Somatic Embryogenesis in Monocotyledonous Plants, State of the Art and Perspectives

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WHY MONOCOTYLEDONOUS PLANTS

A whole range of important agricultural and horticultural species are monocotyledonous plants. These include food, forage, industrial, and ornamental crops (Table 1). Somatic embryogenesis is possible in many monocotyledonous species, but with more or less success. Although our knowledge since 1958, when somatic embryogenesis was first reported, has increased considerably concerning the factors controlling initiation, development, maturation, and germination of somatic embryos, it is still far from routine in most plant species.

WHAT IS SOMATIC EMBRYOGENESIS

Somatic embryogenesis is the development of an embryo from a somatic cell. This is in contrast to the zygotic embryo which develops from a zygote, the fusion product between an egg and a pollen cell. Other cells than the gametes are somatic and cells from any tissue can in theory be the source of somatic embryos. Therefore, somatic embryogenesis is a type of vegetative propagation. Somatic embryogenesis is not a phenomenon restricted to in vitro culture, but is also known from nature in the form of certain types of apomixis.

In principle all somatic cells can develop into embryos, but the best choice of explant tissue is often young and meristematic, e.g., young leaves, immature zygotic embryos, and immature inflorescences. When the explant tissue is exposed to

Table 1. Examples of monocotyledonous plants in which somatic embryogenesis is possible.

Food crops	Cereals (corn, rice, wheat, millet, etc.) Vegetables (yams, leek, onion, garlic) Fruit (banana, date, coco) Sugarcane, oil palm
Forage crops	Cereals (corn, rice, wheat, millet, barley, etc.) Grasses (orchardgrass, rye grass, etc.)
Industrial crops	Fibers (coco, *Miscanthus*, bamboo) Oil (oil palm)
Ornamentals	Pot flowers (*Dieffenbachia, Spathiphyllum,* etc.) Cut flowers (*Alstroemeria*, lilies) Flowering outdoor plants (lilies, tulips, *Yucca*)

growth regulators, most often an auxin and sometimes in combination with a cytokinin, embryos may develop directly (direct embryogenesis) or there may be an intervening callus phase (indirect embryogenesis). Indirect embryogenesis is best suitable for mass propagation because maintenance and propagation of callus is possible. In contrast to the zygotic embryo, somatic embryos are without an endosperm and a seed coat.

PERSPECTIVES

Despite being an interesting tool for investigating the developmental sequence from single cell to whole plant, somatic embryogenesis can in practice be used for mass propagation, as a tool in breeding programs, and for germplasm conservation. Mass propagation through somatic embryogenesis may result in the production of plantlets or artificial seed. Economically, artificial seed will probably never be able to compete with normal seed, however, today somatic embryogenesis can be an alternative to other types of vegetative propagation of high value crops. In the breeding for new culitvars somatic embryogenesis may shorten the breeding cycle or may be used to propagate the parent plants for hybrid seed production. Moreover, mutations may be induced by treatment with mutagenes, or mutations may arise due to the in vitro culture conditions (somaclonal variation). New traits may be introduced to a cultivar either by somatic hybridization or transformation. Regeneration of plants from in vitro cultures is a prerequisite for the current transformation systems.

EXAMPLE 1. SOMATIC EMBRYOGENESIS IN OIL PALM

The French ORSTOM-CIRAD group has for the last 20 years investigated the possibilities of using somatic embryogenesis for commercial clonal propagation of oil palm (*Elaeis guineensis* Jacq.) (Rival et al., 1997). The research started in the beginning of the 1970s and resulted in 1982 in the transfer of knowledge to a pilot-scale laboratory in West Africa followed by additionally three pilot-scale laboratories in Indonesia and Malaysia in 1985-86. Furthermore, in 1987 a production laboratory was opened in France. The pilot-scale labs were designed to produce 250,000 plants per year. To date, 1 million plantlets have been produced by somatic embryogenesis resulting in 2500 ha with cloned material.

Clonal propagation of oil palm through somatic embryogenesis, as outlined in Fig. 1, is restricted by:

- High production costs. The price of clonal-propagated plantlets is five times the price of seedlings from selected material because somatic embryogenesis still involves many labor-intensive steps. The existing protocol is not suitable for large-scale propagation because the propagation rate is too low and it is difficult to fulfill the growers choice of cultivar and delivery time.
- Lack of genetic fidelity. In commercial plantings 5% of the plants develop abnormal flowers, which in severe cases prevent fruit set.

In order to reduce the production costs the possibilities of harvesting somatic embryos from suspension cultures and of processing embryos into artificial seed are currently being investigated. Later such systems may be automated. In addition, research is carried out to develop a quality control for genetic fidelity.

EXAMPLE 2. SOMATIC EMBRYOGENESIS IN ORCHARDGRASS

For a number of years a group of scientists at the University of Florida have been working on somatic embryogenesis in orchardgrass (*Dactylis glomerata*) (Gray et al.,1993). In contrast to oil palm the research in orchardgrass aims at developing more efficient breeding methods and not at mass propagation. Orchardgrass is open pollinated, self incompatible, and can not be cloned efficiently by conventional methods. Therefore, cloning by somatic embryogenesis, in combination with microspore or anther culture, reduces the production time of new cultivars. New cultivars can also be produced by transformation or somatic hybridization followed by plant regeneration through somatic embryogenesis. Another possible use of somatic embryogenesis is propagation of the parents for hybrid seed production (F_1 seed).

The use of somatic embryogenesis in orchardgrass, as outlined in Fig. 2, is like in oil palm limited by relatively high production costs, but also by genotypic differences in ability to form somatic embryos.

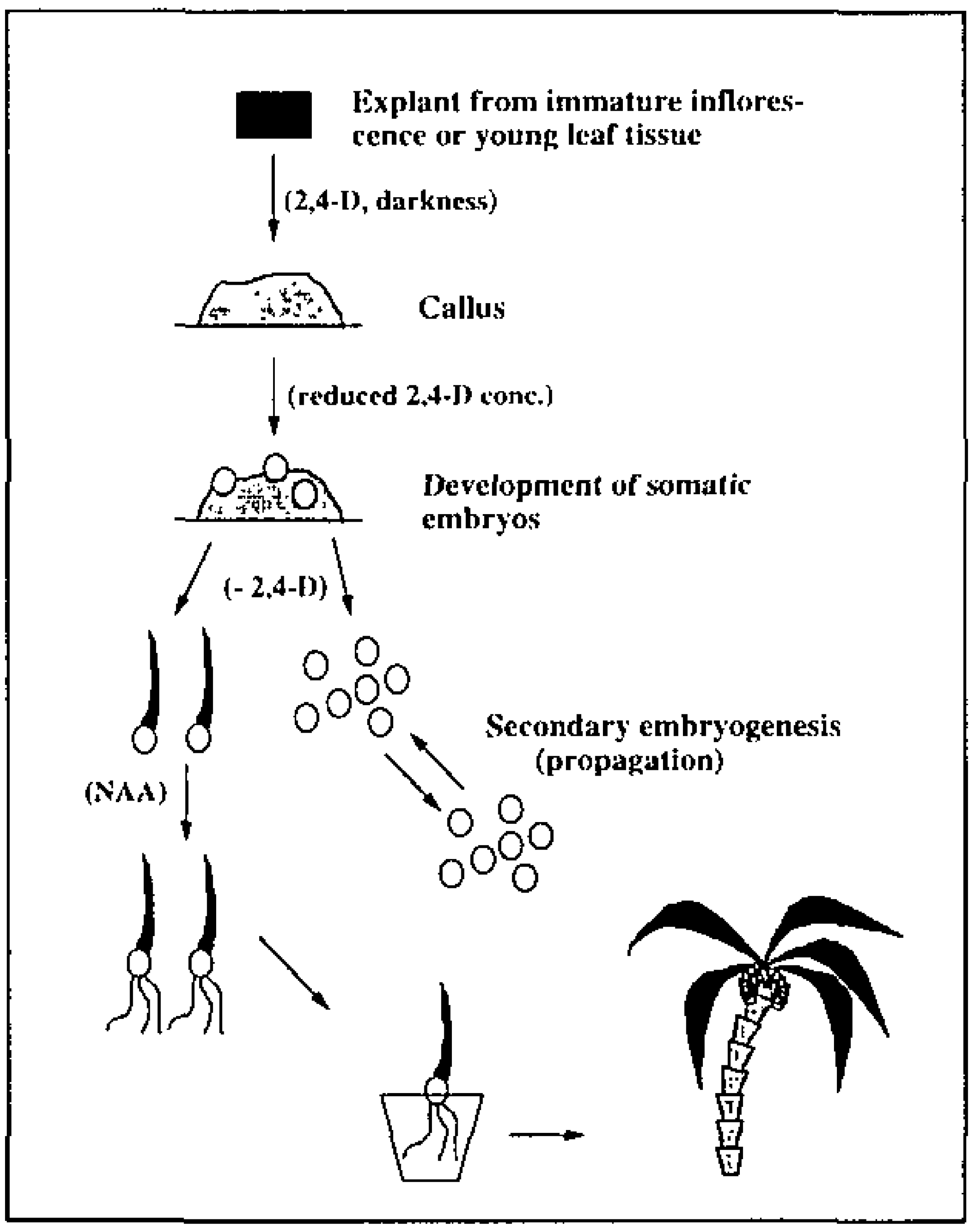

Figure 1. Outline of somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq.).

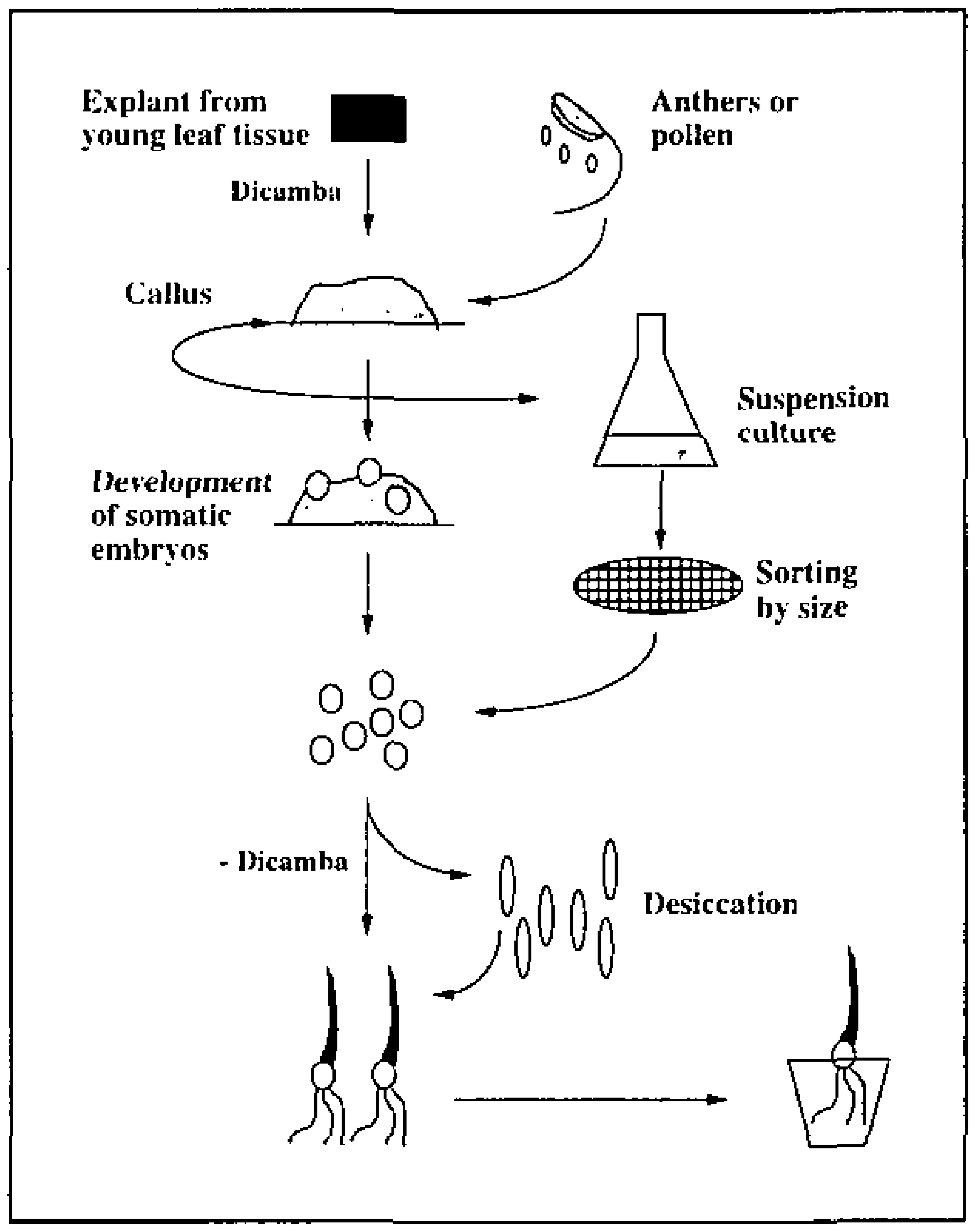

Figure 2. Outline of somatic embryogenesis in orchardgrass (*Dactylis glomerata* L.).

LIMITATIONS IN THE USE OF SOMATIC EMBRYOGENESIS

There are still a number of limitations in the use of somatic embryogenesis:

- Although it is possible to produce somatic embryos from many plant species large genotypic differences exist. This means that many of the most valuable cultivars and hybrids are difficult to propagate.
- Basic research on the induction and development of somatic embryos is not sufficient.
- Somaclonal variation, variation induced by the in vitro growth conditions, is at present not controllable. This is a problem when the offspring need to be true to type.

- Callus and suspension cultures often lose their ability to produce somatic embryos after a shorter or longer period of culture.
- In grass species the regeneration of albino plants (lacks functional chloroplasts) can be a problem in certain genotypes.
- It is very difficult to obtain a complete synchronization of embryo development within a culture, a very important prerequisite in automation of artificial seed production.

CONCLUSION

For some monocotyledonous plants relatively good somatic embryogenesis systems have been developed, but for most species there are still a number of limitations to the commercial application. In high-value crops where plantlet or seed prices are high, somatic embryogenesis may be a way for mass propagation. The use of somatic embryogenesis for mass propagation may be extended if it is possible to control somaclonal variation and to obtain a high degree of automation. Somatic embryogenesis may also be an efficient tool in the breeding of new cultivars.

LITERATURE CITED

Gray D.J., R.N. Trigiano, and **B.V. Conger**. 1993. Liquid suspension culture production of orchardgrass somatic embryos and their potential for the breeding of improved varieties. In: K. Redenbaugh (ed). Synseeds: Applications of synthetic seeds to crop improvement. CRC Press Boca Raton.