Cryopreservation of apple *in vitro* germplasm in China

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Introduction

The Changli Institute of Pomology initiated in the 1980s the establishment of an *in vitro* collection of apple germplasm, in order to progressively duplicate their field collection. Today, 147 cultivars belonging to seven apple species are conserved *in vitro* under slow growth. The intervals between subcultures at standard temperature have been extended from 3–4 months to 10–12 months by increasing the sucrose concentration and by adding mannitol or abscisic acid in the medium.

Cryopreservation research started eight years ago in the institute, with the aim to establish freezing protocols which could be used for the long-term storage of the germplasm collections of apple and other fruit trees maintained by the institute.

Results and discussion

Four different methods were experimented for cryopreserving apple *in vitro* shoot-tips.

Two-step freezing (Chang et al. *1992)*

The experiments showed that the cryoprotective treatment, the prefreezing temperature and the freezing rate had an effect on the survival rate of shoot-tips after cryopreservation. By using a combination of 5% DMSO + 5% glycerol for cryoprotection, prefreezing apices at 0.2°C/min from 0 to –40°C before immersion in liquid nitrogen, the survival rates achieved were around 53% (Table 1).

Table 1. Regrowth rate (%) of control (–LN) and cryopreserved (+LN) apices of four apple cultivars frozen using four different cryopreservation techniques (two-step freezing; vitrification; encapsulation-dehydration; droplet freezing)

	Regrowth (%)							
	Two-step		Vitrification		Encaps.		Droplet freezing	
Cultivar	–LN	+LN	–LN	+LN	–LN	+LN	–LN	+LN
Jonagold	29	20	75	53	100	66	98	70
Baleng Haitang	100	57	100	81	100	69	100	88
Fuji	75	69	100	58	100	67	100	76
Jinbiao	83	67	100	88	100	75	95	87
Average	72	53	94	70	100	69	98	80

Vitrification (Zhao et al. *1994)*

The critical factor was the choice of the vitrification solution. Only the PVS3 vitrification solution (Nishiwaza *et al.* 1993) allowed survival with all cultivars tested. The duration of preculture and pretreatment could also influence the survival rate. However, washing explants with a 1.2M sucrose solution after rewarming did not increase survival, in contrast with what is generally observed with most species (Engelmann 1997).

Encapsulation-dehydration (Wu et al. *1998)*

The water content of beads was the most important parameter to control in order to achieve high survival. When the bead water content was 60%, the survival rate was around 10% only, while it increased to 66–75% when water content was around 30%.

Droplet freezing (Zhao et al. *1998)*

Preculturing apices on culture medium supplemented with DMSO (0.5–5%) increased survival after cryopreservation. Survival of cryopreserved apices was achieved only when a slow, two-step freezing protocol was employed.

Whatever the cryopreservation technique employed, cold-hardening mother-plants for 3 weeks at 5°C had a positive effect on survival. It was also observed that using apices sampled on mother-plants which had not been subcultured for long periods (up to 26 weeks) drastically increased survival after cryopreservation. This could be related to the lower water content of apices after an extended period without subculture.

The cryopreservation method used had an effect on the regrowth pattern and speed of shoot-tips. Callusing was always observed with two-step freezing and vitrification. Callusing was lowest with the encapsulation-dehydration method. The speed of regrowth of cryopreserved apices was higher using encapsulation-dehydration than with two-step and droplet freezing.

Based on all the observations performed during the above experiments, the encapsulation-dehydration technique has been selected for routine cryopreservation of apple germplasm in the Changli Institute of Pomology.

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