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

Secretariat

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COST 252/01

LIMITE

DRAFT MEMORANDUM OF UNDERSTANDING

Subject :	Draft Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action 851 "Gametic cells and molecular breeding for crop improvement"

Please find attached the abovementioned draft Memorandum of Understanding.

DRAFT

Memorandum of Understanding

for the implementation of a European Concerted Research

designated as

COST Action 851

"Gametic cells and molecular breeding for crop improvement"

The Signatories to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above 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", the contents of which are fully known to the Signatories.

2. The main objective of the Action is to discover the biological controls of gametic embryogenesis and to exploit these in plant breeding.

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 EUR 100 million at 2000 prices.

4. The Memorandum of Understanding will take effect on being signed by at least five Signatories.

5. The Memorandum of Understanding will remain in force for a period of five years, unless the duration of the Action is modified according to the provisions of the document referred to in point 1 above.

TECHNICAL ANNEX

COST ACTION 851

"Gametic cells and molecular breeding for crop improvement"

A. BACKGROUND

Gametic cells are those which are involved in sexual reproduction. In plants these include the sperm cells of the pollen (male) and the eggs cells of ovules (female). Gametic cells are haploid (n), having half the genetic constitution of the parent plant, the fusion of a sperm cell with an egg cell produces a new individual in which the diploid (2n) status is reconstituted. Plants generally produce several thousand more pollen grains than that required for sexual reproduction. In gametic embryogenesis the development of gametic cell is diverted to produce embryos rather than mature pollen grains or ovules. In the case of microspores (cells destined to become pollen) there is the potential to produce several hundred plants from a single anther. Induced or spontaneous doubling of the haploid cells produces doubled haploid (2n) plants. These are homozygous, true breeding and are of vital importance in plant breeding either as parental lines or as finished varieties. Doubled haploidy is the fastest route to homozygosity and large numbers of plants can be produced, but not all homozygous lines are of interest. There is a need to select among the doubled haploid populations for individuals carrying desirable genetic constitutions. Genetic markers are used for this purpose, these can be used to tagged specific genes e.g., disease resistance genes, or to examine the genetic background (genetic fingerprinting) of lines. The application of genetic marker techniques to breeding has been termed molecular breeding.

Clearly the more that is known about gametic embryogenesis the more the process can be manipulated. Doubled haploidy is used in many plant breeding programmes with varying degrees of success. The objectives of this proposal are 1) to exploit new ideas and technologies to gain a better understanding of gametic embryogenesis, and 2) to integrate gametic embryogenesis into molecular breeding thereby improving the speed and efficiency of plant breeding. In addition to increased scientific knowledge there are expected to be considerable economic benefits and increased competitiveness of European plant breeding resulting from the COST Action.

Current state of knowledge

The biotechnology of doubled haploidy via gametic embryogenesis has been studied for over 20 years. It has been used as a research tool to investigate fundamental plant processes and has been exploited in developing special genetic stocks for both research and plant breeding purposes. Gametic embryogenesis can be applied with varying degrees of success to most major crops: cereals, oilseeds, potato, vegetables etc. with the notable exception of legumes. For many species doubled haploidy is sufficiently developed for in-depth fundamental research of reproductive biology (pollen, egg, embryo and seed), but commercial exploitation remains limited.

Much past success in developing protocols for gametic embryogenesis has been empirical involving numerous and subtle changes to tissue culture media and culture environment. Our understanding of the genes involved is poor, with only a vague knowledge of their numbers and location. A few genetic loci have been identified, e.g. those controlling *in vitro* embryo and green plant production in wheat and barley lines, but only for a small number of lines. The advent of functional genomics provides an opportunity to place the study of embryogenesis on a more robust scientific basis by discovering the controlling genes. The study of expressed genes via differential display, development of expression libraries etc is now possible and can be targeted towards finding the genes and environments responsible for efficient gametic embryogenesis. Numerous and substantial projects in functional genomics have been financed and set up in Europe (see Section C – 2, below), and more are expected to follow. The national and international support for functional genomics in Europe will ensure a strong and inter-active research base for participating countries of the Action.

With respect to research, the recent successes in doubled haploidy in major crop species has been timely as it coincides with the explosion of knowledge in genetic markers, high resolution maps, QTL analysis, ESTs, candidate genes and marker assisted selection. Doubled haploid populations have been used to establish genetic marker maps in many species, which have been used to identify genetic regions controlling some of the complex traits of economic importance, e.g. yield, quality and disease resistance. Genetic mapping has become routine in many species; the challenge now is to go beyond map locations and to identify the controlling genes present in these regions. The new role for doubled haploidy will be in deployment strategies. Doubled haploidy via gametic embryogenesis is the fastest route to homozygosity (true breeding) and as such is of great interest to researchers and plant breeders. Doubled haploidy offers speed and marker technology precision, by combining these technologies plant breeding can be greatly improved. In addition synergy between these biotechnologies will lead to new options in "molecular breeding".

European competitiveness

Gametic embryogenesis is of research and commercial interest throughout all countries of Europe. The programme aims to move forward from an already well established research base and is expected to attract interest from University research groups, national institutes and commercial breeding companies.

Europe has always been at the leading edge of research and application of gametic embryogenesis. In recent years successful collaborations and networks have been set up, e.g. projects under the "European Plant Embryogenesis Network" (Theme IV of EPBN, Framework IV) and the COST Action "Gametic embryogenesis" (COST 824). The European lead has been threatened in recent years, particularly by multi-national companies who are able to devote substantial inward investments in biotechnologies for agricultural purposes. In some countries, such as Australia, institutes having expertise in the pertinent areas have been formed into a consortium to maximise efficiency in crop development. Because gametic embryogenesis of crop plants is a global issue it is imperative that Europe remains competitive. European cooperation in the form of a new COST Action on "Gametic Cells and Molecular Breeding" would greatly assist in this matter.

B. OBJECTIVES AND BENEFITS

The main objective of this Action is to discover the biological controls of gametic embryogenesis and to exploit these in plant breeding.

It is expected that critical genes and environmental controls of gametic embryogenesis will be discovered in the next 5 years. Some putative candidates have already been identified, e.g. the signal molecule LLP1 in brassica and cell cycle regulatory genes in cereals. These biological controls and their interactions with culture conditions will be harnessed in improving the efficiency of doubled haploid production in a wider number of genotypes and species. Doubled haploids represent unique genetic stocks, which can be tested repeatedly and as such open up interesting areas of research. It is expected that doubled haploid production in Europe will increase in the next 5 years and that the COST Action will be a major vehicle for progress via concerted action. Large multinational biotechnology companies are making investments in this area; the new COST Action provides a means for small and medium size companies and the public sector to maintain an involvement.

C. SCIENTIFIC PROGRAMME

The science will involve: (a) basic research in double haploidy technology; and (b) functional genomics in gene discovery, gene expression and developmental studies. The research will be targeted at a wide range of species including all the major European crops and ornamental species. The model species, barley, brassica and tobacco, will be exploited in transferring results and know how to more recalcitrant species. The demonstration of novel and accelerated breeding programmes using doubled haploidy will provide the motivation for wider application of doubled haploidy, genetic selection and deployment.

The research tasks fall into three main areas:

1) Technology advancement for gametic embryogenesis

The focus here is on basic research on gametic embryogenesis, using excised anthers, isolated microspores or egg cells to regenerate homozygous plants in various *genotypes and* species. Although gametic embryogenesis has been known and researched for more than twenty years many problems remain. The application in many species is hampered by low frequencies of embryo induction, albinism and plant regeneration, and in some species only certain genotypes (lines) respond. In species like barley and brassica gametic embryogenesis works well and has become an integral part of many breeding programmes throughout the world. The main breakthroughs in barley came with the use of maltose in the culture media and the experience in how to handle the donor plants.

Much progress in gametic embryogenesis has been empirical, e.g. small modifications of protocols. Much of this knowledge will never be published, but a COST Action provides a medium for information transfer among scientist and end users. For example, in potato, the main problem is that existing protocols work only for a few genotypes. More experimentation is needed to develop better, more genotype independent protocols. Because of successful European collaboration there have been major breakthroughs, e.g. technology transfer of tobacco to apple microspore culture, and this has had a positive knock-on effect on other woody species. The successful transfer of technology across plant genera highlights a unifying theme in gametic embryogenesis, an example being the preparation and induction of competent cells via pre-treatment. Currently this is achieved by *in vitro* stress treatments of excised anthers or ovules, or by *in vivo* manipulation of the environment of donor plants. Further research is expected to clarify the critical plant developmental stages and processes involved.

The COST Action aims to place the technology of gametic embryogenesis on a firm scientific basis. One means of technological advancement is through the study of gene expression in responding and non-responding cell cultures given alternative treatments (e.g. maltose instead of sucrose). This approach will help elucidate genes and biochemical pathways necessary for successful gametic embryogenesis. The results of functional genomic approaches can then be tested by designing tissue culture protocols with predicted effects. This will in lead to the tailoring of tissue culture methods for specific genotypes for use in molecular breeding. This area of work will therefore be interactive with 2) and 3) below.

2) Functional genomics of gametic embryogenesis

Recent advancements in genomics allow genes involved in specific functions to be identified. The methods include subtractive hybridisation, production of cDNA libraries, expressed sequence tag sequences (ESTs), gene expression on microarrays and proteomics. These techniques are being used in gene discovery for various traits, and the importance of embryogenesis in pure and applied biology places this high on the priority list for research. The major agri-biotech companies (e.g. Du pont/Pioneer, Zeneca, Monsanto, Advanta, Novartis) have invested heavily in "in house" generation of ESTs of major crop plants. This has yielded impressive results in the production of several hundred thousand ESTs. A number of governments have realised the importance of academic based genomics programmes. In Europe the French government in collaboration with industry launched "Geneoplante", a 1,400 million franc plant genome initiative. In Germany a similarly large functional genomics programme has been announced for Arabidopsis research. In Finland there is joint government/industry collaboration of genomics of cereals, and in the UK government funding has supported the Arabidopsis genome sequence initiative and community based cereal and brassica gene function resource. Many of these initiatives address fundamental biological question and include embryo. Major breakthroughs in the identification of genes involved in natural and induced embryogenesis are therefore expected. This work will enable verification of candidate genes and identify the most important ones for further basic and applied research. These funded national and international programmes will help underpin the COST Action. This area of work will have consequences for technological advancement (1 above), in helping to maximise genotype/technology combinations, which can then be exploited in deployment strategies (3 below).

3) Deployment of gametic embryogenesis

Genes of commercial interest fall into two categories, those controlled by single genes, e.g. disease resistance, and those controlled by many genes, e.g. yield. In some species in which good genetic maps have been developed, many major single genes are known and mapped. For other species, and for polygenes in general, genetic mapping remains a basic requirement for which genetic mapping populations in the form of doubled haploids will be required. The development of doubled haploids to map the location of commercially important genes will continue as a research objective as this forms a foundation for their deployment. Once areas of the genome have been identified they can be targeted and manipulated. Detailed investigations include saturation mapping in targeted regions with the aim of getting at the genes of interest, to either develop tightly linked markers or to sequence the important genes and design direct markers for them.

Doubled haploidy is already used in breeding of several crops in Europe, notably brassicas and cereals. As improved protocols are developed through 1) and 2) above the use of doubled haploidy will become more widespread. Doubled haploidy is used by breeders to produce true breeding lines in material developed in their crossing programmes and as such is an important component of conventional breeding.

However, doubled haploidy in conjunction with genetic markers opens up the new strategies of "molecular breeding". It is known, for instance, that doubled haploidy in forage grasses provides a means of producing new allele combinations that are never seen in conventional backcrossing programmes. There will be increasing interest in manipulating specific genes for crop improvement, these can be either single genes or polygenes identified in donor cultivars, wild progenitors of crop or related species, or transgenes from unrelated sources. A number of protocols and variations on protocols will be developed where the logistics of double haploid deployment will depend on the genotype/tissue culture combinations, species, recombination frequency, genetic marker, laboratory facilities and breeding cycle. The development and assessment of these strategies is a major research task.

There are three Working Groups:

1) Technology advancement for gametic embryogenesis

2) Functional genomics of gametic embryogenesis

3) Deployment of gametic embryogenesis in crop improvement

Milestones

Technology advancement for gametic embryogenesis

1. Currently species are classified as completely recalcitrant (e.g. legumes), low-moderately responsive (where efficiency is hampered by problems of embryo formation, albinism an genotypic dependency), or highly responsive. A milestone here is to move more (5 – 10) species into the high efficiency bracket. (Successful application of gametic embryogenesis technology to legumes would be a major achievement).

2. Demonstration of tailoring culture conditions to genotype.

3. Application of automated technology through interaction with COST 843.

4. Development of improved technology for deployment in plant breeding.

Functional genomics of gametic embryogenesis

1. Identification of genes controlling gametic embryogenesis: Functional genomic approaches will produce many gene sequences associated with gametic embryogenesis. These will be used to search data-bases for homologous genes and large numbers of gene candidates will be revealed. This is a relatively easy task, the trick however, is to sift through this wealth of information to find critical genes with main effects, which require great care and effort. The main milestone for members of this working group is to identify these genes. It is anticipated that these will number between 1 and 10.

2. Compare genes and alleles with major effects on gametic embryogenesis across a range of genotypes, genomes and species.

3. Compare gene functions in normal (seed) embryogenesis with that of induced gametic embryogenesis.

Deployment of gametic embryogenesis in crop improvement

1. Provide a demonstration of more efficient plant breeding by combining doubled haploidy with genetic marker technology for at least one monocot. and one dicot. species.

2. Facilitate the transfer of new technologies to plant breeders thereby widening the exploitation of gametic embryogenesis.

3. Develop new doubled haploid populations for genetic mapping.

4. Compare the results of molecular breeding with more conventional methods and to develop innovative methods of producing desired genotypes.

D. ORGANISATION, TIMETABLE AND DISSEMINATION

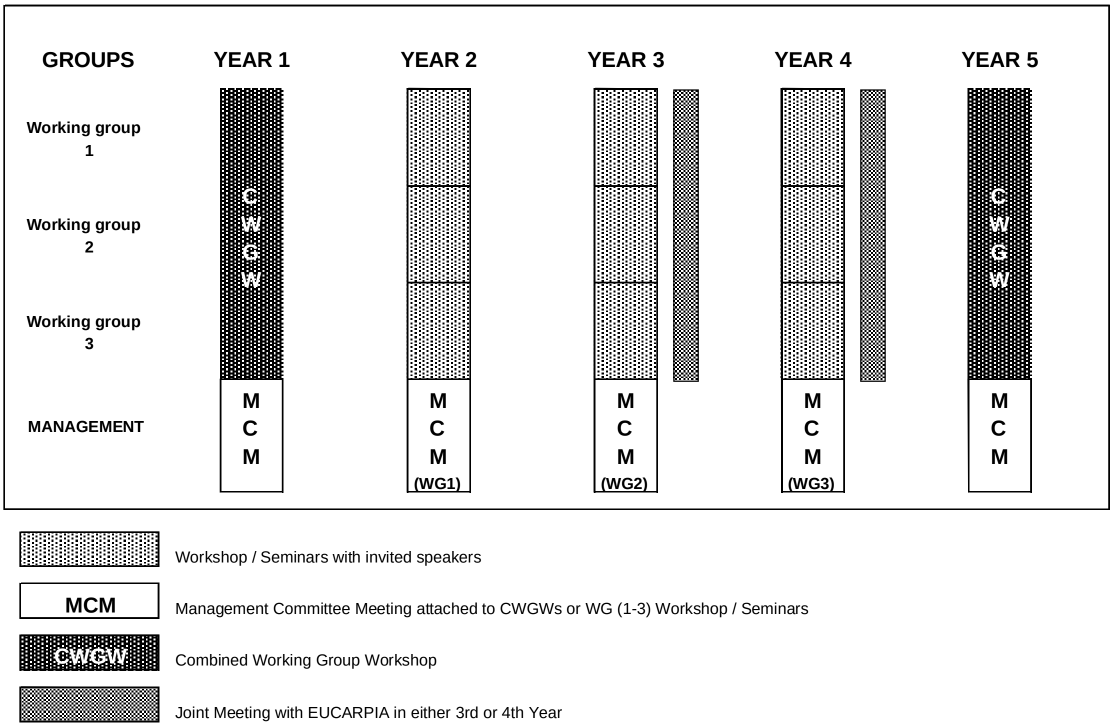
The Action is scheduled for five years. Five years is required for two reasons. Firstly, the work is directed towards improving plant breeding methodologies in a number of species. Plant breeding, by nature, is a long process since it involves the productions of sequential generations from an initial cross of two parents. Depending on the life cycle of the species this can involve several years, in conventional breeding of annual cereals it can take 10-15 years to produce a finished variety. Fifteen years are not required to assess the application of gametic cells in molecular breeding. Doubled haploidy combined with marker assisted breeding provide options for accelerating the breeding process and a five year COST Action is considered sufficient to assess impact. Secondly, it is expected that the newly funded research in functional genomics will produce a wealth of data within the first years of the Action, but their evaluation and application will take longer to assess.

The three Working Groups described in C are interactive and above will run in parallel. Individual and joint meetings of these groups are planned on a yearly basis (Figure 1). The European plant breeding organisation, EUCARPIA have expressed an interest in this Action and a joint meeting is proposed, possibly in 2004 when EUCARPIA holds a congress. The COST Action has some overlap with the proposal on "Disease resistant crops: the necessity for sustainable cereal production in Europe" (compiler, Rients Niks, The Netherlands) in which resistance breeding using new biotechnologies is a component. The main themes of these two COST Actions are quite distinct. In this proposal the aim is to gain a greater understanding of gametic embryogenesis and apply this to molecular breeding of a wide range of species. That of Rients Niks specifically targets cereal diseases, which in part exploits the technologies developed in this proposal. The two are therefore complementary and are not competing. Some collaboration between these COST Actions may arise in the area of targeted breeding and joint meetings or exchange of experts/speakers may be useful.

Similarly there is some common interest between this new COST Action and that of COST 843, "Quality enhancement of plant production through tissue culture". The tissue culture component of the new COST Action is specific to gametic cells, which does not feature in COST 843. However, the development of tissue culture technology in COST 843, particularly automation, will be of considerable interest to members of the new COST Action. Reciprocally, the gene expression approach to formulating tissue culture conditions will be of interest to members of COST 843. The Technical Annex of 843 proposes a joint meeting with COST 824, since COST 824 has now ended, it is suggest that this is replaced with a joint meeting with the new COST Action sometime in Year 2 or Year 3 (see Figure 1), to be negotiated between the two COST Action Management Committees.

figure 1

Five year organisational timetable



In accordance to the "Rules and Procedures for Implementing COST Actions" the Management Committee will include: a chairman, vice chairman, scientific secretary (appointed by EC) and one or two members from each participating country. The Management Committee will be responsible for setting up annual meetings of the Working Groups and assessing and approving short-term scientific missions (STSMs). It will also be active in initiating inter-Action cooperation among the three Working Groups. The Management Committee will oversee the direction and progress of the COST Action in general and the progress of the individual Working Groups. Each of the three Working Groups will be organised by two joint Coordinators who will be responsible for organising meetings and coordinating research activities. The Working Group Coordinators, in conjunction with the Management Committee will be responsible for submitting reports to the EC, and in producing other scientific reports.

Short-term scientific missions: it is expected that the demand for STSMs will vary between years, between 2 – 10 per year. These will fall into three categories:

1) technology transfer, learning techniques in top laboratories

2) access to specialised facilities of host laboratories

3) exchanging, testing and developing new ideas.

These exchanges will take place among participating countries. However, if possible, a small number of STSMs to experts outside Europe will be encouraged.

For fast dissemination of intermediate information and results to participants and other stake holders, a web site on the internet will be developed. A person from the MC will be appointed to develop the website and to coordinate the dissemination of data. Besides the periodic dissemination via the internet, a final report will be written, including an executive summary, final financial statement, and a summary of the results achieved in comparison to initial and updated COST-action plans. Identified stakeholders including potentially interested enterprises and policy makers will be specifically informed about the Action. They will be asked to attend, if appropriate. Dissemination of knowledge between scientists will be further stimulated by short term scientific missions.

E. ECONOMIC DIMENSION

At least 20 countries in Europe have ongoing work in doubled haploidy. It is estimated that this involves 60-70 laboratories in Universities, institutes and private companies.

On the basis of national estimates provided by the representatives of most of these countries and taking into account the coordination costs to be covered over the COST budget of the European Commission, the overall cost of the activities to be carried out under the Action has been estimated, in 2000 prices, at roughly EUR 100 million.

This estimate is valid under 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.