



AGROPOLIS
LES DOSSIERS

Expertise of the Agropolis scientific community

Genetic
resources
Genomics
&
Plant
biotechnology



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Dissemination of innovations

From research to applications

Improved seeds and plants are the traditional and most favoured way of transferring genetic advances to producers.

Plant biotechnologies have greatly accelerated and enhanced this transfer. The research groups of the Languedoc-Roussillon region participate actively in the development of these new technologies. They have fine-tuned methods and processes that are available from Agropolis, and some of these have been transferred to the private sector and the countries of the South.

Facilitating the creation of new varieties

The creation of a new variety is time-consuming and costly. Numerous techniques now improve the performance of traditional methods, and some of these are presented below.

• **Haploid methods**

The use of pure lines is often necessary when the goal of the breeding programmes is to create hybrids. Traditional methods need eight to ten years, whereas haploid methods (haploid-diploidization) rapidly yield hybrid varieties by generating pure lines in a single generation. Plants with only half of the genetic make-up are produced by growing reproductive cells – ovules or pollen – whose genetic make-up is

then doubled to restore fertility. For instance, a cross between such pure lines is currently used to produce hybrids of tropical or temperate rice.

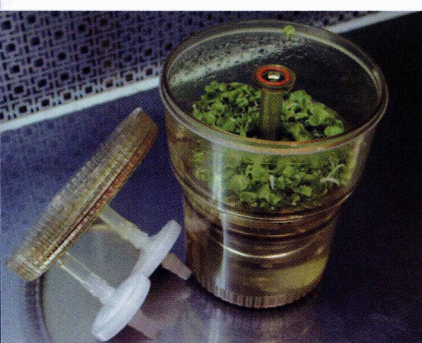
• **Somatic fusions and hybridizations**

The exchange of genetic material between different varieties during reproduction is an important source of variability, but some species hybridize poorly and are therefore difficult to improve by classical methods. Somatic fusions and hybridizations enable mixing in the laboratory of the genetic and cytoplasmic structures of plants by fusing protoplasts, thereby increasing the genetic diversity of certain species. At Agropolis, this technique is used with citrus fruits.

• **Marker-assisted selection**

To ensure that a cultivated hybrid has recovered the gene or genes controlling the agronomic trait of

Marc Lartaud (© Cirad-Amis, Biotrop)



The RITA device with coffee vitroclone

RITA, an apparatus which facilitates in vitro culture

In vitro culture allows routine preparation of thousands of "certified copies" of a plant from a simple tissue fragment from the mother plant. This is achieved through the use of complex mixtures of mineral salts, sugars, amino acids, vitamins, and

immersion recipient). This two-compartment apparatus makes use of the advantages of the liquid medium but does not suffer its drawbacks, since the plant material is only briefly in contact with the liquid. Rita is easy to use because of its design and size and is currently being utilized to micropropagate a large number of plants, notably selected hybrids of *Coffea arabica*.

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growth regulators. Although a liquid medium is considered ideal for the mass production of vitroplants, there are frequent problems of hyperhydricity and of physiological disorder (asphyxia) caused by the presence of residues in the medium. It has long been known that temporary immersion reduces these problems. From 1988, the Biotrop laboratory of Cirad has worked on the use of this technique and the development of a simple, easy-to-use apparatus: Rita (automated temporary

Vitropic: a laboratory for production of vitroplants of tropical species in Languedoc-Roussillon

interest, difficult and often time-consuming field tests were traditionally necessary. Molecular genetics can now be used to identify and locate genes and to follow them during crossing and selection through their association with molecular markers. This technique allows concentration of the most valuable traits and avoids their loss during these operations. This process was first applied to simple characteristics, and is now increasingly used for quantitative traits, which are more difficult to handle.

• Direct transfer of genes by transgenesis

The transfer of an agronomic trait to a hybrid is particularly lengthy and difficult by natural means, but it is now possible to transfer the gene controlling the relevant characteristic directly into the cultivated plant by means of transgenesis.

The first applications of this method are much debated in Europe, yet at this early stage much remains to be discovered. Among the varieties selected, increasing use is made of transfer of a valuable trait to a plant of the same or a similar species. ***

Yvan Mathieu, © Vitropic S.A.



Use of banana vitroclones increases production with less pesticides

Vitropic S.A., a subsidiary of Cirad, is a laboratory at St Mathieu de Tréviers near Montpellier which produces vitroplants (plants obtained by in vitro culture). It was set up in 1986 and now has an annual turnover of 1 million US\$, 15 employees, and produces two to three million vitroplants a year, especially of banana, which are sent mainly to the West Indies and Africa. Vitropic is a leader in these foreign markets and its plantlets production site is one of the more important in the world for banana.

An example of how biotechnology improves production

The production of vitroplants does not involve genetic transformation techniques but rather tissue culture.

Plants selected for their agronomic traits are multiplied rapidly in test tubes. The miniature banana plantlets produced under the protected laboratory conditions are disease-free and are shipped from Montpellier to the production zones. Their utilization as planting material enhances production, since vitroplants give greater yields with less use of pesticide when grown in soil free of nematodes (soil parasites). In the case of banana, vitroplants help promoting a more environmentally-friendly agriculture.

The need for constant innovation

To increase its know-how and to improve and diversify its products, Vitropic must innovate constantly and invest in research and development. Agropolis, and in particular Cirad, provide Vitropic with a favourable environment and expert scientific and technical partners in the pursuit of its goals. Vitropic operates mostly in the Languedoc-Roussillon region, but is also developing collaborations with numerous partners from countries of the South.

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*Multiplying the most interesting
palm tree in large numbers to fix
useful agronomical
characteristics*

In vitro culture to produce true copies of oil palm

The oil palm is the major source of vegetable fat in the humid intertropical zone. With an annual output of ca. 20 millions tons, it accounts for 20% of world oilseed production. Conventional techniques of vegetative propagation (cuttings, shoots, grafts) do not exist for this plant with its obligate cross-pollination and seed-only propagation. The mode of reproduction and the time needed for genetic improvement lead to substantial heterogeneity in the plants grown from selected seeds available to growers. Because of this, the most useful agronomic traits can only be fixed by means of controlled artificial vegetative propagation using cell biology techniques

ensuring large-scale production of true copies of reproduction of the most valuable plants. Large-scale multiplication and regeneration using somatic embryogenesis have been developed since the early 1980s in collaboration between IRD and Cirad, and have been transferred to the main producing countries (Côte d'Ivoire, Malaysia, Indonesia) for pilot testing. Several hundred thousand oil palm plantlets regenerated in vitro have been planted, and various clones have been identified, leading to a 20 to 30% yield increase, thus underscoring the value of this approach. We have also noted the existence of sterile, off-types or variants, resulting from regeneration. This phenomenon is known as somaclonal variation and is now being studied in depth in tandem with research teams from Great Britain and Malaysia, using molecular approaches based on cutting-edge techniques available at Agropolis, notably through the Montpellier Génopole (see page 16).

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Multiplication techniques

In vitro culture of plants in a sterile nutrient medium is a longstanding and widespread technique in microcutting and micrografting. Other techniques are being improved, including:

• Embryo rescue

Similar but poorly compatible species may sometimes be crossed, but the resulting embryo is often unviable or does not develop beyond a certain stage. Yet this type of cross would improve cultivated varieties. Embryo rescue allows the production of hybrids from the first cells of a cross between "incompatible" species. Embryos can be rescued at an early stage, soon after fertilization, cultivated and thus saved. This technique has been used with citrus fruits and rice.

• Somatic embryogenesis

This form of vegetative propagation yields a large number of plantlets genetically identical to the mother plant that gave the explants. It can be used to reproduce millions of identical copies of a plant from a fragment of leaf or stem. This is a highly promising technique as it allows enormous multiplication rates. It is also usable in transgenic systems. It is especially useful when the plant does not naturally propagate vegetatively (oil palm, coconut palm...). Despite certain technical difficulties, hundreds of thousands of palm trees, hevea trees, cotton plants and banana plantlets have been produced by this procedure at Montpellier.

Appropriate and officially approved new equipment for all these techniques is needed, and has been invented or adapted by the research teams at Agropolis. An example is RITA (see page 32 "*Rita, an apparatus which facilitates in vitro culture*"), which was designed to improve the

growth of in vitro cultures. Other equipment has been developed in collaboration with small- and medium-sized local companies, such as confinement greenhouses and culture rooms, several of which have been built in Africa and Asia. Agropolis member institutions possess considerable know-how in this area, particularly regarding in vitro cultures, which have a wide range of potential applications. ■
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