tissue can be thawed and used for mass multiplication of elite plants for commercial reforestation.

### *Temporary Immersion Bioreactors*

To compete with plants coming from countries at low labour costs, new approaches in micro-propagation have recently been explored. Experiences with temporary immersion systems are promising in this respect and deserve to be shared. The technology to produce plants in bioreactors has caught a lot of attention of commercial in vitro laboratories. The main reasons are reduction of space and cutting of labour costs. To avoid problems such as hyperhydricity, other variants were explored. Among those, Temporary Immersion Systems (TIS) become popular as they allow short-term contact of shoot clusters and somatic embryos with the liquid medium. These short immersions are appropriate to take up the nutrients by the complete plant surface, permitting rapid growth under a head space that allows gas-exchange. In the last decades, several TIS bioreactors have been developed, based on single or double containers. They allow automation, as the media can easily be renewed without changing the container. Moreover, the atmosphere can be renewed, providing adequate gas transfer to the cultures. The use of forced ventilation may promote photoautotrophic behaviour, improving plant propagation and acclimation. As rooting and acclimation are still important challenges for woody plant culture, the use of bioreactors for tree propagation may represent a real improvement in this matter. However, there are still some points that should be addressed in order to extend the use of this system, such as the occurrence of hyperhydricity and the risk of loss of explants by fungal or bacterial contamination. The bioreactor model, immersion frequency and duration, phytohormone type and concentration, explant type and the use of support material are some of the parameters that merit careful evaluation.

*Robotics*

The micropropagation process is tedious and labour intensive; thus, limiting the application of this technology due to the high labour cost, which still remains one of the major costs in tissue culture industry in the developed countries. From the 1990s onwards, there was worldwide interest in the development of automated technologies for micropropagation. In addition to reducing labour costs, robotised systems have other advantages: guaranteed uniformity and quality of plants, reduced levels of contamination, elimination of human error and improved control of production. This is why several companies tried to include automation systems in their tissue cultures. Although an automated micropropagation process has been in progress step by step for decades, the technology is only now becoming sufficiently efficient and affordable to implement. The problem is that plant morphology differs a lot, which makes it technically challenging to develop a generally automated process that can be used for multiple species. The flexibility achieved with the human eye-brain combination can only be matched by the current level of artificial intelligence (A.I.) and Machine Learning. In Germany, Robotec's Robocut won many awards. By means of Machine Learning, this robot is taught where to cut the laser cutter.

## Challenge 4: Pro-active management of technological risk, public acceptance and legislation

*Somaclonal variation*

Somaclonal variation has more to do with epigenetic changes (methylation) than with DNA mutations. DNA methylation can be quantified with (i) sodium bisulfite conversion and sequencing, (ii) differential enzymatic cleavage of DNA, and (iii) affinity capture of methylated DNA.

*Public concern and acceptance*

While the production of clonal plants in fruits (rootstocks and cultivars) and ornamentals is today largely accepted and considered economically-important, public acceptance could become very significant if the use of clonal forestry does not adequately address the long-term impact on genetic variation. The main public concern about the use of vegetatively propagated (forest) trees stems from an aversion of man's "manipulation of nature", specifically the concern that vegetatively propagated mate- rials could potentially result in a loss of genetic diversity which could lead to a catastrophic decline of forests. There is indeed a scientific consensus that monocultures with their limited genetic variation are at risk. But it is entirely possible to produce a vegetatively propagated plantation that has a greater genetic diversity than is available even in natural forests by including material from a wide range of populations. An increase in productivity balanced with environmental sustainability will decrease the pressure to allocate more land for plantations, thus mitigating risks as the replacement of native species and potential long-term loss of diversity at the landscape level. A process for addressing environ- mental concerns basically depends on sharing the experience with testing of poly-clonal forestry to demonstrate that public concerns are being addressed. Continued communication is essential in this process. Failure to address these concerns could lead to increased legislation.

*Legislation*

The Council Directive 1999/105/EC on the marketing of forest reproductive material contains definitions of how clones and clonal mixtures are defined. But is the individual member state that can regulate the number and proportion of clones as well as how long clones are to remain in production in forestry. In Germany and France only tested material can be vegetatively propagated and must be planted in clonal mixtures. In Finland, "qualified" mother plants can be vegetatively propagated up to one million copies (two million for birch). More than 11 clones must be used to plant clonal mixtures. In Sweden up to 5 % of a site (up to 20 ha) can be planted with one or more clones. In Norway, a mini- mum of 30 clones from 10 unrelated families are required with a maximum of 50 plants per clone per site. In Denmark only the use of clones of poplar are regulated for planting in Denmark. As for fruit species, the Italian legislation on the production of certified plants imposes a maximum of 12 subcultures before the line must be renewed, starting from genetically- and sanitary-controlled stock plants. In most member states no specific regulations exist. However, this could change due to public pressure (Lelu-Walter et al., 2013).

### Challenge 5: Insufficient commercialization

*Convincing stakeholders and investors*

Pome, stone and berry fruits (cultivars and rootstocks) are largely produced today by micropropagation. European commercial laboratories offer rootstocks of peach, plum, cherry, apricot and walnut, and cultivars of kiwi, blueberry, raspberry, fig, olive, hazelnut, pear and date palm and their commercialization is still growing. But the situation is different for conifers and broadleaved forest trees. Micropropagation of selected well growing and stress resistant phenotypes would speed up reforestation. It gives an opportunity to rapidly deploy selected varieties and to design diversity directly in the field. But In Europe there is no large, well-developed market for genetically improved forestry tree planting stock, especially when price becomes the major factor in deciding what type of planting stock to use.

Unlike tropical forestry, European forestry is generally a very conservative business, where profits seem to be limited because costs have to be borne for many years before a return on investment is achieved. An important problem is that the advantages of planting micropropagated elite material have not been fully and clearly demonstrated, proven and communicated to foresters and landowners. Further efforts are needed here. “Lack of interest in improved material” is a serious bottleneck for the in- creased use of micropropagated trees. (Lelu-Walter et al., 2013).

## 1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

In vitro culture of woody plants is leaving the academic laboratories and is now being developed in a range of commercial applications in horticulture and forestry that respond to the challenges of climate change and changing global food consumption patterns. It is therefore urgent that the research challenges, public acceptance, risk assessment and commercial application are confronted now in order to establish a well-informed scientific community, policy makers and marketplace. This proposal concerns the following questions/challenges, the answers to which will have a significant scientific, social and economic impact:

### How can we overcome recalcitrance in a lot of woody plants?

Our knowledge of plant development, genetics, physiology, biochemistry and molecular biology has expanded exponentially, often through work on mutants of Arabidopsis, and benefited from the breakthroughs in genomics and proteomics. It opened up many new avenues for the plant propagator to explore. However, Arabidopsis is not a tree, and so translation to woody plants just started. There is a need for outlining and sharing knowledge about the key regulatory genes and proteomics concerning in vitro shoot, root and somatic embryo induction, growth and development. New compounds, LEDs and imaging systems should be introduced to the toolbox of every academic and commercial laboratory.

### What are the best tools for diagnosis and sanitation?

Detecting and eliminating viruses and bacteria has enormous potential as a therapeutic tool. The field is currently at a tipping point and, with prices falling, it can easily be integrated into woody plant re- search and production,

### How can the production of elite clones be scaled up at an acceptable price

Since the 1980s, micropropagation of plants has grown into a multi-billion-dollar industry, mainly focused on ornamental plants, a market that now seems saturated. In recent years, however, the demand for superior planting material for fruit and nut orchards, commodity and biomass plantations and timber wood has steadily increased. Therefore, in Europe, companies and researchers in the field of micropropagation translate the new insights and technologies into practical applications. The dissemination of the results of this action is vital for efficient, reliable and competitive in vitro production of woody plants.

What are the real risks of this technology and how can the public be informed so that they appreciate and accept the applications ?

There is already a large public acceptance for the clonal production of fruit and ornamental woody species. Nevertheless, the situation is different for forest species. Lack of public acceptance of clonal forestry can become a very important issue if we do not communicate clearly about the risk to genetic variation and how we want to reduce it.

How can foresters and landowners be persuaded to invest in planting poly-clonal forests

Not only the sharing of scientific results and technical progress is important to make investment decisions in forestry, but the sharing of the results of long-term, multisite growth performance field trials is at least as important.

## 1.2. PROGRESS BEYOND THE STATE OF THE ART

### 1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

We will address the five challenges by integrating our different skills and know-how and by sharing the expertise, opinions and experiences of all stakeholders, from scientists to the general public and foresters. We believe the following innovations are very important to meet the challenges:

Recalcitrance

- New molecular insights regarding the initiation and development of shoot and root meristems and somatic embryos
- Recently gel-free separation and tag-free quantitation technology that will considerably enhanced insight in protein networks
- DNA methylation quantification with (i) the very promising sodium bisulfite conversion and sequencing, (ii) differential enzymatic cleavage of DNA, and (iii) affinity capture of methylated DNA
- A chemical toolbox recently expanded to include new in vitro chemicals: new classes of cytokinins, auxin-like compounds and signal amplifying drugs
- Phytochrome and cryptochrome manipulation by means of monochromatic light (blue, red and far-red LEDs)
- Advanced bioimaging technologies and imaging software (Artificial Intelligence)

Diagnosis, sanitation and conservation

- High-throughput new generation sequencing (HTS) has become affordable and is extremely powerful in combination with robust bioinformatics for diagnosing plant viruses, viroids and bacteria.
- Additional tools to remove microorganisms, such as thermotherapy, chemotherapy, electrotherapy and cryotherapy were developed in combination with tissue culture techniques but have yet to prove their validity.
- New encapsulation-, vitrification-, and droplet-based techniques for the long-term storage of mother plant stocks that were sanitized with great efforts, as well as ancient and endangered fruit germplasm

Scaling up and automation

- Methods are being developed to enable cryopreservation and subsequent thawing of embryonic tissue for an increasing number of tree species. They allow 'on demand production' when, for example, the results of field trials are finished.
- Various temporary immersion systems are industrially produced and compete for the interest of commercial enterprises
- Fully automatic micropropagation systems are currently for sale for ornamental plants. They combine mini-robots, laser cutting, machine learning and artificial intelligence. It is obvious that these robotized systems will also be usable for woody crops.

Technological risk assessment and public acceptance

- Vulgarization of the technology of micropropagation and somatic embryogenesis
- translating the results of long-term, multisite growth performance field trials
- modern communication tools (appealing website, facebook, twitter, youtube, blogs and pod-casts)

Commercialization

- Creating a platform to share valuable results of long-term, multisite growth performance field trials with the forester community
- Networking events with elevator pitches by Ph.D. students to convince venture capitalists and business angels to invest in cost effective and innovative business models for in vitro production
- Introducing the concept of crowdfunding projects by scientists and foresters for polyclonal forestry, nut plantations, cloning special historical and cultural heritage individual trees for the public, etc.

## 1.2.2. OBJECTIVES

We propose this Action to collect, share and disseminate the available information and experience in all aspects of the micropropagation of woody plants. We want to move to the next level of this technology in order to address the challenges 'Recalcitrance', 'Diagnosis and sanitation' and 'Scaling up and automation'. Concerning challenges 'Technological risk and public acceptance' and 'Commercialization' we want to summarize and communicate the relevant field trial data and experience so that legitimate steps can be taken to minimize risks and to convince all stakeholders of the economic benefits of planting cloned elite trees and shrubs. In this way, in-vitro technology can achieve its full eco nomic potential for supporting European forestry and horticulture. In summary, our main objectives and results of this Action:

### 1.2.2.1 Research Coordination Objectives

A. Improving the comprehension of the already defined challenges and the possible strategies to address them

B. Comparison and performance assessment of emerging technology and tools

C. Encouraging open access publication of joined research results

D. Vulgarization of breakthroughs and their impact, tailored to stakeholders including the general public and input on future market application for investors

E. Informing and raising awareness of the possible risks of the technology

#### 1.2.2.2 Capacity-building Objectives

F. Bridging in vitro technology with other disciplines to achieve breakthroughs that require an inter-disciplinary approach

G. Promoting knowledge exchange and the development of joint projects and doctoral research themes around new or emerging research areas

H. Building a stakeholder platform for exchanging information and stimulating interaction

I. Stimulating investments in cost effective and innovative business models for in vitro production of woody plants.

# 2. NETWORKING EXCELLENCE

## 2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

### 2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

In the last 20 years, there have been three relevant COST Actions dedicated to Plant Tissue Culture, but they were never specifically dedicated to woody plants:

- 822 - Development of integrated systems for large scale propagation of elite plants using in vitro techniques (23/5/1995-23/5/1999)
- 843 – ‘Quality Enhancement of Plant Production through Tissue Culture’ (7/12/00-7/12/04)
- 871 – ‘Cryoplanet, Cryopreservation of crop species in Europe’ (12/12/06-11/12/10)

As the research community of researchers is both limited and fragmented, a major objective of this COST Action is therefore to strengthen collaboration in the field of biotechnology applied to woody plants at the European level. This will be achieved by sharing research tools (e.g. embryogenic cell lines, in vitro model plants, compounds, innovative micropropagation technologies) and know-how (experimental protocols, procedures and practical experience) to tackle common challenges. This platform will integrate in vitro protocols, diagnostic needs (identification of virus, viroids, phytoplasma and bacteria) and production needs (automatisation, temporary immersion bioreactors) by integrating the skills of the different groups.

This is not the first networking initiative at international level since a large number of researchers in this field are already federated within the IUFRO 2.09.02 Unit entitled 'Somatic embryogenesis and other vegetative propagation technologies' (686 researchers and stakeholders from 67 countries). Every two years an international conference is organized and dissemination of results is done by its proceedings/books. We will try to combine the activities of IUFRO and COST to create a synergy for the European in vitro community of woody plants. This will boost the dissemination of results, notably through the temporal coordination of meetings or the organization of side events whenever possible.

## 2.2. ADDED VALUE OF NETWORKING IN IMPACT

### 2.2.1. SECURING THE CRITICAL MASS AND EXPERTISE

The challenges in woody plant cloning in terms of techniques and know-how can only be tackled on a European scale, as it is impossible to find all expertise in one country. Although the European re- search in this area can be considered being of high level, it nevertheless suffers from fragmentation in small research groups and a lack of coordination. This implies a poor integration of interdisciplinary research and spreading of results towards forestry and horticulture. This project is designed to over- come the shattering of the European research field, stimulate mobility, and improve links with less re- search-intensive countries across Europe. Thus, it will be possible to maintain its level on a world-leading stage.

All participants have extensive experience in the in vitro culture of woody species, but while some of the members lead projects related to the fundamental questions of plant morphogenesis, others focus mainly on more applied aspects, such as the sanitization of the starting material, cryopreservation and the use of bioreactors for large-scale propagation. The interaction and feedback between fundamental and applied strategies will ultimately allow cutting-edge research to reach companies and society as a whole. The complementation based on expertise extends to the type of species with which each group works and their main uses (hardwoods, conifers, currently productive species, endangered germplasm...), as well as the type of challenges their native forests and plantations suffer in the current climatic change scenario. The creation of a diverse and interactive group that detects the problems that are currently affecting forests and plantations at the local level will allow scientific solutions to be sought before they become global.

### 2.2.2. INVOLVEMENT OF STAKEHOLDERS

Following stakeholders should be made aware of the progress of this technology and the new opportunities it offers to contribute to the bioeconomy:

- Scientific community
- Commercial laboratories: In Europe, many large commercial laboratories are producing high-quality woody plants
- Centers for woody plant germplasm conservation, elimination of pathogens, and genetic engineering
- Nurseries and producers of fruit, ornamental and forest tree planting material
- Suppliers of chemicals and equipment for In vitro laboratories
- Educational institutions and training in the sector of in vitro technologies
- Land owners and associated foresters,
- Policymakers and risk assessors (EU or national, regional institutions and government authorities)
- NGO's: IUCN
- Organisations for biodiversity: BIOVERSITY INTERNATIONAL, CGIAR, FORESTS FOR FUTURE, EVOLTREE
- Media: journalists

These main stakeholders will benefit greatly from the innovations produced by this COST Action. Their involvement will take place through active participation in meeting initiatives organized within the Action (workshops, one-day meetings on specific topics, "traditional" seminars and webinars), as well as a specific website supported platform of the Action, through which operators will be able to interact with the participants in the COST Action, propose their problems, get in touch with operators from other Countries and be informed about the potential benefits and risks so as to enable multi-perspective discussions on the sociological and ethical aspects of the technology.

### 2.2.3. MUTUAL BENEFITS OF THE INVOLVEMENT OF SECONDARY PROPOSERS FROM NEAR NEIGHBOUR OR INTERNATIONAL PARTNER COUNTRIES OR INTERNATIONAL ORGANISATIONS

There is a tendency for research groups to work in networks dedicated to temperate or tropical fruit rootstock, berry shrubs, ornamental tree, conifers or forest tree rather than tool or process specific networks. As such, isolated networks have to "reinvent the wheel" and cannot benefit from the learning of others working on other plants. Given the size of the problems, and the current dynamics of the field, a concerted and aligned effort is required, also involving research from tropical or arid regions groups, to learn from each other's new fundamental insights and experiences with new technical tools.

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# 3. IMPACT

## 3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAKTHROUGHS

### 3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

By joining complementary forces, COPYTREE will serve as a network of excellence in innovation based woody plant production for food, timber, feed, fuel, fiber, ornamental and medicinal use and thus provides a long term and sustainable service to society. The impact of this network on the academic quality of the participants will be highly positive. During the annual meetings, not only the COST Action members, but also external specialists in other scientific fields will be invited to talk about their expertise which will stimulate new connections, creativity and inspire innovation. Indeed, meetings, staff exchange, shared PhD supervision and training programs will allow cross fertilization of ideas and methods and building a critical mass. Improved access to each other's state-of-the-art facilities and technologies and multidisciplinary expertise will further stimulate the academic quality and career of all involved participants.

The immediate results of this action will be scientific and technological advances, including a better understanding of the initiation and development of shoots, roots and somatic embryos, as well as the genes, proteins and signalling pathways involved. In the short to medium term, it is expected that the European plant production industries will rapidly absorb and benefit from the practical results of the action, given the potential economic advantage over traditional plant production methods.

## 3.2. MEASURES TO MAXIMISE IMPACT

### 3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

COPYTREE intends to become a platform that centralizes the skills and resources of researchers and companies to tackle the challenges in mass in vitro cloning of woody plants by knowledge transfer. Through concerted pursuit for extra funding, the network will further improve its impact. The members of the network will make use of each other's expertise during conferences, workshops, summer schools and Short-Term Scientific Missions (STSM). They will be preferred partners in research proposals, PhD and master thesis student exchange and assessment and jointly work on publication. This will help in scientific and educational career development of the participants. The frequent meetings and communications will further shape a true sense of community that will survive the COST Action for a long time ahead.

### 3.2.2. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

We foresee a specific, focused, and structured plan for dissemination, based on clever online strategies, life presence at stakeholders' events and complementary activities. Furthermore, we are interested in education of the broad public on innovative plant biotechnologies. In order to carry out this task, the following activities will be coordinated by a dedicated workgroup: WG5.

Online strategies

**Website**: Conceived as a key element for the communication strategy, a dedicated and independent project website with a distinctive URL will be developed, published, updated, and maintained for the duration of the Action and running even after its end. The website will be organized in the following sections: General description of the action, consortium composition, progress update, news and events, documents, news, links and contact. It will be comprehensive and attractive, enhanced with photography, video, graphs and audio presentations. The Action website will serve throughout the duration of the project as a constant point of reference for the partners as well as for current and potential stakeholders. The homepage will be clearly and visibly marked with the COST and COPYTREE's logo, together with all the partner´s logos and will acknowledge the European Community contribution. The web site will also host links or sub-pages where databases, datasets, e-manuals. Link with others COST Actions and European Projects with similar aims will be provided in the Action web page to mutually increase the audience of interested parties. Scientists involved in the project will continue to peri- odically update the site and respond to the questions arising. The visitors of the website will have the possibility to submit questions and clarifications directly to the scientists/technicians who have been involved in the COST Action.

**Facebook, Twitter, YouTube, blogs and podcasts:** In order to improve the dissemination to a wide range of stakeholders and eventually, to maintain a two-way and interactive communication, the project will offer linking widgets to social media tools such as Facebook page and Twitter account. In particular, an Action´s Facebook profile will be created to target the most dynamic audience and facilitate the rapid information diffusion and to stimulate discussions. A YouTube channel will be dedicated to video related to the COPYTREE development. YouTube movies will be recorded at the laboratories with interviews and attractive images of in vitro culture practices and cloned trees. This will be placed on the website, available for online visitors for many years. Twitter will also be effectively used to create a hashtag for the Action. Each article posted on the website will have a share button below to promote a regular sharing and promotion of the news. The URL will also be promoted in other relevant forestry stakeholders' webs (forestry research institutions, Universities, nurseries, wood processing industry, forestry associations, etc.). Podcasts will be recorded in interview format. A blog about the conference will appear on the website.

**TV:** Actions will be taken to allow scientists/technicians, participating in the COST Actions, to be hosted by local/national radio/tv channels to give information, details and, essentially, to raise the awareness of the public on the Action topics.

**Video:** A video/documentary will be produced in English, explaining the most relevant characteristics of the COPYTREE Action in a simple and direct language. The video length won´t exceed the 15 minute duration and will also be uploaded on the Action´s website and on YouTube. It will be broadcasted on screens during events (Conferences, Congresses, Symposia) related to plant propagation and in vitro culture that will accept to offer a space to the COST Action.

**Scientific E-community:** To maintain informed the scientific community, the scientists involved in the COST action will provide information about the project also in their Researchgate profiles.

### Information stands

A demonstration and information stand will be installed at selected events where stakeholders are present, such as important professional forestry and horticultural trade expositions in Europe. Panels and posters will present the Action, in vitro material will trigger the passing visitors to start an informative talk. This will also contribute to advertise and promote the website and social media activities.

### Annual reports

A scientific report will be made before the annual meeting to explain all the activities carried out.

### Promotional materials

Production and distribution of digital brochures in pdf format will be made.

### Scientific dissemination of the project

- Specialized papers in peer-reviewed open access journals.
- Presentation of the Action by means of talks and a stand at International Conferences/Symposia such us IUFRO Unit 2.09.02 (biotechnology section of the International Forestry Research Organization), IAPB (International Association for Plant Biotechnology) meeting, ISHS Symposia on plant biotechnology and tissue culture

(International Symposium on Production and Establishment of Micropropagated Plants, IS on In Vitro Culture and Horticultural Breeding, IS on Micropropagation and In Vitro techniques) SLTB (Society for Low Temperature Biology) Annual Conferences.

- Furthermore, participants will inform about initiatives and outcomes resulting from the COPYTREE Action also in National events (Conferences, Congresses).

### Complementary actions

- Exchange of technology between participants: all questions regarding IPR, material exchanges, responsibilities and further scientific disseminations shall be regulated in a detailed specific agreement document according to COST policies.
- Establishing links with International Associations (IUFRO, EVOLTREE, EFI, SLTB, etc…).
- Invitation of different stakeholders, especially of the host country of the annual conference, to participate in the event.

# 4. IMPLEMENTATION

## 4.1. COHERENCE AND EFFECTIVENESS OF THE WORKPLAN

### 4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

Taking into account the aforementioned challenges, we are planning four working groups (WG).

#### WG1: Recalcitrance

Tasks: Outlining knowledge, sharing and communicating insights in genetics, epigenetics and protein networks. Re- viewing new chemical and physical tools that might break recalcitrance. Demonstrating the power of bio-imaging systems.

#### WG2: Diagnosis, sanitation and conservation

Tasks: Updating, sharing and communicating the latest technology for the diagnosis of viruses, viroids and bacteria, sanitation protocols for clean stock production and identifying new breakthroughs in mid- and long-term storage of clean stocks and germplasm.

#### WG3: Scaling up and automation

Tasks: Sharing and communicating breakthroughs in the initiation, multiplication, preservation, germination and conversion of somatic embryos of new species. Critical comparison of the results of micropropagation in different models of temporary immersion systems. Performance assessment of advanced fully automatic micro- propagation systems combining robotics, laser cutting, machine learning and artificial intelligence. To provide a forum for industrial suppliers of this technology.

#### WG4: Technological risk assessment, public acceptance, legislation and commercialization ( transversal working group)

Tasks: Fostering knowledge ex- change dealing with risk assessment, developing a strategy to stimulating technology awareness and acceptance of stakeholders, stimulating investment and commercialization.

### WG5: Communication, dissemination and technology transfer (transversal working group)

Tasks: Coordination of the reports, organizing a stakeholder platform for communication and feedback, dissemination and technology transfer by website, social media, information stands, promotional materials.

### Working groups interact at the yearly conferences

The yearly conferences represent the heydays of the Action. The four working groups will have strong ties and interactions that will bring mutual benefits. To promote new ideas and exchange of information among different WGs, ensure the specific needs of each WG and to guarantee dissemination towards the stakeholders, the yearly conference will be organized as follows. During Plenary sessions, each WG provides a key-note speaker who gives an overview of the state of the art and the challenge. However, there will also be a forum for external scientists in new evolutionary domains such HTS for virus and bacterial load detection, large throughput robotic screening of chemicals, big data algorithms, artificial intelligence, and image analysis. During parallel sessions, the working groups organize specific lectures, including an in-house poster session. The working groups come together to set up joint experiments in which each lab examines the same tool (connection, technique, apparatus, service) with specific model plants (ring test). There will also be an opportunity to visit stakeholders in the vicinity of the conference: woody plant propagators, commercial laboratories, etc.

## 4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

| ID | Deliverable | Output | Objective | WG | Timing |
|---|---|---|---|---|---|
| A1 | current state of the art | White paper | Better comprehension of the challenges | WG1-4 | Y1, Q3 |
| B1 | 'Ring test' with new hormonal compounds | Report | assessment of emerging technology | WG1 | Y2, Q2 |
| C1 | Open Acces Publications | 10 papers per year | Encouraging open access publication | WG1-4 | Y1-Y2-Y3-Y4 |
| D1 | Contributions in forestry and horticulture journals | 4 papers | Dissemination for foresters | WG1-4 | Y1-Y2-Y3-Y4 |
| D2 | articles in investor magazines | 4 articles | Dissemination for investors | WG1-4 | Y1-Y2-Y3-Y4 |
| D3 | Website including video's | Website, 4 videos | Dissemination for general public | WG5 | Y1-Y2-Y3-Y4 |
| D4 | Social media | Blog, twitter account, facebook group | Dissemination for general public | WG5 | |
| D4, E1 | Book chapters | 4 book chapters | Dissemination, raising awareness of the possible risks | WG1-4 | Y1-Y2-Y3-Y4 |
| H1 | Website | Website | stakeholder platform | WG5 | Y1-Y2-Y3-Y4 |
| I1 | articles on investor forums. | 4 articles | Stimulating investments | WG4 | Y1-Y2-Y3-Y4 |

**Milestones**

- M1: Website is running
- M2: each WG publishes a white paper describing the current state of the art, bottlenecks and gaps
- M3: First Ring tests started
- M4: First conference
- M5: 50% of the STSM is accomplished

**TIMEFRAME (see also GANTT diagram)**

- The Action would last **4 years**.
- A **first general meeting** in which the WG leaders will be selected will **Kick-off** the action. The members will present themselves and choose one or more working groups.
- A **homepage** will be created shortly after the Kick-off and will be regularly updated
- Each WG will gather at the yearly conference and will edit a **report**, coordinated by WG4 .
- **STSMs** can be requested after the first WG meeting has taken place, in consultation with the
- Management committee
- **Workshops** will be organized every year, in between the yearly conferences
- **Plenary Management Committee meetings** will coincide with the yearly conference
- **Restricted MC meetings** (Chair and Vice-chair(s) + WG coordinators) will take place be- tween the yearly conferences
- The action will be closed with a **Final Conference** at which the outcomes will be presented by each WG for all Action members and stakeholders.

## 4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

Following potential risks could be identified, and contingency plans to address those risks are anticipated.

**IP issues** - The members may be hesitating to share sensitive pre-published or pre-patented data. This will be addressed by:

- using codes for chemical compounds with special effects. When somebody want to apply them, bilateral agreements have to be signed
- signing of a non-disclosure agreement before the annual meetings. This will allow the attendees to also share their newest results to the benefit of all attendees. Best practices as de- fined in 'Guidelines for the communication, dissemination and exploitation of COST Action results and outcomes' will be followed.

**Illness, retirement or death -** All members in the management structures must identify a deputy who can take over their role in the project.

**Financial losses -**The coordinator/chairman will establish strict financial rules and provide a reserve for possible losses.

**Management -** Due to the high number of participants likely to be involved, the management of the Action may be difficult. This will partly be addressed through part-time employment of an experienced secretary to manage the administration of the Action using 15% of the funds.

# 4.1.4. GANTT DIAGRAM