European Cooperation in the field of Scientific and Technical Research
- COST -
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Secretariat

Brussels, 11 July 2006

COST 264/06

MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding (MoU) for the implementation of a European Concerted Research Action designated as COST Action 871 'Cryopreservation of crop species in Europe'

Delegations will find attached the Memorandum of Understanding for COST Action 871as approved by the COST Committee of Senior Officials (CSO) at its 165th meeting on 27/28 June 2006.

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MEMORANDUM OF UNDERSTANDING
FOR THE IMPLEMENTATION OF A EUROPEAN CONCERTED RESEARCH ACTION
DESIGNATED AS

COST ACTION 871

'Cryopreservation of crop species in Europe'

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 400/01 'Rules and Procedures for Implementing COST Actions', or in any new document amending or replacing it, the contents of which the Signatories are fully aware of.

2. The main objective of the Action is to improve and apply technologically advanced techniques for plant genetic resources conservation of crops that are grown and/or conserved in Europe with the main emphasis on long-term conservation through cryopreservation.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at approximately EUR 38 million in 2005 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 four years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter 6 of the document referred to in Point 1 above.

COST ACTION 871

Cryopreservation of crop species in Europe

A. ABSTRACT

Plant germplasm stored in liquid nitrogen (-196°C) does not undergo cellular divisions. In addition, metabolic and most physical processes are stopped at this temperature. Therefore, plant germplasm preserved under cryogenic storage can be maintained for very long periods and problems that are typical for storage in the active growth state, such as genetic instability and the loss of accessions due to contamination, loss of vigour and totipotency and human error during continual subculturing are overcome. So far, cryopreservation procedures have been developed for the in vitro tissues and non-orthodox seeds of about 200 plant species. However, there are still a very limited number of examples in Europe where cryopreservation is used routinely for plant germplasm conservation. This is mainly because of: (1) the unavailability of efficient and robust cryopreservation protocols applicable to many plant species and diverse germplasm types, (2) limited awareness of plant researchers unacquainted with recent developments in cryogenic storage methods, and (3) lack of coordinated research on plant cryopreservation.

To address these shortfalls, the goal of this COST Action is to create a network that brings together European scientists with an expertise and/or interest in plant cryopreservation with the main aim of developing efficient cryopreservation procedures. Emphasis will be placed on using this approach as a complementary technique for the preservation of crops that are vegetatively propagated and/or produce non-orthodox seeds with a focus on under-utilised crop species grown and/or conserved in Europe, and their wild relatives. The network, once formed, will also alert and inform stakeholders in plant breeding and conservation practitioners who require cryopreservation to implement and underpin sustainable crop plant breeding programmes.

Keywords: plant biodiversity, germplasm conservation, under-utilised crop species, plant physiology, food security.

B. BACKGROUND

It is estimated that up to 100 000 plants, representing more than one third of all the world's plant species, are currently threatened or face extinction in the wild. In Europe, particularly, biodiversity is seriously threatened as indicated by the following figures:

- 64 endemic plants of Europe have become extinct in recent decades
- 24% of the species/subspecies of certain groups of European plants are in danger of being lost

- agricultural intensification has reduced the area under wetlands in Europe by some 60% in the last decades with consequent threats to biodiversity.

In the past, most plant species and varieties were grown and improved because of their high adaptability to local microclimates and environments. Breeding projects developed during the 20th century were mainly aimed at increasing production capacities, and often other intrinsic qualities (e.g. nutrients, healthy molecules and secondary metabolites, social and environmental enrichment traits) were secondary to this objective. Focusing breeding programmes across a narrow genetic base has consequentially relegated many potentially valuable plants to a marginal position with respect to the conservation of their genetic resources. So much so that many under-utilised crops, wild relatives and ancient and archived cultivars are now at high risk of genetic erosion. Recently, interest has been manifest in prospecting for new and rediscovering older utility crop plant species, many of which have important potential applications in human and animal health and well-being and environmental stability. The importance of conserving a wide crop genetic base has been realised by many European Governments which have taken initiatives to promote the establishment of plant germplasm collections. As a consequence since the 1970s, large numbers of landraces and wild relatives of cultivated crops have been sampled and stored in ex situ gene banks. It is estimated that 6 million samples of plant genetic resources are now held in national, regional, international and private gene bank collections around the world (IPGRI (2004) In: http://www.ipgri.cgiar.org/themes/human/economics.htm). While significant advances in ex situ crop conservation have been made, the process is still limited by the special needs of difficult-to-preserve germplasm types.

Storage of desiccated seeds at low temperature, the most convenient method of preserving orthodox plant germplasm, is not applicable to crops that do not produce seed (i.e. vegetatively propagated) or produce seed that is either non-orthodox or intermediate in storage behaviour. Non-orthodox seed cannot be dried to moisture contents low enough to permit low temperature storage; typically they are produced by tropical species. Moreover, in the case of plant breeding programmes, and especially those implementing biotechnology, plants are vegetatively propagated in order to preserve their unique genomic constitution. Examples include cultivars of soft and top fruits, timber and ornamental trees. Preservation of field collections is the present and often only option for many categories of crop plants, but this is a high risk strategy, especially if it is not underpinned by more secure conservation measures. Field genebanks holding rare and valuable actively growing plant germplasm can be lost (genetic erosion) as a result of pests, diseases and adverse weather conditions and human-made impacts (conflict and changes in land use). Moreover, maintenance of clonal orchards is labour-intensive and expensive. On balance, while maintenance of in vitro collections is labour-intensive, it provides a more secure contingency option to field genebanking. But there are also risks of accession loss through contamination, facilities failure, human error and somaclonal variation. The latter arises from mutations that occur spontaneously in tissue culture, with a frequency that increases with repeated subculturing and as it is heritable in regenerated plants it has important implications for breeding programmes.

In the context of the present options available for germplasm conservation, cryopreservation is the method of choice for the long-term ex situ conservation of plant genetic resources and it provides a complementary approach and additional security for germplasm already held in the ‘active growth state’. The longevity of cryopreserved germplasm is well proven by other

sectors. Bull sperm can be successfully stored in liquid nitrogen for more than 50 years without impairing its viability. The advantage of cryopreservation is that the material can be stored: (1) in a stable way, (2) for the long term at relatively low cost, and (3) in disease-free conditions. In addition, cryopreservation has important applications for plant germplasm used in the biotechnology and health care industries. It is extremely useful for the safe, long-term storage of plant cells and tissues that have specific metabolic characteristics; for example, phyto-medicinals, alkaloid-producing cell lines, hairy root cultures, genetically transformed and transformation-competent culture lines. Recently, it has been proved that cryotherapy can be successfully applied to eradicate viruses from, for example, plum, banana and grape.

While plant cryopreservation offers considerable advantages as compared with the cryogenic storage and banking of microbial, mammalian and medical cells, plant cryopreserved genebanks are still in their infancy. This is largely because plant conservationists are required to cryopreserve a very broad range of genetic diversity which behaves differentially under cryopreservation. Even within one species, different varieties and tissue types behave heterogeneously under cryopreservation. In addition, European research in cryopreservation of plant germplasm is lagging behind that of other regions. This is because it is limited, dispersed and lacks the focus of the more integrated strategies of other countries. For example, the National Seed Storage Laboratory (NSSL) of Fort Collins, USA; the National Clonal Germplasm Repository (NCGR) of Corvallis, USA; the National Institute of Agrobiological Resources (NIAR) of Japan and the National Bureau of Plant Genetic Resources (NBPGR), India apply cryopreservation on a larger and concerted scale for respective plant species and crop plant groups. Initiatives in Europe are currently limited, fragmented and uncoordinated. Therefore a collaborative research infrastructure designed to assist plant cryopreservation through networking agendas is urgently required in Europe. This is essential in order to ensure that the plant cryopreservation sector plays 'catch up' with (1) other European areas in the animal and medical sciences which use cryopreservation on a routine basis, and (2) other countries and economic regions (USA, India, SE Asia, Japan) that already have large-scale, nationally and regionally coordinated cryogenic genebanking facilities that support their crop plant and phyto-products industries.

To address these needs and shortfalls a first initiative was taken within the EU Fifth Framework Programme project CRYMCEPT: Establishing Cryopreservation Methods For Conserving European Plant Germplasm Collections that ended October 2005 (see also http://www.agr.kuleuven.ac.be/dtp/tro/home.htm). This project, coordinated by K.U.Leuven (Belgium) was carried out in collaboration with the University of Abertay, Dundee (UK), University of Derby (UK), IRD (Institut de Recherche pour le Développement, France), ISF (Fruit Tree Research Institute, Italy), DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen (Germany) and IPGRI (International Plant Genetic Resources Institute, Italy). The aim was to develop more efficient and generally applicable plant cryopreservation protocols based on fundamental research. In this project, biochemical, biophysical and physiological aspects of cryopreservation/cryoprotection in a wide variety of plant species was studied in order to unravel the processes of cryoprotection. Moreover, at the end of this project two workshops were organised allowing trainees of 20 different nationalities to be trained and policy-maker participants informed about prospects/applications of plant cryopreservation.

The European Community Biodiversity Strategy (1998) aims to anticipate, prevent and counter the causes of significant reduction or loss of biodiversity at source (http://europa.eu.int/comm/environment/docum/9842sm.htm). This strategy will help to reverse present trends in biodiversity reduction or losses and to place species and ecosystems, including agro-ecosystems, at a satisfactory conservation status, both within and beyond the territory of the European Union. This is the EU's response to the Convention on Biological Diversity (http://www.biodiv.org/default.shtml). The strategy is supported by the European Community Clearing-House Mechanism (EC CHM) (http://biodiversity-chm.eea.eu.int/) and has been activated through four Biodiversity Action Plans (2001). They are fundamental for the sixth Environmental Action Plan, which sets as its target: no further loss of biodiversity by 2010. It is clear that these actions will cause an increase of tasks and put increased pressure on existing facilities and organisations for the preservation of biodiversity. Therefore, there is a need to develop user-friendly and safe plant preservation protocols applicable to European plant collections. These protocols will facilitate the implementation of plant conservation efforts at the European level. However, it will also have major ramifications at a global level and in doing so will support the wider international obligations of the EU and its member countries in obviating the loss of the Earth's biological resources.

In the Stakeholder Proposal for a Strategic Research Agenda 2025 (Including Draft Action Plan 2010) of the European Technology Platform: Plants for the Future (http://www.epsoweb.org/catalog/TP/docs/SRA-I.PDF), Challenge Two is defined as 'Sustainable agriculture, forestry and landscape'. Within this challenge, *Goal number three* is the pertinent challenge 'Enhance biodiversity' and the third sustainability priority is to enhance and utilise plant biodiversity. First, we need to characterise and maintain the biodiversity that exists in the field. Also *Goal number four* within Challenge Two, 'Enhance the aesthetical value and sustainability of the landscape', deals with the conservation of plant genetic resources: "Ornamental and indigenous plants are an important component of our everyday landscape. Their tremendous diversity needs to be preserved." From this we can conclude that conservation of plant genetic resources will remain a priority in future European research actions.

The Projects database from CORDIS was searched for all documents containing the word 'cryopreservation'. As such, 32 documents were found. The large majority is related to cryopreservation of animal and human tissues and fungal strains. Only three are dedicated to the conservation of plant tissue through cryopreservation. Two of them pertain to the collection and development of conservation methods (using a trial and error approach) of a limited species base (hardwood and kiwi). The third one is CRYMCEPT (see above). This CORDIS Projects database was also searched for 'conservation, biodiversity, plants' and resulted in 34 documents. These deal with molecular screening techniques and other tools to assess biodiversity, calculation of extinction risks, protection of forests and taxonomy. Projects focusing on the development of methods for plant germplasm conservation are thus scarce.

The flexibility of the COST Actions allows the coordination of nationally funded research on a European level. COST is therefore the instrument of choice to bring together European plant cryopreservation specialists. Moreover, the number of researchers on plant cryopreservation is too limited to effectively perform this research at a national level. Only through a consortium of different institutes situated in different European countries bringing together their

collective experience on: (1) cryobiology and cryopreservation techniques, (2) cryoconservation of different plant species, (3) experience on plant physiology, and (4) experience of plant culture collection management, can a critical mass be obtained.

C. OBJECTIVES AND BENEFITS

C.1. Objectives

The **main objective** of this Action is to improve and apply technologically advanced techniques for plant genetic resources conservation of crops that are grown and/or conserved in Europe with the main emphasis on long-term conservation through cryopreservation.

The Action also aims to deepen cooperation in Europe by regular meetings, scientific contributions to international conferences and collaboration with industries, in order to comply with the **secondary objectives as follows.**

***Objective 1*: To screen in detail the current utilisation of plant cryopreservation in Europe**. Current research on plant cryopreservation in Europe is limited, fragmented and not coordinated. Therefore a listing of all initiatives that are currently ongoing is essential.

***Objective 2*: To screen and compare the efficiency of existing plant cryopreservation protocols**. Many cryopreservation protocols are used today. The most commonly applied ones are the vitrification, encapsulation-dehydration and classical slow cooling.

***Objective 3*: To improve fundamental knowledge about cryoprotection through the determination of physico-biochemical changes associated with tolerance towards cryopreservation.** A multidisciplinary approach evaluating simultaneously multiple variables and including orthodox as well as recalcitrant plant species will lead to an understanding of the processes of cryoprotection.

***Objective 4*: To develop new plant cryopreservation protocols**. These will be based on existing protocols (Objectives 1 and 2) and findings generated in Objective 3. The resulting cryopreservation protocols will be:

- efficient (at least 50% post-thaw recovery)
- user-friendly (technically not too demanding and with a limited number of steps)
- fast (and thus applicable to large amounts of germplasm)
- broad (applicable to a wide variety of plant species and tissues).

***Objective 5*: To assure the genetic stability and 'true to typeness' of plants after cryopreservation.** It is desirable to assess the genetic integrity of plants surviving cryogenic storage to determine if they are 'true to type' after cryopreservation. This can be done at the morphological, histological, cytological, biochemical and molecular levels.

***Objective 6*: To apply cryopreservation to European plant germplasm collections.** This will be promoted through workshops and technical guidelines.

***Objective 7*: To prove the environmental, social and economic impact of plant cryopreservation.**

The main **outcomes** of this COST Action will thus be:

- The establishment of a network of European plant cryopreservation specialists
- Proceedings of technical recommendations for cryopreservation
- Proceedings of proof of conformity after cryopreservation
- The establishment of a tentative virtual lab
- Long-term plant germplasm collections

C.2. Benefits

It is virtually impossible to assign monetary values to the benefits generated by crop genetic resources conserved in ex situ collections. It is simply the basis for agriculture. Private- and public-funded breeding programmes need new germplasm to improve current cultivated crops which are demanded by the industry and the consumers. The value of crop genetic resources conserved in ex situ collections includes not only current use value and expected future use value but also option value, associated with the flexibility to respond to some unknown, future events (http://www.ipgri.cgiar.org/system/page.asp?theme=1)

Benefits to the scientific community

A better understanding of the mechanisms involved in tolerance to cryopreservation can lead to the detection of genes/proteins involved in resistance to freezing, cold and drought stress, which are essential components for developing future breeding strategies for marginal areas and their crops. Especially understanding the mechanisms that induce tolerance to drought conditions is of the utmost importance in view of the ongoing climate change/global warming.

Benefits to society

The Action will provide alternative and efficient methods for preserving plant genetic resource for the long term. This safe, long-term conservation of European plant collections is the assurance that European industries, farmers and consumers will have continuous and ready access to new plant germplasm. Because of the development of better plant biodiversity conservation systems, farmers will have ready and swift access to more varieties which are pest/pathogen free and which can have unique features, such as flower colour, disease resistance, cold tolerance. As such they can respond rapidly to disease outbreaks as well as to specific market needs.

Benefits to consumers and the environment

Currently, agriculture in Europe relies on the continuous production of pest/disease-resistant plants produced by private- and public-funded breeding programmes. These breeding

programmes therefore need to rely on a collection of interesting source material which provides the genes for resistance. In consequence, the provision of resistant breeding lines guarantees that crops need less pesticide (environmentally safer and more sustainable) and result in fewer residues in the food chain. It is expected that these processes of introgression will move to processes of incorporation; i.e. the cultivation of species that are currently out of fashion or whose potential is not properly discovered.

Benefits for European agriculture

The incorporation of more germplasm of under-utilised species will further increase biodiversity on farms, and variability of products for consumers. The trend towards organic farming in Europe can be supported more if biodiversity becomes available from safe collections.

Benefits for employment

Continuing the development of better plant preservation tools, and provided the Biodiversity Action Plans are followed, existing facilities and organisations for the preservation of biodiversity will soon have an increased workload. In addition, straightforward safe-storage methods will open prospects for companies to provide storage facilities/services. A company in Canada (Forest Biotechnology Center at BC Research Center) serves as an example. Access to large collections where plant material is safely stored will open prospects to companies for a regular and quick launch of new material. This will also generate job opportunities.

Benefits for reaching the Millennium Developmental Goals

The challenge of the Millennium Development Goals is not only to address the problem of hunger with respect to food availability but also to ameliorate the incipient issues caused by unbalanced diets. This is due, in part, to a lack of a nutritional diversity, which is an indirect and often overlooked consequence of plant diversity erosion. The traditional crops, hitherto neglected by research assigned to crops of the industrialised nations, are often more nutritious than exotic imported species. Additionally and most importantly, traditional, indigenous crops are better able to grow in marginal areas, for which there is less need for irrigation, pesticides and fertilisers. An important target for European conservationists is to assist rural third world farmers, who are usually women, as they can boost local income generation at the basic level and hence fight poverty. Cryopreservation will thus become an increasingly important tool to preserve global agricultural biodiversity and will be an important tool in meeting millennium development goals.

D. SCIENTIFIC PROGRAMME

This COST Action will focus on the scientific understanding of the effects of cryoprotection, the development of efficient cryopreservation protocols and the dissemination of the findings for application in plant germplasm collections. The scientific programme has been developed with the input of researchers from 17 different COST countries. Taking into account their research priorities, two main Working Groups (WGs) have been identified: (1) a Working

Group on fundamental aspects of cryopreservation/cryoprotection and genetic stability, and (2) a working group on technology implementation, transfer, application and validation in plant genebanks, culture collections and research groups.

These two Working Groups will have strong links and interactions between them (Figure 1). The development and applications of new, efficient cryopreservation technologies will benefit from the elucidation of the physico-biochemical background of cryoprotection and cryopreservation. Research on the genetic stability aspects of plant tissues and regenerated plants as a consequence of the cryopreservation protocol and the storage in liquid nitrogen itself will have a major impact on technology that will also be developed (Arrow down). On the other hand, germplasm curators (that will be mainly involved in WG2) will define the requirements of the new cryopreservation protocols; they will need to be efficient, user-friendly, fast and broadly applicable. Moreover, it is essential that a feedback to WG1 is given with respect to the field performance of plants regenerated after cryopreservation (Arrow up).

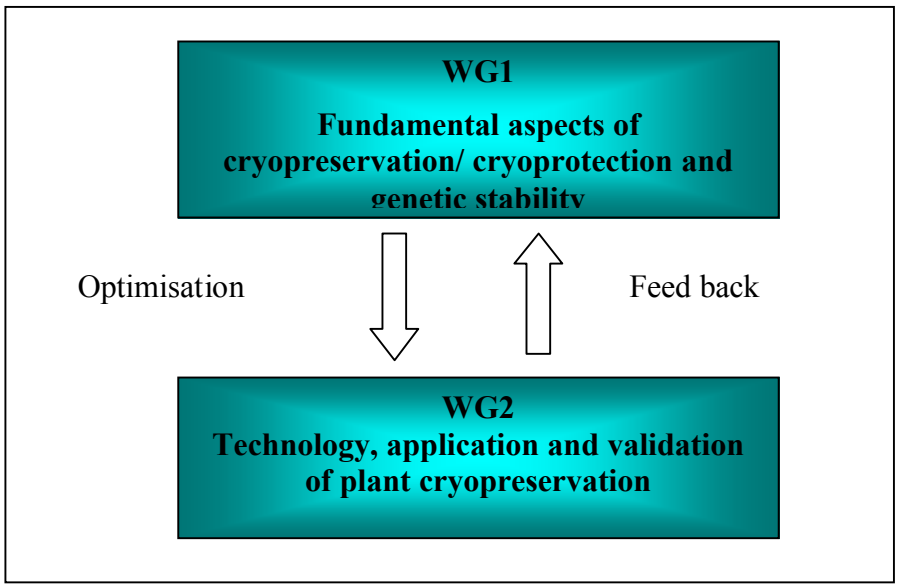

Figure1. Interaction between WG1 and WG2

WG1: Fundamental aspects of cryopreservation/cryoprotection and genetic stability

Within this Working Group, two major research topics can be distinguished: (1) fundamental aspect of cryopreservation and cryoprotection, and (2) genetic stability and traceability.

1.1. Fundamental aspect of cryopreservation and cryoprotection

This part mainly aims at the elucidation of the physico-biochemical background of cryoprotection and cryopreservation. For this, physico-biochemical changes associated with cryoprotection will be studied and correlated with post-thaw viability rates.

Currently, for each species and tissue type, a cryopreservation protocol needs to be developed/ adapted to the natural cold, freezing and desiccation resistance of the species, explant size and type. Cryopreservation protocols are largely developed through empirical studies and care is thereby taken to avoid ice intracellular crystallisation during the freezing process causing physical damage to the tissue. The only way to prevent ice crystal formation at ultra-low temperatures without an extreme reduction in moisture content is through vitrification; i.e. non-crystalline solidification of water (Sakai et al., 1990). To obtain a vitrified solution, it needs to be sufficiently concentrated and/or cooling rates need to be high (Fahy et al., 1984). The existing cryogenic strategies rely on freeze-dehydration, addition of cryoprotective substances including the recently developed plant vitrification solutions, desiccation and acclimatisation or combinations of these processes. It is now generally accepted that the critical step to achieving post-thaw survival lies in the dehydration step and not in the freezing step per se. The key for successful cryopreservation thus lies in the induction of tolerance to dehydration/ desiccation. In practice, this tolerance is induced by sugar treatment, osmotic treatment, cold acclimatisation, ABA treatment, etc., depending on the plant species, tissue type and research group.

The induction of freezing tolerance in nature is now more and more subject to intensive investigations. A wide range of studies indicates that the cell membrane systems are the primary site of freezing injury in plants. Freeze tolerance mechanisms depend on the membrane stabilisation (through changes in lipid composition, production of membrane protecting polypeptides), the water status in the plant cell, the change of the proteins and cytoskeletal proteins, the accumulation of sugars, polyamines and the induction of anti-oxidative mechanisms (Figure 2).

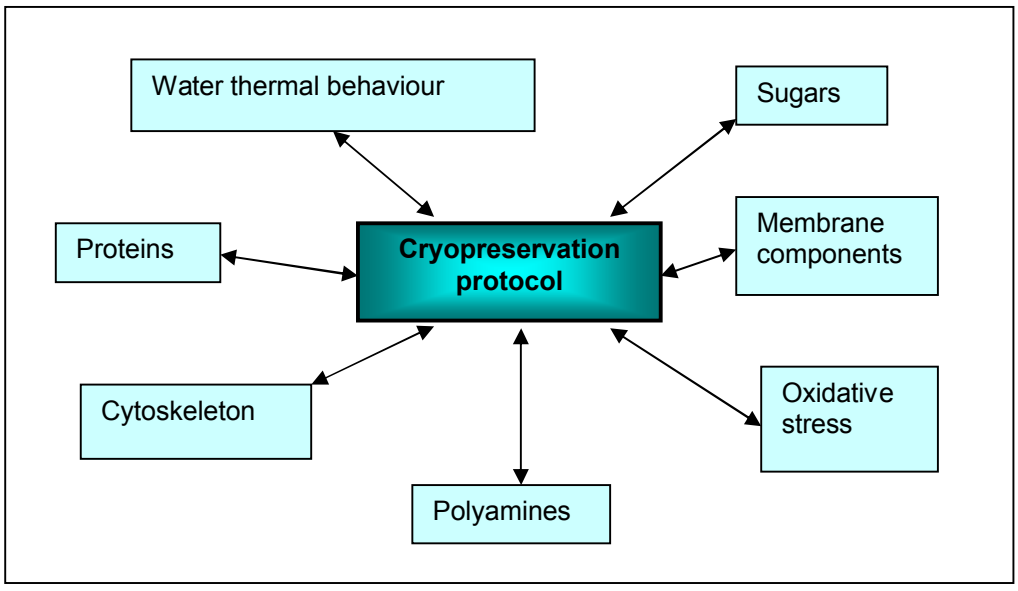

Figure2. Key parameters involved in cryoprotection.

The most damaging physical event during cryopreservation is caused by the development of intracellular ice crystals during the cooling/thawing process. This can be prevented through vitrification; i.e. solidification of water in an amorphous (non-crystalline) form. Vitrification of intracellular water can be achieved by reducing the amount of freezable water in cells and/or the use of very high cooling/thawing rates. Differential Scanning Calorimetry (DSC) is a well-established method for characterising and identifying the ***water thermal behaviour*** (phase transitions) over a large temperature range. While the potential of this technique in the understanding of mechanisms involved in the tolerance of plant materials to ultra-low temperature exposure has been demonstrated (Wesley-Smith et al., 1992, Dumet et al., 1993, Vertucci et al., 1995, Benson et al., 2005, Dussert et al., 2001, Hor et al., 2005, Sherlock et al., 2005), its use for plant cryopreservation studies is still rare and should be expanded.

It has been observed that cold acclimatisation in nature often leads to the accumulation of ***proteins*** such as heat shock proteins, cold regulated proteins and dehydrines. Many of these proteins would play an important role in the stabilisation of membranes against freeze-induced injury (Thomashow, 1999). A change of the protein pattern through cryoprotection using a sugar treatment was described for somatic embryos of *Daucus carota* (Thierry et al., 1999). The most critical aspect of this approach is to find methods which are sensitive enough to detect such proteins, and to establish their applicability to the different plant tissues/species of this project. In many cases immunological methods have been used to detect those proteins among other cell proteins. However, immunological methods often identify only a specific protein, and antisera do not always show the cross-reactivity between different plant species. Using a 2DE protein analytical method and cross-species tandem mass spectrometry identification, it is possible to identify proteins that are significantly up-regulated and down-regulated due to a cryoprotective treatment for banana meristems (Carpentier et al., 2005). The identified proteins represent a broad range of pathways. Down-regulated proteins deal with cell growth, cellulose and storage protein biosynthesis while up-regulated proteins are among others related to glycolysis, cell rescue and defence, secondary metabolisms and signal transduction.

Soluble ***sugars*** (di- and oligo-saccharides) have been proposed as playing an important role in plant tolerance to desiccation and freezing (Crowe et al., 1992; Hoekstra et al., 1997; Thomashow, 1998; Wolfe and Bryant, 1999) because of their stabilisation of membranes and the vitrification of the intracellular aqueous phase. During the last 15 years, numerous studies, using liposomes, pollen and animal tissues, have shown that desiccation or freeze-induced desiccation can lead to at least two types of membrane damage which cause cell death: lateral phase separation of membrane components and formation of non-lamellar phases such as the inverse hexagonal phase. The protective effect of soluble sugars on membrane stability during desiccation and freeze-induced desiccation relies on their ability to form hydrogen-bonds with the polar headgroups of membrane phospholipids and thus to replace water molecules bound to phospholipids in the hydrated state. This would prevent the reduction of the spacing between phospholipids thus (1) maintaining membranes in a fluid phase, and (2) avoiding lateral phase separation. The second mechanism relies on the fact that sugars are excellent glass formers. Since vitrification of the intracellular aqueous phase can be crucial for survival to ultra-low temperature exposure, this second mechanism could also be involved in the differences in sensitivity to liquid nitrogen exposure observed between different plant materials and after various pre-treatments (Zhu et al., in press).

Besides proteins, sterols and phospholipids are the major components of plant ***membranes***. Plant sterols are not only able to regulate membrane fluidity and permeability, but also can modulate the activity of membrane-bound enzymes (Hartmann, 1998). It is now also recognised that phospholipids are not only just structure components of membranes, but they can also act as co-factors for membrane enzymes, signal precursors or signalling molecules themselves (Laxalt and Munnik, 2002). Fatty acids of phospholipids influence membrane flexibility and permeability. The unsaturation degree of phospholipid fatty acids is closely associated with abiotic stress resistance in plants (Kodama et al., 1994). The proportion of non-bilayer phospholipids and destabilising lipids, such as phosphatidylethanolamine (PE) and phosphatidic acid (PA), and destabilising lipids, such as free fatty acids (FFA), is supposed to be important regarding the deleterious formation of non-bilayer structures, such as inverse hexagonal phase, which follows lateral phase separation of membrane PL according to their calorimetric properties during desiccation or freeze-induced dehydration (Gordon-Kamm and Steponkus, 1984; Webb and Steponkus, 1993; Zuidam et al., 1995). In recent years, there has been increasing evidence that lipids also function as mediators in many plant processes including signal transduction, cytoskeleton rearrangements and membrane trafficking (Wang, 2004). For example, free fatty acids (and more specifically oleic acid) are considered as stimulators of the signalling enzyme PLDδ, which has an anti-cell death function (Zhang et al., 2003) suggesting that free oleic acid is a signalling messenger and that PLDδ is one of its potential targets. In addition, it was also proved that the saturated fatty acids, palmitic and stearic acid were involved in cell regulation (Bonaventure et al., 2003).

Polyamines are polycations and thus bind readily to important cellular polyanions such as DNA, RNA, phospholipids and acidic protein residues and cell wall components. Their involvement in various cell functions such as cell division, growth, differentiation and senescence has widely been widely recognised (Kakkar and Sawhney, 2002). There are more and more indications that polyamine metabolism is also strongly involved in the plant response to environmental challenges such as osmotic stress, salinity, hypoxia, cold stress and environmental pollutants (Bouchereau et al., 1999; Hummel et al. 2004). It was shown that cold acclimatisation (Kushad and Yelenosky, 1987) as well as an osmotic shock (Flores and Galston, 1982) induces the accumulation of polyamines. The mode of action, however, is still far from understood. Binding of polyamines to membranes renders them more stress-resistant and reduces leakage (Zheliaskova et al*.,* 2000). Also aromatic amines may also respond to several abiotic stresses (Bouchereau et al., 1999; Szopa et al., 2001; Dufeu et al., 2003). A correlation between the presence of specific polyamines and cryopreservation ability was described by Ramon and coworkers (2002).

Published evidence indicated that a modified ***cytoskeleton*** can improve cell survival after cryopreservation (Morisset et al., 1992; 1993) and other studies indicate that both cytoskeletal stability and the activity of Ca2+ dependent protein cross-linking enzyme transglutaminase are both affected differently by the above cryopreservation pre-treatments (Harris et al., 2004). Since such treatments involve physical stresses, they are also likely to affect stress-related cell signalling pathways. Hence studies are ongoing towards the identification of key molecular events associated with exposure of cells to different cryopreservation pre-treatments and post-thaw recovery, with particular emphasis on stress-related changes in the cytoskeleton and associated enzyme activities involved in its regulation.

Since free radical-mediated ***oxidative damage*** is a generic stress response in aerobic organisms, it is also a key factor in cryoinjury. Metabolic uncoupling occurs leading to the production of toxic free radicals (molecules with unpaired electrons), which cause lethal incipient cellular damage by extracting electrons from essential macromolecules. For example, hydroxyl radical adducts of DNA (e.g. 8-hydoxyguanosine) are correlated with mammalian cancers and mutagenesis (Grollman and Moriya, 1993). Similarly, free radicals can attack proteins and lipids forming highly toxic reaction products (Esterbauer et al., 1998) such as the unsaturated aldehydic compounds 4-hydroxyalkenals and malondialdehyde. These extremely reactive compounds cross-link with enzymes and proteins (via Michel additions and Schiff's base reactions) and impair metabolic and regulatory processes. Medical cryobiologists have used a fundamental knowledge of free radical damage and antioxidant protection to assist the development of improved low temperature storage protocols (Fuller and Green, 1986; McAnulty and Huang, 1997). These are based on the avoidance of free radical reactions and the amelioration of oxidative stress through the application of antioxidants and their pharmacological analogues; these approaches have also been successfully applied to freeze-recalcitrant algae (Fleck et al., 2000). It is now accepted that free radical mediated oxidative stress can potentially occur in all components of plant cryopreservation protocols, including in vitro manipulations, cryoprotection and desiccation (Benson and Bremner, 2004). For example, it has been shown that desiccation of coffee seeds to optimal water status for cryopreservation (0.2 g $H_2O.g^{-1}$ dw) leads to extensive loss and oxidation of antioxidants, lipid hydrolysis and phospholipid loss (Dussert et al., 2006).

Studies of all parameters described above and their relation to cryopreservation ability thus offers considerable potential for the improvement of conservation methods.

Specific objectives of this part are to:

- define changes of the key parameters during the application of different cryopreservation protocols;
- determine the relation between these changes and cryopreservation ability;
- find treatments which improve the cryopreservation ability of plant tissue through a manipulation of the key parameters.

1.2. Genetic stability and authenticity

This part mainly deals with the assessment of the genetic integrity of plants to determine if they are 'true to type' after cryopreservation.

There are many issues and concerns regarding investigations into the genetic stability of plant germplasm after recovery from cryopreservation. One central theme is the lack of international agreement and consensus in approaching generic solutions acceptable to a wide range of conservation activities. Although it is recognised that the effects of cryoinjury upon the genome are often unknown, and any accumulative DNA polymorphisms may *not* be induced by cryopreservation per se but as the result of the whole culture-cryoprotection-regeneration process, it is generally experimentally difficult to detect what specific stages generate genomic instability. Higher plant cells exhibit the phenomenon of somaclonal variation, which can be manifest as manifold plant variations. Consequently, successful cryopreservation is often judged by the survival of the tissue and ability to regenerate into

complete plants; therefore it is desirable to assess the genetic integrity of plants to determine if they are 'true to type' after cryopreservation

Techniques to assess genetic stability

The genetic integrity of plants at the phenotypic, cytological, biochemical and molecular levels and its relevance to stability investigations have been reviewed (Harding, 2004; Harding et al., 1997). Briefly, these include:

- Assessments of phenotypic variation with defined morphological descriptors for a given species, whereby stem, leaf, root, flower, fruit and growth habit can be analysed collectively by a range of multivariate statistical techniques.
- Cytological techniques to detect various types of chromosomal instability. Variant cells with gross chromosomal changes may include: polyploidy, aneuploidy and other mitotic abnormalities.
- Biochemical metabolite/protein (isozyme) profiles that are useful to compare changing patterns in gene expression in plants recovered from cryopreservation.
- Genomic DNA sequences that can be analysed using a range of hybridisation and Polymerase Chain Reaction (PCR) techniques for which several investigations report evidence of stability after cryopreservation.
- Epi-genetic variation in chromatin and DNA methylation of gene sequences which have been found in plants after cryopreservation, suggesting altered patterns of gene expression.

Cryobionomics

The concept of 'cryobionomics' was first introduced and explored by Harding (2004). It addresses the issues and inherent weakness in current genetic stability investigations leading to concerted action for the basis of an acceptable international agreement in the characterisation of cryopreserved germplasm. The conceptual impact of 'cryobionomics' on European germplasm repositories and culture collections is fundamental for this COST Action in order to fully explore its implication.

Regarding the application of investigative tools, there are criticisms following the advancement of techniques in molecular biology, where the analysis may be 'technique' driven. Analytical technology proceeded RFLPs, to RAPDs, SSRs and AFLPs, but the advancement from RFLPs to PCR-based RAPDs is not without its problems or criticism (Harding, 2004, Harding et al., 2005; Lowe et al., 1996; Jones et al., 1997). Implicit here, is the assumption that a molecular technique will analyse and produce a DNA fragment profile linked to instability; that is, a marker-assisted-selection for instability. Such molecular analyses, however, examine only a fraction of the total genome. For example, in the analysis of potato with ~10^9bp per (tetraploid) genome, assume a mean number of 10 fragments and an average fragment size of 1000bp, therefore any given analysis will cover approximately [(10 x 1000)/ 10^9bp] x 100 = 0.001% of the genome. Given this small fraction of the total genome analysed, DNA fragment polymorphic profiles from cryopreserved plants may not detect genetic instability but more likely ***stability*** in specifically selected sequences (Harding, 2004).

There is a need for guidance, before the application of specific techniques; guidelines exist for the selection of markers to assess genetic diversity in conservation. Moreover, there is also a need for more robust and stringent analytical procedures (Johnston et al., 2005). Challenges remain regarding the adequacy of investigations for stability prior to the export of germplasm, where clearly there is not one single approach adequate to judge genetic stability. To date, there are no established criteria available for germplasm repositories that are adequate to evaluate genetic stability in plants regenerated from cryopreserved germplasm. Therefore, regarding the concept of genetic stability, an accurate definition is lacking and this is an issue that needs to be addressed at the international level to ensure germplasm stability is maintained, especially for endangered species. For these species, cryopreservation provides the only long-term storage preservation security, which precedes their re-introduction into the environment. Taking these factors into consideration an appropriate definition for an integrative approach to genetic stability would be 'cryo-bionomics' (Harding 2004, Harding et al., 2005), defined as the biological science dealing with cryopreserved organisms' behaviour and habitats following their re-introduction into their natural environment. Clearly, the term 'cryobionomics' can be described as interdisciplinary requiring phenotypic, histological, cytological, biochemical and molecular biological knowledge of the organism to assess possible cellular/biochemical damage (cryoinjury), impairment of metabolism. It may also describe loss of reproductive functions, and can examine temporal shifts in gene expression that may cause disruption of normal regulatory mechanisms, growth and developmental sequences.

Specific objectives of this part are to:

- develop recommendations and techniques to assess genetic stability of plants and plant tissues after cryopreservation; and
- give a guidance to cryobiologists on how to limit mutational events and genetic changes during the cryopreservation procedure as well as during storage in liquid nitrogen.

WG2: Technology, application and validation of plant cryopreservation

Within this Working Group two major research topics can be distinguished: (1) the technology aspects of the different cryopreservation techniques; and (2) impact and applications: links to and integration with conventional plant resources and genebanks, establishment of cryobank, dissemination of results.

2.1. Technology aspects of cryopreservation

This part mainly deals with the applications of cryopreservation protocols to different plant species and tissues. The cryopreservation technologies that will be used are based on existing protocols that are mainly developed through empirical studies, knowledge of traditional controlled rate freezing and contemporary protocols based on the multidisciplinary fundamental approaches aimed at understanding cryoinjury and tolerance as described under WG1.

Two requirements must be met to prevent lethal ice crystallisation during cryopreservation: (1) rapid freezing rates, and (2) a concentrated cellular solution. The cell cytosol can be

concentrated through air drying, freeze dehydration, application of penetrating or non-penetrating substances (cryoprotectants), or adaptive metabolism (hardening). For a solution to be vitrified at high cooling rates, a reduction in water content to at least 20-30% is required. Most hydrated tissues, however, do not withstand dehydration to moisture contents needed for vitrification (20-30%) because of solution and mechanical effects. Exceptions are pollen, seeds, dormant buds and somatic embryos of most temperate seed species. The key for successful cryopreservation is thus shifted from freezing tolerance to dehydration tolerance. This tolerance can be induced by chemical cryoprotection with substances such as sugars, amino acids, DMSO, glycerol, etc. but can also be induced by adaptive metabolism such as cold hardening in temperate species and/or for less cold tolerance species. All this is the subject of investigations executed under WG1. The following cryopreservation protocols can be distinguished.

Air drying (evaporative drying, flash drying)

This method is directly applicable to seeds, dormant buds, zygotic embryos and pollen of many common agricultural and horticultural species. Flash (or ultra-rapid) drying proved to be beneficial for recalcitrant zygotic embryos of some plant species (Berjak et al., 2000).

Classical slow-cooling (or slow-freezing) protocol

This was the first 'standard' protocol that was developed for hydrated plant tissues. It is based on slow cooling of specimens (at a rate of 0.5-2°C/min) in the presence of a cryoprotectant solution, generally containing DMSO at a 5-15% concentration. When during the slow-cooling process a temperature of about –40°C is reached, the intracellular solution is considered to be concentrated enough to vitrify upon a subsequent liquid nitrogen plunging. This method is mainly used for cryopreservation of dormant buds and non-organised tissues, such as cell suspensions and calli. This 'more traditional' freezing method still has a vitally important place in plant cryopreservation and more specifically in biotechnology, cereal breeding and forestry (e.g. conifers) where they are used extensively, particularly in the commercial sector (Cyr, 2000).

Encapsulation/dehydration

In this method, developed by Fabre and Dereuddre (1990), explants (usually meristems or embryos) are first encapsulated in alginate beads (which can also contain mineral salts and organics), thus forming 'synthetic seeds' ('artificial seeds' or 'synseeds'). Then, the synseeds are treated with a high sucrose concentration, dried down to a moisture content of 20-30% (under airflow or using silica gel) and subsequently rapidly frozen in liquid nitrogen. Although the procedure can be considered rather lengthy and labour-intensive, it is observed that the presence of a nutritive matrix (the bead) surrounding the explant can promote its regrowth after thawing.

Vitrification

First reports on the use of a vitrification solution with plant tissues appeared in 1989 (Langis et al., 1989; Uragami et al., 1989). The technique relies on treatment of explants with a concentrated vitrification solution for variable periods of time (from 15 minutes up to 2

hours), followed by a direct plunge into liquid nitrogen ('vitrification/one-step freezing'). This results in both intra- and extracellular vitrification. Freezing rates of about 6°C/sec are normally obtained by plunging explants enclosed in a cryovial into liquid nitrogen. Higher cooling rates can be obtained by enclosing the meristems in semen straws, resulting in cooling rates of about 60°C/sec, or using a 'droplet freezing protocol' where the material is placed on aluminium foil strips that are plunged directly into liquid nitrogen, giving rise to cooling rates of 130°C/sec (Panis et al., 2005; Schäfer-Menuhr et al., 1997). The vitrification solution consists of a concentrated mixture of penetrating and non-penetrating cryoprotectant substances. The most commonly applied solution, named 'PVS2' (Plant Vitrification Solution No. 2), consists of 30% glycerol, 15% ethylene glycol, 15% DMSO (all v/v) and 0.4 M sucrose (Sakai et al., 1990). Fifteen years after its first report, vitrification is today a widely used cryopreservation protocol. The success of the procedure can be attributed to its simplicity, high reproducibility and to the fact that it can successfully be applied to a wide range of tissues and plant species.

Other protocols

Other available methods are the 'droplet freezing' (Schäfer-Menuhr et al., 1997), the 'pre-culture method' (Panis et al., 1996), the 'encapsulation/vitrification' (Sakai., 2000) and the 'pre-culture/dehydration' (Dumet et al., 1993). Up to now, these techniques have been applied to only a limited number of plant species.

Specific objectives of this part are to:

- develop efficient cryopreservation protocols partially based on the elucidation of the mode of action of cryoprotection /tolerance to cryopreservation; and
- apply the cryopreservation protocols to a wide variety of plant species/tissues derived from species exhibiting different natural tolerances to cold, freezing and desiccation stress.

2.2. Impact and applications of cryopreservation in plants

This part mainly deals with making links between the more technical aspects of cryopreservation (see previous parts) and plant genetic resources and genebanks, establishment of cryobank and dissemination of results

In Europe, there is a large number of crops, including roots and tubers but also fruit trees, as well as forest tree species for which cryopreservation represents the only option for safe and cost-effective long-term conservation of genetic resources. However, if in vitro slow growth storage is used in a number of genebanks for medium-term conservation of different species, cryopreservation is currently used in a few exceptional cases only (Frison and Serwinski, 1995; Turok et al., 1995). There is an urgent need to disseminate information on this technology and to train scientists and technicians in genebanks in the implementation of cryopreservation protocols. In this part, the new cryopreservation methods will be applied to the germplasm collections of the collaborating institutes. At the same time the results will be validated and disseminated through a plant cryopreservation manual and specialised training workshops which will be attended by end users (European industries and scientific and technical staff of relevant research institutes and genebanks) as well as by decision makers.

In this part the choice of IPGRI (International Plant Genetic Resources Institute) as a partner is obvious. It is the largest international agricultural research institute involved solely in research on conservation of plant genetic resources. Its role in this COST Action is to help disseminate the results and in translating them into practical applications to improve cryopreservation procedures for crops other than those incorporated in this COST Action. In Europe, IPGRI has two main programmes: the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) and the European Forest Genetic Resources Programme (EUFORGEN).

Specific objectives of this part are to:

- disseminate the improved cryopreservation protocols developed by the COST Action participants to relevant European industries, research institutes and genebanks through the development of a plant cryopreservation manual and the organisation and implementation of specialised training courses;
- provide policy makers with first-hand information on the role of existing, and the potential of the new, cryopreservation protocols in the sustainable use of Europe's plant genetic resources in key application sectors and in meeting the needs of international conservation agendas.

E. ORGANISATION

The **Management Committee (MC)** will coordinate the Action. Management Committee meetings will take place once a year, usually linked with the scientific meeting of a Working Group or with a workshop. Meanwhile, restricted MC meetings (chairperson and vice-chair(s), WG coordinators) will take place to ensure an efficient coordination of the activities, to review critical points and to maintain a clear focus on the objectives and milestones. In principle, decisions are taken unanimously. In the case of disagreement, the majority vote will be conclusive. The tasks of the Management Committee will be the following:

- Appointment of Action chairperson, vice-chairperson(s) and Working Group coordinators.
- Planning of Management Committee meetings and of scientific meetings and workshops.
- Assessment and report of the progress made by the different Working Groups to meet their respective objectives, in the framework of the focus and direction of the Action.
- Coordination and critical appraisal of the previous, ongoing and planned activities (meetings; short-term scientific missions, STSMs; publications, etc.) to meet the general objectives of the Action.
- Promotion of cooperation and of data exchange between the Working Groups.
- Promotion and approval of STSMs, according to the recommendations of an ad hoc evaluation committee.
- Preparation of the Annual Reports.
- Establishment and update of a web site for internal communication and results dissemination.
- Organisation of contacts and common workshops with the appropriate ongoing COST Actions and other relevant technology or scientific platforms, to address problems of common interest (e.g. possible COST Actions on Plant Biotechnology).
- Preparation of an EU Seventh Framework Programme project with respect to plant cryopreservation.

Two **Working Groups** will be established and each will be managed by two coordinators**.** The Working Groups are:

WG1: Fundamental aspects of cryopreservation/cryoprotection and genetic stability

WG2: Technology, application and validation of plant cryopreservation

The tasks of **WG coordinators** will be:

- Planning the appropriate scientific meetings.
- Coordination of the activities within the Working Group, to meet the objectives.
- Promoting the set-up of joint research (for example in the framework of an STSM) and the writing of common publications.
- Report on the WG progress to the Action chairperson and Management Committee.
- Participation in the plenary and restricted MC meetings.

Working Group meetings will be organised on a yearly basis separately or combined with the other WG. Separate meetings will enhance the exchange of information and ideas, stimulate the synergy between scientists, institutes and countries and are more appropriate to address specific topics and to plan joint experimental work. Combined meetings and scientific workshops will enhance the integration of activities, addressing primarily the interfaces between the different fields. The information obtained in one WG can have important implications for the other WG; for example the identification of mode of actions of cryoprotection (WG1) will lead to improved and more efficient cryopreservation protocols (WG2). Also feedback from plant germplasm curators on the behaviour of cryopreserved plants (WG2) will aid the research on genetic stability (WG1).

Inter-COST workshops with appropriate ongoing COST Actions will be organised, to address topics of common interest. The following COST Actions will benefit from the development of efficient cryopreservation protocols:

- COST Action 864: Combining traditional and advanced strategies for plant protection in pome fruit growing
- COST Action 863: Euroberry Research: from Genomics to Sustainable Production, Quality & Health

Short-Term Scientific Missions (STSMs) within and also between the Working Groups, will help to coordinate the research by the establishment of adequate cooperation between the participating institutes. Research will be strengthened and intensified by an **exchange of young scientists** between organisations from the participating countries, with a special focus on scientists from the new EU-memberstates. An ad hoc STSM evaluation committee will be appointed by the Management Committee, with one coordinator and one representative from each Working Group.

F. Timetable

- The duration of the Action is 4 years.
- The **kick-off meeting** will start the Action and at this meeting the WG leaders will be selected.
- The **web site** will be created soon after the kick-off meeting and will be updated twice a year.
- The timetable of the Action is spaced out by the **meetings of each WG** which will be held once a year. **Combined meetings** and workshops of the two WGs will take place every two years and, possibly, **inter-COST workshops** will be held to address problems at the interface of WGs and ongoing COST Actions, and will allow for the cross-fertilisation of outputs and ideas.
- **Plenary Management Committee meetings** will take place once a year, linked with the scientific meeting of a Working Group or with a workshop. Meanwhile, **restricted MC meetings** (chairperson and vice-chair(s), WG coordinators) will take place to ensure an efficient coordination of the activities, to review critical points and to maintain a clear focus on the objectives and milestones.
- **STSMs** can be requested after the first WG meeting has taken place.
- The Action will be closed with a **Final Conference** combined with all WG meetings.

Table 1. Timetable of events

	Year 1				*Year 2*				*Year 3*				*Year 4*			
Coordination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Kick-off meeting	x															
Web site		x		x		x		x		x		x		x		x
Reporting				x				x				x				x
MC meeting	x					x					x					x
WG1 meeting			x				x			x						x
WG2 meeting				x			x				x					x
Workshop*																
STSMs				x	x	x	x	x	x	x	x	x	x	x	x	x
Final Conference																x

MC meeting: Management Committee meeting;
WG meeting: Working Group meeting;
STSMs: Short-Term Scientific Missions;
Workshop***:** Timing of the inter-COST workshops will be defined in agreement with the Management Committee of that specific Action.

G. ECONOMIC DIMENSION

The following 17 COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Italy, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Switzerland, Turkey and the United Kingdom.

On the basis of national estimates provided by the representatives of these countries, the economic dimension of the activities to be carried out under the Action has been estimated, in 2005 prices, at approximately EUR 38 million. This estimate is valid on the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

In addition, participants from the non-COST countries Morocco, Russia and Tunisia have shown an interest in participating.

H. DISSEMINATION PLAN

The consortium that results from this COST Action will provide the following **services and expertises**:

- service and consulting to private collections (industry). Indeed, many companies involved in agriculture have their own collections of germplasm in support of their specific breeding activities. Companies will then be able to drastically reduce costs if their germplasm is cryopreserved. This will make them more competitive.
- service and consulting to breeders (intermediate storage of breeding lines).
- service and consulting to molecular biologists (reduced costs and labour-free maintenance of transformed plants until safety issues are solved).
- service and consulting to researchers (medium-term and long-term storage of research objects).
- service preservation of large-scale stocks of plantlets and embryogenic tissue for planting (ornamentals are subject to fashion; by storing large amounts one can respond faster to the market).

These services and expertises will be advertised on the web site of the COST Action.

It was also shown that cryopreservation of virus-infected plants resulted in disease-free plants. Cryopreservation thus offers much promise for phytosanitation and the provision of healthy planting material, without the use of pesticides.

What will the consortium do to disseminate the results?

- Scientific results of the project will be disseminated in **refereed scientific journals** provided that publication does not impair IPR.
- **Common review articles** are also expected, whereas the publication of **book**(s) would be welcome when appropriate and timely.
- A **public web site** will be installed to inform the scientific community about the project and achievements. The web site will also facilitate the communication of project outputs related to services/consultancies offered and it will be used to announce training workshops.
- Information about the COST Action will also be available on the official **web pages of the collaborating institutions**.
- The consortium will organise **workshops** to inform interested scientists, regulatory bodies and policy makers about the results of the project and about new technologies developed throughout the project. During these workshops hands-on practical and training theoretical information will be given to germplasm curators.
- At the end of the Action, the consortium will offer its **expertise as a service to the EU**. The acquired knowledge can then guide the EU in the implementation of the European Community Biodiversity Strategy and related Action Plans.
- Knowledge and data resulting from the COST Action activities will be integrated and presented in **international conferences**, for promoting the European know-how and increasing the international collaboration.
- **Teaching activities** in universities at undergraduate and post-graduate level will also take advantage of the knowledge and experience acquired during this COST Action. Young scientists and engineers will thus be trained and informed on the latest developments in cryopreservation.