First results on cryopreservation by dormant bud technique of a set of *Malus* and *Pyrus* cultivars from the INRA Biological Resources Centre

Arnaud Guyader[1], Rémi Guisnel [1], Fabienne Simonneau [1], Bernard Rocand [2], Camille Le Bras [3], Agnès Grapin [3], Philippe Chatelet [4], Stéphane Dussert [5], Florent Engelmann [5], Laurence Feugey [1]

[1] INRA, UMR GenHort, 42 rue Georges Morel, BP 60057, 49071 Beaucouzé cedex - France
[2] INRA, UE Horti, 42 rue Georges Morel, BP 60057, 49071 Beaucouzé cedex - France
[3] AGROCAMPUS OUEST, UMR GenHort, 2 rue André Le Nôtre, 49045 Angers cedex 01 - France
[4] INRA, UMR AGAP, 2 place Viala, 34060 Montpellier cedex 1 - France
[5] IRD, UMR DIADE, 911 avenue Agropolis, 34394 Montpellier cedex 5 - France

1. Introduction

The Pip Fruit Biological Resources Centre of INRA (National Institute for Agricultural Research) located in Angers (France) is in charge of the preservation, management, characterization and promotion of traditional and scientific genetic resources of apple (8,493 accessions), pear (825 accessions), quince (64 accessions) and related species. The creation of a cryobank should allow us to optimize the security of long term preservation of these germplasm collections.

The development of a cryopreservation program for apple and pear germplasm was initiated in 2010 in the UMR GenHort (Angers) thanks to our participation in the national project CRYOVEG, funded by the French public body IBISA (Biology Infrastructure Health and Agronomy), which aims at developing or optimizing cryopreservation techniques for different plant species maintained in French Biological Resources Centres, and at establishing at the national level a scientific and technical cryopreservation network.

Two training periods in USDA NCGRP, Fort Collins USA (25th January to 29th January 2010) and in JKI Dresden Germany (1st February to 5th February 2010) funded by IBISA and COST respectively allowed us to improve our skills and knowledge about this technique.

The aims of this project were two-fold:

- to validate the reference protocol in our experimental environment (equipment, plant material and climatic aspects),
- to evaluate the response of different genotypes of our germplasm collections.

2. Materials and Methods

2.1. Plant material

The plant material used was a set of diverse genotypes of *Malus* and *Pyrus* from our germplasm collections:

- 15 *Malus* varieties: ancient (11) and modern (2) varieties of dessert apple, ancient varieties of cider apple (2).
- 15 *Pyrus* varieties: ancient varieties of European pear (12), varieties of nashi (3).

2.2. Cryopreservation protocol employed at INRA UMR Genhort

The experimental protocol used in Angers was adapted from reference protocols developed in USDA NCGRP, Fort Collins (Towill *et al*, 2004; Towill and Ellis, 2008). The different steps of protocol were:

- Sampling of graftwoods: the budsticks were harvested in January 2010 in cold conditions (-3 °C). Three consecutive days of negative temperatures before harvesting were observed, which corresponds to the optimal conditions for the reference protocol.
- Storage of graftwoods at -0.5 °C in airtight bag during 4 to 12 weeks.
- Preparing of samples: single nodal sections, 3.5 cm long, with the bud in central position were prepared from budsticks at -0.5 °C.
- Desiccation of nodal sections at -5 °C in an incubator (Sanyo ® MIR 254). The objective was to reach 30 % moisture content (fresh weight basis).
- Slow cooling: Sections were packaged in plastic tubes for slow cooling in a climatic chamber (Binder ® MK53) at 1 °C/h to -30 °C with an additional step at -30 °C for 24 h.
- Storage in plastic tubes in the vapour phase over liquid nitrogen in a liquid nitrogen freezer (Taylor-Wharton ® 750 RS) for 72 h.
- Slow rewarming in plastic tubes at +3 °C for 24 h and rehydration in plastic bags filled with moist peat moss at +3 °C for 8 to 15 days, depending on the variety.
- Regeneration: grafting of the buds by the chip budding technique on MM106 rootstocks for apple and Kirschensaller rootstocks for pear. For each test date and variety, at least 12 buds from cryopreserved material and 6 buds from fresh material were grafted. The rootstocks were planted in pots in greenhouse for 4 to 6 weeks before grafting.

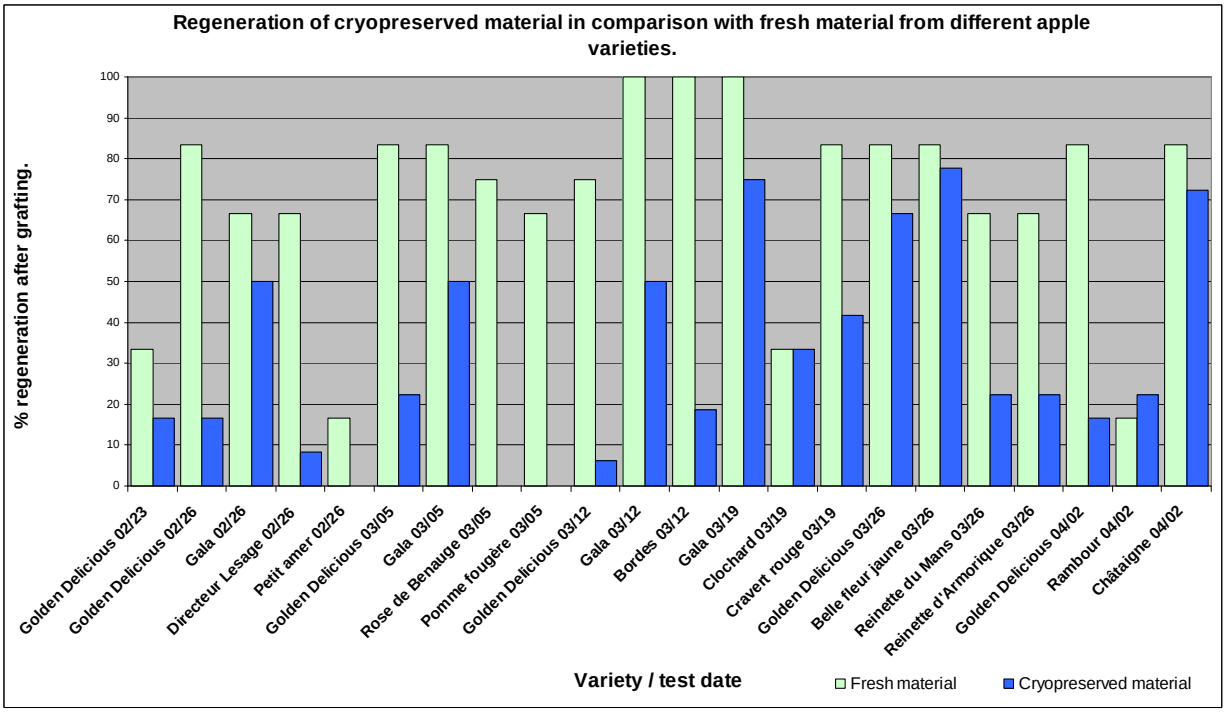

Figure. 1: Regeneration of cryopreserved material in comparison with fresh material from different apple varieties (percentages were calculated from at least 12 buds from cryopreserved material and 6 buds from fresh material).

3. Results

Considering all the experiments, the average regeneration percentage after grafting of fresh material was 69.6 % for apple and 54.6 % for pear, while the average regeneration percentage after grafting of cryopreserved material was 31.8 % for apple and 26.9 % for pear.

For apple (Figure. 1), the regeneration percentages of cryopreserved material ranged from 0 to 77.8 %. Three genotypes ('Petit amer', 'Pomme Fougère', 'Rose de Benauge') did not respond to the technique. When using the same protocol in different series of tests at different dates, regeneration after cryopreservation fluctuated between 6.3 % and 66.7 % for 'Golden Delicious' and between 50.0 % and 75.0 % for 'Gala'.

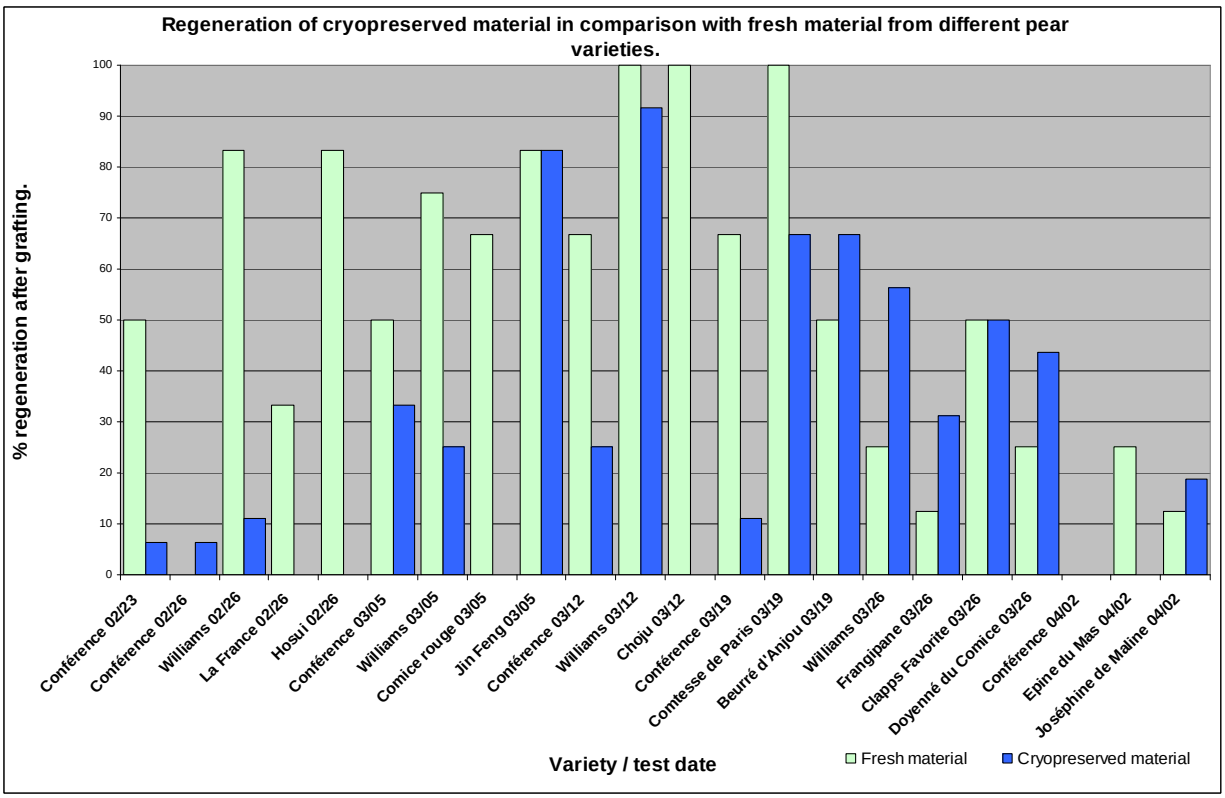

Figure. 2: Regeneration of cryopreserved material in comparison with fresh material from different pear varieties (percentages were calculated from at least 12 buds from cryopreserved material and 6 buds from fresh material).

For pear (Figure. 2), the regeneration percentages of cryopreserved material ranged from 0 to 91.7 %. Five genotypes ('La France', 'Hosui', 'Comice rouge', 'Choju', 'Epine du Mas') did not respond to the technique. When using the same protocol in different series of tests at different dates, regeneration after cryopreservation fluctuated between 11.1 % to 91.7 % for 'Williams'.

4. Discussion

The dormant bud cryopreservation protocol could be successfully applied in our experimental conditions and on our plant material, both with *Pyrus* and *Malus*. These results are very encouraging, especially for *Pyrus* which is reportedly more recalcitrant to the method. However, the current protocol does not yet guarantee a satisfactory regeneration percentage for all genotypes, nor a satisfactory reproducibility of the results for a given genotype. Several factors, which seem to significantly influence the results have been identified: bud

morphotypes, rehydration phase (technique used and duration), rootstock calibre, grafting technique, etc. In 2011, the key points which need to be further examined are the optimal bud residual moisture content, the slow cooling, rewarming and rehydration phases, as well as some technical questions related to grafting.

5. Acknowledgements

André Peyrière, INRA UMR AGAP Montpellier, France; Gayle Volk, Remi Bonnart, John Waddell, USDA NCGRP Fort Collins, Colorado, USA; Monika Höfer, JKI Dresden-Pillnitz, Germany.

6. References

Towill LE, Forsline PL, Walters C, Waddell JW, Laufmann J (2004) Cryopreservation of *Malus* Germplasm using a winter vegetative bud method: results from 1915 accessions. CryoLetters 25:323-334

Towill LE, Ellis DD (2008) Cryopreservation of dormant buds, *in* Plant Cryopreservation, a Practical Guide, Reed BM (ed) Springer, USA, pp. 421-435

Guyader A., Guisnel R., Simmoneau F., Rocand B., Le Bras C., Grapin A., Chatelet P., Dussert Stéphane, Engelmann Florent, Feugey L., Engelmann Florent (2011)

First results on cryopreservation by dormant bud technique of a set of Malus and Pyrus cultivars from the INRA biological resources centre

In : Grapin A. (ed.), Keller E.R.J. (ed.), Lynch P.T. (ed.), Panis B., Revilla Bahillo A. (ed.), Engelmann Florent (ed.). Cryopreservation of crop species in Europe : COST Action 871 : CryoPlanet : proceedings of the final meeting

Bruxelles (BEL) ; Luxembourg : COST Office ; European Union, 141-144

COST Action 871 : CryoPlanet : Cryopreservation of Crop Species in Europe : Final Meeting, Angers (FRA), 2011/02/08-11

ISBN 978-92-898-0051-8