

Brussels, 12 January 1994

COST 331/94

**Memorandum of Understanding**  
**for the implementation of a**  
**European Concerted Research Action**  
**designated as COST Action 824**  
  
**"Gametic embryogenesis"**

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

1. The Action will be carried out in accordance with the provisions of document COST 400/94 "Rules and Procedures for Implementing COST Actions", the contents of which are fully known to the Signatories.
2. The main objective of the Action is to promote and coordinate precompetitive research on gametic embryogenesis.
3. The overall cost of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at ECU 10,2 million at 1994 prices.
4. The Memorandum of Understanding will take effect on being signed by at least five Signatories.
5. The Memorandum of Understanding will remain in force for a period of five years, unless the duration of the Action is modified according to the provisions of Chapter 6 of the document referred to in Point 1 above.



## ACTION 824

### A. General background

The value of obtaining doubled haploid plants for plant breeding has been extensively acknowledged. The introduction of *in vitro* techniques for the induction of androgenesis or gynogenesis has significantly facilitated the production of doubled haploids as a support to plant breeding programmes, resulting in the early release of varieties.

The application of this new approach to plant breeding (and propagation) is encountering many difficulties, particularly in the field of whole plant regeneration from a microspore. It will not be possible to benefit from, and fully exploit, this new technique and opportunity without a fuller understanding of the processes involved.

The primary aim of this proposed Action is to coordinate research and development in Europe aimed at overcoming the various bottlenecks which restrict the full application of haploid culture technique to crop breeding and propagation. Breeding, including haploid production and genetic engineering, will have an impact only after 20 to 30 years.

The new COST Action will bring together scientists from several European laboratories. The participants hope for a well-established network, cooperation and strong connections among the laboratories, and for a strong cooperation between the official and private laboratories.

Links have to be established with the International Association for Plant Tissue Culture, the International Association for Plant Reproduction, the International Society for Horticultural Science and the European Association for Research on Plant Breeding (EUCARPIA).

The new COST action group will establish a joint working group with COST 822 (Development of integrated systems for large scale propagation of elite plants using *in vitro* techniques).

## **B. Objectives for the Action**

Foster increased cooperation in Europe, in part through yearly meetings, in research on gametic embryogenesis in order to:

### (a) Primary objectives:

Increase the use of gametic embryogenesis in plant breeding, including the extension of this technology to additional crops. Obtain quality control of regenerants (albinos, aneuploids, spontaneous diploidisation). This will be achieved by joint experiments and annual meetings.

Develop systems for the genetic transformation of gametic cells as a method for obtaining genetically modified crops.

Further the understanding of the molecular, biochemical and physiological processes underlying gametic embryogenesis.

### (b) Secondary objective:

An important secondary objective is that the understanding of gametic embryogenesis will increase our understanding of embryogenesis in plants in general (zygotic as well as somatic embryogenesis).

## C. Scientific content for the Action

The three proposed primary objectives form the basis for the organization of the Action into three working groups.

In Working Group 1 "Anther, microspore and ovule culture" the focus is on the establishment of optimized *in vitro* culture systems using excised anthers, isolated microspores or pollen, excised ovules or isolated embryosacs or egg cells to regenerate haploid plants in various species.

The application of anther culture in species of importance in Europe is hampered by the rather low embryo induction and plant regeneration frequencies as well as by strong genotypic effects. This lack of efficiency is mainly caused by strong genetic control which can be modified by environmental factors such as donor plant conditions, temperature, media composition and culture conditions. The success of the relatively simple anther culture technique depends on the complex interactions of a high number of inducing factors. Further investigations are necessary to analyse the factors determining the balance of gametic to sporophytic development. Anther culture may be the technique of choice for the breeder if a sufficient number of doubled haploid plants can be produced from the various genotypes of interest.

The ultimate central goal of this COST Action will be to set up protocols in various species for the high-efficiency production of green, haploid plants in anther culture as well as in isolated microspore or pollen cultures, by exchanging and using protocols and know-how from the established model species *Brassica napus*, *Nicotiana tabacum* and *Hordeum vulgare*. Since the requirements for setting up successful anther or microspore cultures differ from species to species, subgroups will focus on dicots, cereals, non-cereal monocots and woody species. This subdivision is, however, preliminary. Depending on the progress, it may be necessary to regroup the activities and include for instance legumes or *compositae*.

Ovule culture is a less frequently used technique for haploid production but with great potential. An extension of this technique is the isolation and culture of embryo sacs and even egg cells.

Since microspores and embryo sacs are products of meiosis, a population of plants derived from these structures represents some of the genetic variability produced by this meiosis. Selection can therefore be performed during *in vitro* culture of "gametes" from plants produced by specific crosses. There is little experience with such a type of selection, a procedure that is quite unlike the *in vitro* selection utilizing somaclonal variation. Since large numbers of individuals are required for selection, the microspores, i.e. the male "gametes", rather than the ovules, are the cellular targets of choice.

An important, and as yet insufficiently investigated aspect of doubled haploid breeding, is the quality of the regenerated plants. In some species, such as cereals, spontaneous diploidisation takes place. Although beneficial at first sight, there is evidence that spontaneous diploids are sometimes less fertile. Somaclonal variation, mostly as chromosome losses, may be the cause of this reduction in fertility. Again in cereals, albinos are produced, often in high frequency. In tetraploid wheats, such as *Triticum durum*, hardly any green plants are produced. In other species not much is known about the effect of somaclonal variation (sometimes called gametoclonal variation) on the quality of the doubled haploid plants, but such effects cannot be excluded. The *in vitro* culture systems have, therefore, to be adapted in such a way as to minimize these and other forms of somaclonal variation. To do this, techniques have to be developed that allow early detection of variant forms during the plant production process. These techniques will be developed within the working group "Fundamental aspects of gametic embryogenesis". On the basis of these detection techniques, the *in vitro* systems will be optimized for efficient and non-deleterious methods of diploidisation.

In Working Group 2 "Transformation of gametic cells" the progress obtained in Working Group 1 "Anther, microspore and ovule culture" will be utilized to produce transgenic plants. At present, the optimized systems of pollen culture existing in tobacco, rape seed and barley are being used to produce transgenic plants. Potentially, transformation via microspores or ovules may be more generally applicable, even in those species that cannot easily be regenerated from somatic explants. Transformants obtained via haploids are homozygous in one step, saving time for the breeder. A further bonus: once a breeder is using anther and – in the future – pollen culture for doubled haploid production, it makes sense for him/her to use the same regeneration technique also for gene transfer, particularly since he/she should be able to rely on the quality of the regenerated plants.

There are two specific problems with transformation of "gametes" (microspores and ovules). The microspore wall and even more so the tissue layers surrounding the ovule represent barriers to DNA delivery that may require specific adaptation to the various DNA delivery devices. Second, since there is little or no experience with selection during gametic embryogenesis (see working group on "Anther, microspore and ovule culture"), the conditions for proper selection procedures have to be worked out. This pertains e.g. to the use of appropriate constructs (promoters, selective agents/resistance genes) and to the timing of the application of selection pressures. No subgroups will be formed initially, but such groups may later be formed on the basis of groups of plant species.

In Working Group 3 "Fundamental aspects of gametic embryogenesis" two different aspects will be considered. In a subgroup called "Cytogenetic and molecular analysis of events leading to genetic variation", the problems with the quality of the regenerants mentioned in connection with the working group "Anther, microspore and ovule culture" will be addressed. Cytogenetic techniques such as flow cytometry, *in situ* hybridisation, classical chromosome analysis, but also molecular techniques (e.g. PCR) will be required to detect various forms of somaclonal variation. Of particular interest will be the timing and mechanisms of spontaneous diploidisation, chromosome losses and the occurrence of chloroplast deletions. A functioning feed-back is required to enhance the exchange of information between the two working groups.

A second general topic within this working group is the molecular analysis of embryogenic induction and embryo development. Since it is generally recognized that gametic embryogenesis requires shock treatments to initiate embryogenesis, the molecular events of stress-induced gene expression are of particular interest. Various strategies will be used for isolating early and late genes of microspore and haploid ovule embryogenesis. These genes can be compared with genes isolated from somatic and zygotic embryos. A specific problem for late development is the arrest of already formed embryos in some species. Basic physiological research may be necessary to analyse the underlying causes.

In addition to these primary objectives, a secondary objective is to add to our understanding of plant reproduction in general, specifically of gametogenesis and embryogenesis. An understanding of microsporogenesis and ovule development is required since these processes are the starting points for the respective induced embryogenic pathways. Embryogenesis *in vitro* has phenotypically a striking resemblance to zygotic embryogenesis. Whether and to which degree this is reflected at the molecular level has still to be shown. A further field to which this Action will contribute is stress research. Starvation, cold and heat treatments are all able to induce embryogenesis in one species or another. A molecular analysis of embryogenic induction will inevitably yield an understanding of stress-induced gene expression.

## **D. Timetable**

The Action is scheduled for five years. It is anticipated that the research programmes described above will run in parallel, building on previous achievements of each laboratory and on international communication.

## **E. Organization, management and responsibilities**

The Management Committee will be responsible for initiating the inter-Action cooperation with appropriate working groups. An annual planning and evaluation session will be organized for each working group, and the Management Committee will oversee the general direction and progress of the interaction between working groups and their relationships with other COST Actions. Each working group will be organized with two Joint Coordinators who will be responsible for coordinating the research programme and organizing meetings of their working group. It is expected that each working group will meet annually. The Group Coordinators, in conjunction with the Management Committee, will be responsible for the Annual Report for submission to the COST Senior Officials.

Proposed working groups:

- (1) Anther, microspore and ovule culture
- (2) Genetic transformation of gametic cells
- (3) Fundamental aspects of gametic embryogenesis

## Implementation and publishing

Each working group will be organized with two coordinators who will be responsible for coordinating the research programme and organizing workshops, conferences and seminars of their working groups. Coordination will be required also to organize conferences together with other COST Actions. Within the working groups, a group leader will organize meetings and workshops of his/her subgroup.

The final results will be published by the preparation of reports from the group leaders, supplemented by reports from the coordinators of the working groups. In conjunction with the Management Committee the coordinators will submit the final report to COST Senior Officials.

### **F. Economic dimension of the Action**

So far, 66 laboratories in 17 different countries have expressed their interest in this COST Action.

It is estimated that the yearly total personnel costs will be ECU 8 500 000, with ECU 1 700 000 for running/operational costs. These costs will be covered from national resources.

This estimate is on the basis of information given in COST scheme No 2 and information from national experts. It does not include the United Kingdom, Denmark, Ireland and Iceland, although there is information that they also plan to take part in this COST Action.

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