Cryopreservation of *Ajania pacifica* (Nakai) Bremer et Humphries via encapsulation-dehydration technique

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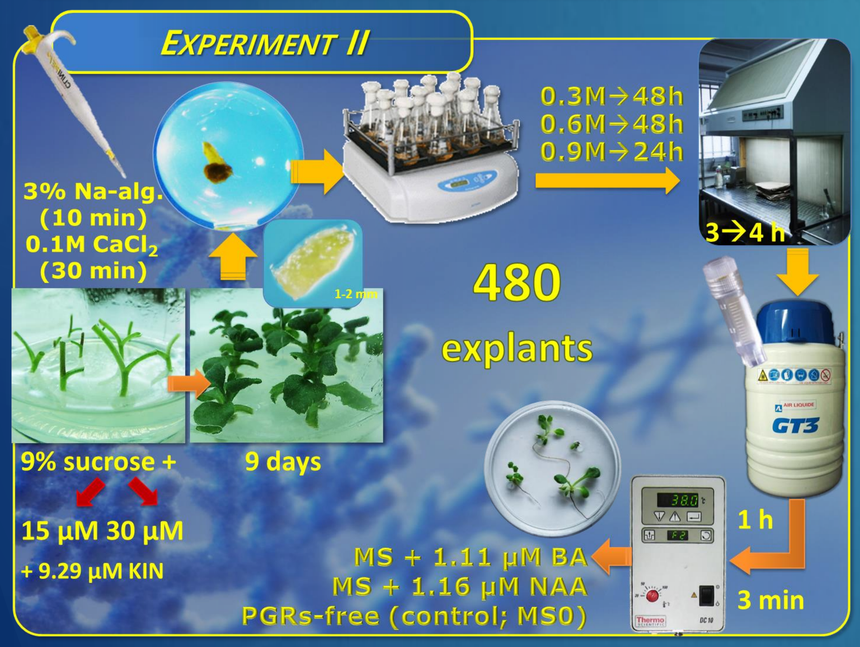
Introduction

Ajania pacifica, a novelty on the horticultural market, is valued both as an ornamental and a medicinal plant. Establishment of numerous breeding programs led to the creation of many cultivars. Therefore, it is important to develop efficient storage methods of the species. **The aim of this study was to develop an encapsulation-dehydration cryopreservation protocol of *Ajania pacifica* shoot tips.**

Materials and methods

Shoot tips of ajania 'Bengo' were precultured on media with different sucrose and ABA concentrations, encapsulated in 3% sodium alginate and dehydrated osmotically. Subsequently, the beads were desiccated in sterile air flow for various periods and immersed in liquid nitrogen. After thawing the explants were inoculated on various recovery media.



Results

Higher (9%) sucrose concentration and addition of ABA (15 µM) during preculture, followed by 4-hour desiccation, as well as, application of cytokinins in the *post*-thawing recovery medium were necessary to provide high survival of ajania 'Bengo' shoot tips.

Table 1. Survival [%] of ajania 'Bengo' shoot tips 7 days after thawing.

Sucrose cp [%]	Recovery medium			Mean
	MS0	BA+ NAA	KIN+ NAA	
3	0.0 c*	5.4 c	1.8 c	**2.4 b**
6	0.0 c	0.0 c	0.0 c	**0.0 b**
9	3.3 c	32.5 b	56.2 a	**30.7 a**
Mean	**1.1 b**	**12.6 a**	**19.3 a**	

Table 2. Regrowth capacity [%] of ajania 'Bengo' shoot tips 90 days after thawing.

Sucrose cp [%]	Recovery medium			Mean
	MS0	BA+ NAA	KIN+ NAA	
3	0.0 b*	0.0 b	0.0 b	**0.0 b**
6	0.0 b	0.0 b	0.0 b	**0.0 b**
9	0.0 b	0.0 b	8.3 a	**2.8 a**
Mean	**0.0 b**	**0.0 b**	**2.8 a**	

Table 3. Influence of ABA concentration [µM] during preculture, desiccation period [h] and the recovery medium composition on the survival [%] and regeneration [%] of *Ajania pacifica* 'Bengo' shoot tips 7 and 90 days after thawing, respectively.

Recovery medium	15 µM ABA				30 µM ABA			
	3-hour desiccation		4-hour desiccation		3-hour desiccation		4-hour desiccation	
	Surv.	Reg.	Surv.	Reg.	Surv.	Reg.	Surv.	Reg.
MS0	30.6 a*	10.0 c	15.6 a	0.0 c	10.0 a	0.0 c	24.7 a	0.0 c
BA	40.0 a	40.0 bc	50.0 a	55.0 b	15.0 a	0.0 c	50.0 a	75.0 a
KIN	52.3 a	57.3 b	50.0 a	77.8 a	10.0 a	45.0 b	12.5 a	30.0 bc

*means marked with the same letter do not differ significantly at $P = 0.05$; according to the Newman-Keuls test; surv. – survival; reg. - regeneration

Shoot tips 10 days after thawing (1 bar = 1 mm)

Fig. A-C. Longitudal section through shoot tips of ajania 'Bengo' – control (A), five (B) and ten (C) days after thawing. A1 – ultrastructure of control L1 histogen layer. A2 – ultrastructure of control L2 and L3 layers. B1 – ultrastructure of entirely viable cells of tunica (L1 and L2) 5 days after thawing. B2 – corpus cells with visible inclusions in vacuoles. C1-C2 – viable leaf cells 10 days after thawing. CW - cell wall, K - cuticle, Mt - mitochondria, N - nucleus, Nu - nucleolus, Pd - plastid, V - vacuole (TEM magnification A1: 2000×, A2-C2: 1000×).