Cryopreservation of pineapple (*Ananas comosus*) apices by vitrification

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Introduction

Pineapple is a fruit crop of major importance in many tropical countries. Pineapple is vegetatively propagated and crosses between varieties produce botanical seeds. However, these seeds are highly heterozygous and therefore of limited interest for the conservation of specific gene combinations.

Cryopreservation of apices is the most relevant strategy for long-term conservation of vegetatively propagated crops. The freezing methods employed are principally encapsulation-dehydration and vitrification, which do not require sophisticated equipment for freezing and produce high recovery rates with a wide range of materials (Engelmann 1997).

As reported in this paper, vitrification and encapsulation-dehydration were used to freeze apices of *in vitro* plantlets of pineapple. The most efficient protocol was applied to apices of three different varieties.

Materials and methods

The plant material consisted of apices sampled on *in vitro* plantlets of three pineapple [*Ananas comosus* (Stickm.) Merr.] varieties (Puerto Rico, Perolera and Smooth Cayenne). Plantlets were subcultured every 30 d and apices (up to 3 mm in length) dissected 15 d after the last subculture.

For cryopreservation experiments, the encapsulation-dehydration technique and the vitrification procedure were applied. The experimental approach has been described in detail by González–Arnao *et al.* (1998).

Results and discussion

Encapsulation-dehydration did not allow successful cryopreservation of pineapple apices under the conditions tested. These negative results can be related to the high sensitivity of pineapple apices to sucrose and dehydration. Indeed, pregrowth in media with sucrose concentrations higher than 0.5M was detrimental to survival and a prolonged treatment in 0.5M sucrose was required to improve survival after desiccation. The viability loss observed after freezing may be due to the crystallization of remaining intracellular freezable water upon freezing. This detrimental effect might be avoided by slowly cooling the encapsulated apices to allow freeze-induced dehydration to take place. Several plant species such as potato, grape and citrus cryopreserved by the encapsulation-dehydration technique have required a slow freezing regime to achieve optimal survival (Fabre and Dereuddre 1990; Plessis *et al.* 1993; González–Arnao *et al.* 1998).

In contrast, survival of pineapple apices after cryopreservation was achieved using the vitrification technique, which has been successfully employed for freezing apices of a large number of different crops (Niino *et al.* 1992a, 1992b; Matsumoto *et al.* 1994; Kuranuki and Sakai 1995; Tagaki *et al.* 1997). Optimal conditions for vitrification of pineapple apices included a 2-d preculture on semi-solid MS medium supplemented with 0.3M sucrose, loading treatment for 25 min in medium with 0.75M sucrose + 1M glycerol and dehydration at 0°C for 7 h with the PVS2 vitrification soultion before rapid immersion in liquid nitrogen.

The originality of the protocol developed with pineapple apices was the extended duration (7 h) of treatment with PVS2 required to achieve optimal survival of apices after freezing, in comparison with the much shorter optimal durations (around 1–1.5 h) suggested for most materials (Sakai *et al.* 1990; Matsumoto *et al.* 1994). This result is certainly due to the large size and compact structure of the pineapple apices employed in our experiments: the apices were around 3 mm long, and the apical dome was tightly covered by 2–3 leaf primordia with a very thick cuticle. Extended treatment durations were therefore needed for the vitrification solution to sufficiently dehydrate these very compact structures.

The vitrification protocol developed was sucessfully employed for cryopreserving apices of a total of eight pineapple varieties (Table 1).

Table 1. Effect of vitrification protocol on survival of apices from eight different pineapple varieties before (–LN) and after (+LN) cryopreservation. After loading with 1M glycerol and 0.75M sucrose for 25 min, apices were treated with PVS2 solution at 0°C for 7 h before freezing. (Reprinted from Gonzalez–Arnao *et al.* 1998, with permission).

	Survival (%)	
Variety	**–LN**	**+LN**
Puerto Rico	80	65
Perolera	50	35
Smooth Cayenne	50	25
Cabezona	63	30
Piña Blanca	60	27
P3R5	53	20
Bromelia	33	10
Española Roja	47	13

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