Integration of cryopreservation in French plant genetic resource collections: the CRYOVEG project

Florent Engelmann[1, 2], Emilie Balsemin[3], Teresa Barreneche[3], Philippe Chatelet[4], Jean-Eric Chauvin[5], Emmanuel Couturon[6], Franck Curk[7], Marie-Ange Dantec[5], Jean-Paul Dantec[5], Thibault Decourcelles[3], Stéphane Dussert[1], Laurence Feugey[8], Yann Froelicher[7], Lydie Fouilhaux[3], Franciane Gamiette[9], Agnès Grapin[10], Michel Grisoni[11], Philippe Guérif[8], Arnaud Guyader[8], Alain Label[5], François Luro[7], Bernard Moulin[5], Martine Muller[5], André Peyrière[4], Yvon Prigent[5], Michel Renard[5], Michel Roux-Cuvelier[11], Danièle Roques[12], Suzia Rubens[9], Jocelyne Sapotille[12], Catherine Souchet[5], Isabelle Sylvestre[1], David Teyssedre[11]

1 IRD, 911 avenue Agropolis, BP 64501, 34394 Montpellier cedex, France.
2 Bioversity International, Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Rome, Italy.
3 INRA, Domaine de la Grande-Ferrade, BP 8133883, Villenave d'Ornon cedex, France.
4 INRA UMR AGAP, Equipe DAVEM, 2 place Viala, 34060 Montpellier, France.
5 INR INRA, Domaine de Keraïber, 29260 Ploudaniel, France.
6 IRD Réunion, Parc Technologique Universitaire, 2 rue Joseph Wetzell, BP 50172, 97492 Sainte Clotilde cedex, France.
7 INRA-CIRAD, 20230 San Giuliano, France.
8 INRA, 42 rue Georges Morel BP 6005749071 Beaucouzé cedex, France.
9 INRA, Domaine Duclos, 97170 Petit Bourg, France.
10 AGROCAMPUS OUEST – Angers, Institut National d'Horticulture et de Paysage UMR GENHORT (INHP/INRA/UA), 2, rue Le Nôtre - 49045 Angers, France.
11 CIRAD, Station de Ligne-Paradis, Pôle de Protection des Plantes - 3P, 7 chemin de l'IRAT, 97410 Saint-Pierre, France.
12 CIRAD, Station de Roujol, 97170 Petit Bourg, France.

1. Introduction

Cryopreservation (liquid nitrogen, -196 °C) is currently the only technique ensuring long-term, safe and cost-effective conservation of vegetatively propagated plants and of non-orthodox seed species (Engelmann 2011). In France, cryopreservation is presently used only for the conservation of genetic resources of a limited number of forestry species but it is not used for food crops. With the aim of improving the long-term safety and security of national plant germplasm collections through the increased utilisation of cryopreservation, the Consultative Committee for Biological Resources / Infrastructures in the Biology, Health and Agronomy sectors (CCRB/IBiSA) opened in 2008 the Call for Projects "Biological Resource Centers" and funded the CRYOVEG (Cryopreservation of French plant genetic resources collections) project, which had been submitted to this call by a group of French researchers and curators of plant germplasm collections.

The CRYOVEG project aims at 1) developing or optimizing cryopreservation techniques in a range of selected species; 2) establishing a national scientific and technical network of plant biological resource centers (BRCs) using cryopreservation. The project has a network organization, with IRD/INRA Montpellier as the cryopreservation expertise centre and partners in continental France and overseas departments in charge of genetic resource conservation for various species: INRA Petit Bourg, Guadeloupe (yam); INRA San Giuliano, Corsica (*Citrus*); INRA Bordeaux (*Prunus*); INRA Angers (apple and pear); INRA Montpellier (grapevine); INRA Ploudaniel (potato, *Brassica*); IRD La Réunion (coffee); CIRAD Roujol, Guadeloupe (sugarcane); and CIRAD La Réunion (vanilla, garlic).

The project started in September 2009. After a launching meeting held in IRD in October 2009, participants from all BRCs involved performed a training period in Montpellier on cryopreservation of seeds, *in vitro* shoot tips and/or dormant buds, depending on their species of interest, and then implemented the experimental programme established with Montpellier colleagues in their respective laboratories. Several participants also benefited from STSMs funded by COST Action 871, which allowed them to receive additional training in laboratories of European partners. In this paper, we present a brief summary of the results obtained by project participants regarding cryopreservation of dormant buds, *in vitro* cultures and seeds during the first year of the project.

2. Results

Dormant buds

Apple and pear: For the first set of experiments, the cryopreservation technique developed by the NCGRP (National Centre for Genetic Resources Preservation, Fort Collins, USA) was employed. A total of 15 *Malus* and 15 *Pyrus* accessions have been tested. With apple, the mean regeneration percentage was 32 %, with results varying between 0 and 78 %, depending on the accession. With pear, the mean regeneration percentage was 28 %, with results between 0 and 92 %. These results are extremely satisfactory, particularly with, pear, which is considered very difficult to cryopreserve.

Prunus: The experiments have been performed with sweet (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.), using the protocol established by Towill and Forsline (1999). Dormant buds of five *Prunus* genotypes were first dehydrated to various moisture contents, cooled slowly or rapidly and regenerated either through direct grafting on rootstocks or through *in vitro* culture of apices extracted from rehydrated dormant buds. Until now only a limited percentage of regeneration has been obtained, only from *in vitro* cultured apices.

Grape: Experiments were performed with dormant buds of the variety 'Muscat', using various combinations of dehydration (slow or rapid) and cooling (slow or rapid) methods. After rewarming and rehydration, apices were extracted from the buds and introduced *in vitro*. Only apices sampled from buds dehydrated to 25 % moisture content and cooled slowly showed signs of regrowth. This may indicate that there is a difference in the reactivity of shoot tips, depending on the dehydration and cooling procedure.

Citrus: preliminary experiments were performed with *Poncirus* dormant buds. No regrowth has been obtained yet after cryopreservation.

In vitro cultures

Potato: Experiments focused on several parameters of the encapsulation-dehydration technique, including the size of the apices used for cryopreservation, pre-treatment of mother-plants and composition of regeneration medium. The optimal stage of development of shoot tips was between "open leaf primordia" and "closed leaf primordia". As regards pretreatment, there was no positive effect on post-cryopreservation recovery of sampling shoot tips on single node cultures. A culture of mother-plants on medium with high sucrose content had different effects on recovery, depending on the cultivar. Finally, recovery was generally better on medium containing Tendille and Lecerf (1974) mineral elements.

Sugarcane: the encapsulation-dehydration technique was tested on two sugarcane varieties. Recovery of cryopreserved shoot tips varied between 25-54 % for one variety and between 10-30 % for the other.

Yam: the encapsulation-dehydration and droplet-vitrification were compared using *in vitro* shoot tips of one yam variety. Survival was higher with encapsulation-dehydration, reaching

30 %, and less than 20 % with droplet-vitrification, due to the high toxicity of the vitrification solutions employed.
Garlic and vanilla: positive results were achieved with garlic during an initial training period in IPK, Germany. However no positive results were obtained during additional experiments performed in Réunion island, because the plant material employed was not at the right physiological stage. Only preliminary results were performed with vanilla, which did not produce positive results.

Seeds
Traditional vegetable species from Réunion Island: seeds of accessions belonging to various families including Fabaceae, Cucurbitaceae and Solanaceae and of several maize accessions could be successfully cryopreserved using the protocol developed by Dussert *et al.* (1997) for coffee seeds.
Citrus: seeds of 33 varieties belonging to three genera and 14 species were cryopreserved. The materials tested displayed different degrees of tolerance to desiccation and cryopreservation, with some species showing high seed germination after cryopreservation (>80%), others intermediate germination (40-70 %), and others low germination (<18 %).
Brassica: seeds of 17 *Brassica* varieties were employed for cryopreservation experiments. Germination of cryopreserved seeds could be achieved with all materials tested, after partial desiccation of seeds using saturated salt solutions and slow or rapid cooling.
Coffee: seeds of 138 accessions belonging to four species, *C. arabica* (117 accessions), *C. pseudozanguebariae* (5), *C. costatifructa* (6) and *C. racemosa* (5) were cryopreserved. The results showed that processing of the seeds after harvest (moisture content, duration of storage) is of critical importance. They also indicated that it is possible to establish a cryobank of *C. arabica* and of a range of wild coffee species.

3. Conclusion
Very encouraging results have already been achieved during the first year of the project. The experimental programme of all participants for the second year of the project has been established, which should lead to improved results at the end of the project. The establishment of cryopreserved collections in a near future appears as a foreseeable reality for many species included in the CRYOVEG project.

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