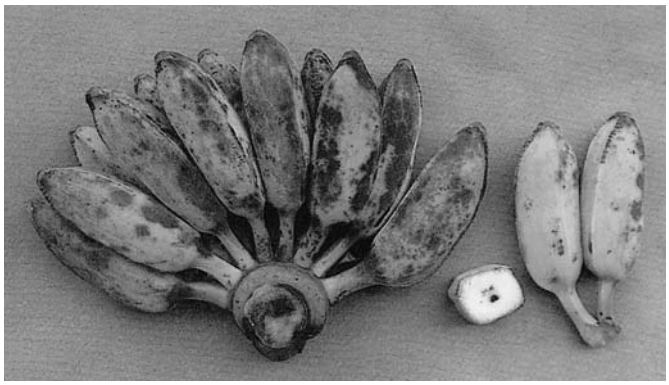

Figure 8. *Ripe fruits of Chuoi Mat, a cooking banana with triploid* balbisiana *genome composition*

esting and highly ornamental germplasm of Vietnam.

To safeguard the banana germplasm of the country, the duplicated field collection at Long Dinh Fruit Research Centre was established in South Vietnam. But field germplasm collections are vulnerable to disease infection, floods, drought and entry of pests such as nematodes. To further ensure the conservation of Vietnam's indigenous *Musa* genetic resources, a duplicate *in vitro* collection was also established at the Department of agro-biotechnology of VASI by Dr Ho Huu Nhi. Vietnam is sharing with INIBAP/IPGRI a large number of its *Musa* germplasm accessions and the materials are being sent to the INIBAP Transit Centre of INIBAP in Leuven, Belgium where it is held in trust for the benefit of the world community.

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Improvement | Embryogenic cell suspension techniques

Establishment of embryogenic callus and initiation and regeneration of embryogenic cell suspensions from female and male immature flowers of *Musa*

Agnès Grapin, Juan-Luis Ortíz, Régis Domergue, Jacqueline Babeau, Sophie Monmarson, Jean-Vincent Escalant, Claude Teisson and François Côte

In *Musa*, there are two main reasons for developing an efficient cell regeneration process: (i) as a new mass micropropagation technique and (ii) to have a cell regeneration system useful in the development of genetic engineering techniques.

During the last ten years, four methods of somatic embryogenesis in *Musa* have been published, each one using a different type of explant. The first results were obtained using immature zygotic embryos (Cronauer and Krikorian 1988, Escalant and Teisson 1988, 1989). However, this technique is limited to wild seminiferous genotypes, and is not suitable for cultivated genotypes. Embryogenic cell suspensions were obtained from leaf tissues and rhizomes (Novak *et al.* 1989), and from highly proliferating meristem cultures (Dhed'a *et al.*, 1991). For these latter two techniques, little quantitative data on the regeneration efficiencies are available. The fourth technique for somatic embryogenesis, using immature male flowers, was initially developed by Ma (1991). Using temporary immersion, an increased level of embryogenesis was obtained by Escalant *et al.* 1994, while researchers at CIRAD also obtained embryogenic cell suspensions with a high regeneration rate using immature male flowers (Grapin *et al.* 1996, Côte *et al.* 1996). This technique was however limited to genotypes having a persistent male bud. Recently, due to the development at CATIE of a process using immature female flowers, this technique has potentially been extended to all types of *Musa* (Grapin *et al.* 1997).

A synthesis of the results obtained to date by CIRAD and CATIE on callogenesis and embryogenic cell suspensions initiated from immature flowers is reported here. Full details of the composition of the culture media used and the four stages of the methodology are provided in Grapin *et al.* (1996) and Côte *et al.* (1996).

Initial explants

Male flowers

After bunch development, the male buds are collected. The cultured tissues consist of immature male flowers removed from the bud under sterile conditions. Of the hundreds of floral rows present in the bud, only the hands from the rows nearest to the meristem are cultured. These hands are smaller than 3mm and contain 15 to 20 flowers each.

Female flowers

Plants are cut at their base when they are apparently at the transition between the vegetative and the floral stage. After eliminating the leaves, the pseudostems are opened lengthwise to extract the young bud. For the cv. Curraré, the buds contain less than 20 differentiated hands and those formed after the sixth or seventh row contain only one flower.

Development of callus

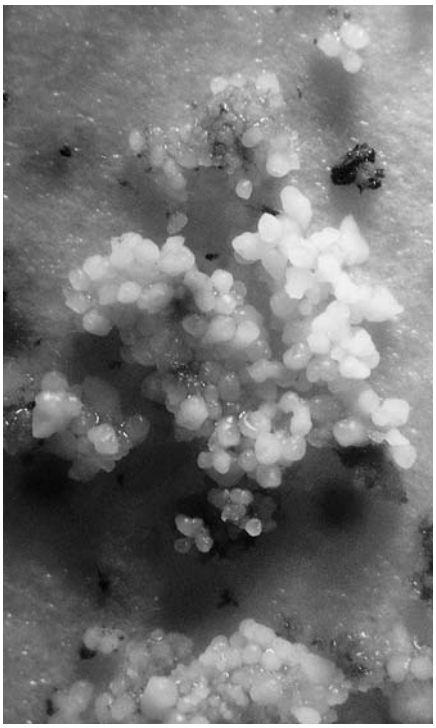
During culture, the development of the explants is relatively similar whether they originate from female or male flowers. The largest hands of flowers (more than 2mm) continue their growth without forming callus tissues. Necrosis occurs in the smallest hands. Yellow nodular callus appears on medium size hands. After the third month in culture, the first callus with proembryos is visible. This embryogenic callus can vary from callus bearing one to a few embryos to white friable callus with multiple proembryos. Plants can be regenerated by transferring somatic embryos removed from an embryogenic callus onto a germination medium.

The percentage of male buds forming embryogenic callus depends on the genotype. At CATIE, the average is 40% and 5% for cv. Grande Naine and cv. Gros Michel respectively. For female flowers, 24% of the cultured buds form embryogenic callus for cv. Curraré. Only the hands of the first to the forth row, showing at least three flowers, form embryogenic callus.

Embryogenic cell suspensions

Cell suspensions are established by transferring embryogenic callus to liquid medium. The development and composition of such cell suspensions are relatively similar, whether they originate from female or male flowers, and whatever the genotype used. Cytological studies were carried out on various genotypes to determinate their cellular composition and their mode of multiplication.

After several months of subculture, the suspensions contain mainly cell aggregates with a friable structure. These essentially consist of cells with a large nucleus, usually with a single nucleolus. The cytoplasm is rich in soluble proteins, and contains small vacuoles with some protein reserves. Starch reserves are rare or even absent. Internal cell divisions occasionally occur within the aggregates leading to the formation of proembryos. However, these culture conditions do not allow ontogenesis to be completed. They evolve in compact nodular masses with a ring of cells rich in starch. Isolated cells, containing large vacuoles, are rare.



Somatic embryons of Curraré (AAB False Horn type) formed by establishment of cell suspensions (primary explant: immature female flowers).

The increase of cell volume basically depends on the growth and the fragmentation of the cell aggregates. Multiplication rates of two to six in terms of cellular volume are observed each month.

Embryogenic cell suspensions regeneration

Regeneration is achieved by plating the cell suspensions onto a semi-solid medium rich in cytokinin. With a zoom microscope, the first proembryos are visible on the aggregates fifteen days after plating. Cytological studies of somatic embryo ontogenesis demonstrated that a unicellular origin was more than likely. After 45 days, the embryos present an epidermis, a caulinary meristem, a root pole and a provascular system. They have very few reserves, even after a longer period of culture.

The regeneration efficiency quantification was reproduced several times. For the cv. French Sombre, plating 1 ml of packed cells led to the formation of 10^5 embryos of which 10 to 40% could be converted into plantlets.

For the cv. Grand Naine, plating 1 ml of packed cells led to the formation of 3.7×10^5 embryos with an average rate of germination of 5%. For the cv. Curraré Enano (cell suspension initiated from female flowers), 1 ml of packed cells could result in the formation of 10^5 to 5×10^5 embryos.

Conclusion

Immature flowers cultivated *in vitro* can result in embryogenic callus formation and embryogenic cell suspension initiation for many genotypes of *Musa* belonging to different groups and different levels of ploidy (Table 1). The development of a methodology for the culture of immature female flowers means that genotypes without male buds, such as AAB False Horn types, can also be included. It is interesting to note that the culture methodology was appropriate for all genotypes studied and modifications were not required. Moreover, this technique of initiating embryogenic cell suspensions has proved to be repeatable in several different laboratories.

Every genotype from which more than 20 to 30 male buds have been cultured

Plants of Dominico (AAB French type) from somatic embryos formed by establishment of cell suspensions (primary explant: immature male flowers).

has produced embryogenic callus followed by cell suspensions and plantlet regeneration. For the genotype Col 49, the culture of only two buds led to the formation of two calli and then two embryogenic cell suspensions.

Some cell suspensions have been subcultured for more than two years, during which time they have maintained their embryogenic capacities. In order to simplify cell suspension management, it is possible to store suspension cultures by cryopreservation using a protocol adapted from Panis *et al.* (1990).

It has been demonstrated on various genotypes that this embryogenic cell suspension regeneration technique can be very efficient. The technique using immature male flowers has already been successfully used for the development of a genetic transformation method using particle bombardment. It is also being used in an EU-INCO project, one aim of which is the creation of triploid hybrids by somatic fusion. Finally, this regeneration technique is being tested to evaluate its potential as system for mass propagation. ■

Table 1. Cultivated genotypes with obtained results.

Explants	Type	Genotype	Embryogenic callus formation	Subculture and regeneration of embryogenic cell suspensions
Male flowers	AA	903	✓	✓
		col 49	✓	✓
		SF 265	✓	✓
	AAA	Grand Nain	✓	✓
		Gros Michel	✓	✓
		Yangambi	✓	not tested
	AAB	French Sombre	✓	✓
		Dominico	✓	✓
		Mysore	✓	not tested
		Silk	✓	not tested
	AAAB	FHIA 1	✓	initiation in progress
		FHIA 2	✓	initiation in progress
Female flowers	AAB	Curraré	✓	✓
		Curraré Enano	✓	✓

✓: yes

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Improvement | Improved banana varieties reach Tanzania

Propagation and diffusion of improved banana varieties in the Kagera region

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The most important staple food crop for around 1,000,000 people living in the Kagera region of Tanzania is banana. In recent years yields have declined, mainly as a result of increasing pest and disease attack, declining soil fertility and drought. Severe flooding in some areas, attributed to the El Niño phenomenon, has coumpounded the affects of poor yields in several parts of the region (Figure 1). Poor banana yields are causing increasing food insecurity and declining health and living standards throughout the region.

***Figure 1**. Effects of El Niño*

Due to their weak financial position, farmers are unable to afford the pesticides and fertilizers necessary for im-