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Research Note

Cryopreservation of seeds and embryonic axes of wild apricot (*Prunus armeniaca* L.)

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Summary

Wild apricot (*Prunus armeniaca* L.) is an economically important fruit crop with rich germplasm variability existing in the high altitude and cold desert areas of India. Due to the vast economic importance of this fruit species and availability of diverse genetic resources, there is an urgent need to collect, characterize and conserve the existing genetic variability of apricot for safe protection and utilization. A cryopreservation protocol for seeds, half seeds and embryonic axes has been successfully devised using desiccation-followed by fast-freezing with high recovery growth percentages. Whole seeds showed viability in the range of 82.5 to 92.5%, half seeds from 85 to 100% and embryonic axes from 98.5 to 100%. The excised embryonic axes showed the highest values of germination in both unfrozen and frozen samples. A total of 250 accessions belonging to 28 folk cultivars have been successfully cryostored as a base collection in the National Cryogenebank at NBPGR, New Delhi, India. The conserved germplasm represents the invaluable variability existing in both cultivated and wild populations of apricot present in India.

Experimental and discussion

Wild and cultivated apricot (*Prunus armeniaca* L.) belonging to family Rosaceae is an important temperate fruit crop of India. The seed storage behaviour and germination characteristics of *Prunus* species has been described by Ellis and Hong (1985) and seeds have been designated as intermediate. Seeds of this category although they tolerate desiccation do not store well at subzero temperatures (de Boucaud *et al*., 2002). The genetic diversity conservation of *Prunus* species has been largely attempted via *ex situ* conservation as *in situ* and field genebank conservation strategies are costly and lack appropriate methods (Chaudhury and Chandel, 1995 and de Boucaud *et al*., 1996). Cryopreservation of such species, using seeds and embryonic axes, has been found to be a cost effective and reliable method of genetic resources conservation (Chandel *et al*., 1995 and Engelmann, 2000). To date cryopreservation studies have been limited to two species of *Prunus*; Chaudhury and Chandel (1995) successfully cryopreserved embryonic axes of *Purnus amygdalus* and de Boucaud *et al.* (1996) worked on *Prunus persica*.

In large seeded non-orthodox fruit species excised embryonic axes being an organized structure and small in size are ideal cryo-propagules exhibiting high recovery growth after cryopreservation (Chandel *et al.,* 1995, Cho *et al*, 2002 and Malik and Chaudhury, 2006). In *Prunus* species removal of the endocarp has been reported to break dormancy and increase germination (Ellis *et al*., 1985). Mechanical removal of the endocarp and part of the cotyledons in *Prunus* species avoids the need for any type of scarification treatment (Garcia-Gusano *et al*., 2004). In the present investigation cryopreservation of seeds, half seeds and embryonic axes of *Prunus armeniaca* has been undertaken. Using the developed protocol collected diversity has been successfully cryopreserved as a base collection in the National Cryogenebank at NBPGR, New Delhi.

Fruits of wild apricot were collected during various exploration missions in the high altitude areas of Himachal Pradesh and Jammu and Kashmir states of India. Whole fruits or stones were brought to the laboratory at NBPGR, New Delhi for various cryopreservation experiments and cryobanking. Whole fruits were depulped and stones were cracked to collect seeds (kernel). Moisture content and viability of seeds was measured before processing for cryostorage. Moisture content of seeds was determined gravimetrically using low constant temperature oven method of 17h drying at 103 ± 2°C temperature (ISTA, 1985). Moisture content was obtained from 2-4 independent determinations using parts from 5-10 seeds each and expressed on fresh weight basis.

For viability testing the seeds were surface decontaminated by treating with 1% NaOCl (Sodium hypo-chlorite) for 10 min followed by three rinses in sterile deionised water before placing for germination. Investigations were carried out to determine a suitable pretreatment and germination method for evaluation of seed viability. In view of the requirement of stratification (pre-chilling) for expressing full germination potential of apricot seeds, in present studies stratification of seeds for 20 days at 4°C was conducted before germination. Three explants were used for germination testing these were whole seeds, half seeds with embryonic axes and excised embryonic axes. The whole seeds and half seeds were germinated in rolled paper towels (between paper). The material was maintained at 25 ± 2°C in a BOD incubator with 16/8h-light/dark photoperiod. Germination was scored as the extrusion of the radical. Embryonic axes were excised aseptically from the surface decontaminated seeds in the laminar air flow cabinet. The embryonic axes, fresh as well as after cryopreservation, were cultured on Murashige and Skoog's medium (1962) supplemented with 1g l^{-1} activated charcoal, 0.17 gl^{-1} NaH_2PO_4, 1mgl^{-1} each of 6-Benzylaminopurine (BAP) and α-Naphthalene acetic acid (NAA) as defined by Chin *et al.* (1988). Cultures were maintained at 25 ± 2°C with a 16 h photoperiod under light intensity of 35 μEm^{-2} s^{-1}.

Germplasm accessions which showed satisfactory germination (above 80% germinability) were further processed for cryostorage. Germplasm of wild apricot was cryostored in the form of whole seed and as half seeds. Half seeds 25-30 and whole seeds 8-10 were packed in 5ml polypropylene cryovials and fast frozen by plunging in liquid nitrogen. The cryostored samples were thawed in a water bath maintained at 37°C after 48h of storage. The explants were retested for viability by the appropriate germination method standardized for each explant.

For cryobanking of germplasm, a minimum of 50 half seeds of each accession were packed in 5 or 50ml cryovials depending on the number of seeds. These cryovials were cryostored after placing in the aluminium canisters in the extra large capacity cryotanks of 960 litres capacity (XLC-1830, MVE Cryogenics, USA) in the vapour phase of liquid nitrogen at -180°C. Each cryovial and cryocontainer was appropriately labelled. Passport data of individual accession along with storage conditions and retrieval requirements was entered in the cryogenebank database.

Whole seeds, half seeds and excised embryonic axes were tested for viability before and after 48 hours of cryostorage. The representative data for 5 cultivars are shown in table 1. Moisture content of seeds varied from 4.79 percent to 6.01 percent in the five accessions. The germinability of unfrozen controls of whole seeds ranged from 80 to 90%, half seeds from 80.5 to 90% and of excised axes from 90 to 100% (table 1). After cryostorage no significant change in viability was noticed. Whole seeds showed viability in the range of 82.5 to 92.5%, half seeds from 85 to 100% and embryonic axes from 98.5 to 100% (table 1). The excised embryonic axes showed the highest values of germination in both unfrozen and frozen samples. The seedlings obtained from cryostored seeds and half seeds were visually normal and healthy (figure 1A). Plantlets obtained *in vitro* from cryostored axes were the result of direct regrowth without any intervening callus (figure 1B). A total of 250 accessions belonging to 28 folk cultivars from 10 districts of Himachal Pradesh and Jammu and Kashmir states of India have been successfully cryostored as base collection in the cryogenebank. The conserved germplasm represents the invaluable variability existing in cultivated and wild populations of wild and cultivated apricot in India.

Table 1. Cryostorage of apricot germplasm using seeds and excised embryonic axes.

S. No.	Accession No.	Moisture content (%) of seeds (±SE)	Viability (%) before cryopreservation (±SE)			Viability (%) after cryopreservation (±SE)		
			Whole seed	Half seed	Embryonic axes	Whole seed	Half seed	Embryonic axes
1	IC-537700	4.79 (±0.07)	85.0 (±2.8)	85.5 (±2.4)	100.0 (±0.0)	82.5 (±3.9)	85.0 (±7.6)	100.0 (±0.0)
2	IC-537760	5.85 (±0.06)	80.0 (±5.7)	85.5 (±4.7)	95.0 (±5.0)	82.5 (±3.6)	85.0 (±2.8)	98.5 (±0.2)
3	IC-537758	6.01 (±0.04)	90.0 (±5.0)	85.0 (±2.8)	100.0 (±0.0)	92.5 (±1.6)	100.0 (±0.0)	100.0 (±0.0)
4	IC-537681	5.36 (±0.04)	82.5 (±3.9)	90.0 (±5.0)	90.0 (±2.8)	85.0 (±10.0)	90.0 (±2.8)	100.0 (±0.0)
5	IC-553608	4.88 (±0.08)	85.0 (±3.6)	80.5 (±7.6)	100.0 (±0.0)	87.5 (±6.7)	93.3 (±3.3)	100.0 (±0.0)

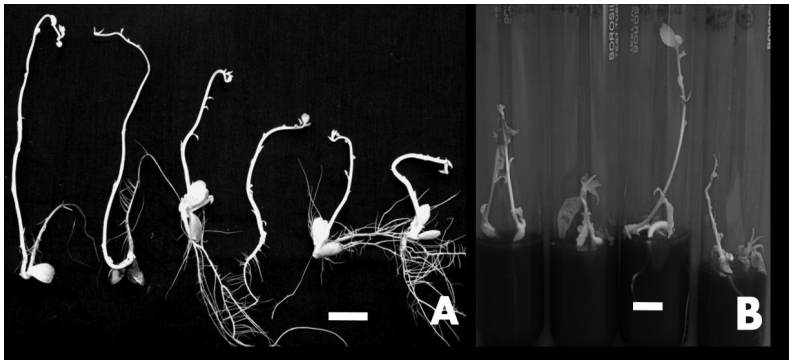

Figure 1A. Healthy seedlings of wild apricot raised from cryopreserved whole and half seeds, Bar-15mm. Figure 1B. *In vitro* regenerated plantlets of wild apricot from cryopreserved embryonic axes after 45 days of culture, Bar-10mm.

Conservation of genetic variability using seeds is the most efficient, reliable and globally acceptable method of genebanking. In such species like *Prunus persica*, *Prunus amygdalus* and several other large seeded species (e.g. almond) whole seeds, a part of the seed or isolated embryonic axes have been used for cryopreservation (Pence 1990; de Boucaud *et al.,* 1991, 1996; Chaudhury and Chandel, 1995). In the present study air-desiccation of the explants to low water contents, rapid freezing and rapid rewarming was found to be the most advantageous for high recovery. Embryonic axes being an organized structure and smaller in size showed highest regeneration after cryopreservation in comparison with whole seeds and half seeds. The advantage of cryopreserving half seeds and embryonic axes over whole seeds of wild apricot is that it saves space in the cryogenebank and also avoids the need for stratification during the germination of the cryopreserved material (Ellis *et al*., 1985; Garcia-Gusano *et al*., 2004). This has further confirmed the amenability of intermediate seeds of *Prunus* species for cryopreservation and particularly in *P. armeniaca* using half seeds and embryonic axes. Use of simple air-desiccation followed by fast-freezing method and high recovery percentage after cryopreservation indicates the applicability of this technique for routine cryobanking of genetic resources of *P. armeniaca*. Cryogenic storage prolongs the viability to the theoretically infinite periods. Hence, the apricot genepool stored is expected to retain viability with maximum phenotypic and genotypic stability.

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