0960-2585

Cryopreservation of seeds of four coffee species (*Coffea arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora*): importance of water content and cooling rate

Stéphane Dussert*[1], Nathalie Chabrillange[1], Florent Engelmann[2], François Anthony[3], Jacques Louarn[4] and Serge Hamon[1]

[1]ORSTOM, Laboratoire des Ressources Génétiques et Amélioration des Plantes Tropicales, BP 5045, 34032 Montpellier Cedex 1, France
[2]IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy
[3]CATIE, Apartado 59, 7170 Turrialba, Costa Rica
[4]ORSTOM, BP 434 Man, Côte-d'Ivoire

Abstract

In the range of water contents studied (0.1–0.4 g H_2O g dw^{-1}), *Coffea arabica* seeds were less sensitive to desiccation than *C. costatifructa*, *C. racemosa* and *C. sessiliflora* seeds. At 0.20 g H_2O g dw^{-1}, 53% of *C. arabica* seeds germinated after direct immersion in LN (rapid cooling, 200°C min^{-1}), but none of them developed into normal seedlings. By contrast, in *C. costatifructa*, *C. racemosa* and *C. sessiliflora*, when seeds were dehydrated to the optimal water content (0.19, 0.28 and 0.31 g H_2O g dw^{-1}, respectively), the percentages of seeds which developed into normal seedlings after LN exposure were 26, 78 and 31% of the desiccation control, respectively. Normal seedlings could be recovered from cryopreserved *C. arabica* seeds only if they were desiccated to 0.20 g H_2O g dw^{-1} and precooled slowly to –50°C prior to immersion in LN. Precooling seeds at 2°C min^{-1} allowed 25% of seeds to develop into normal seedlings. The thawing rate had no effect on the survival of cryopreserved *C. arabica* seeds. In all cryopreservation experiments, the total germination did not reflect the percentage of seeds which developed into normal seedlings. Examination of excised embryos indicated a partial explanation of this difference since only the shoot apex was destroyed in abnormal embryos, whereas the hypocotyl and radicle were normal.

Keywords: *Coffea*, cooling rate, cryopreservation, desiccation, genetic resources, seeds.

*Correspondence
E-mail: stephane.dussert@mpl.orstom.fr

Abbreviations: CATIE = Centro Agronomico Tropical de Investigacion y Enseñanza; IDEFOR-DCC = Institut Des Fôrets - Département Café Cacao; LN = liquid nitrogen; ORSTOM = Institut français de recherche scientifique pour le développement en coopération; IPGRI = International Plant Genetic Resources Institute.

Introduction

Even though *Coffea arabica* seeds can withstand desiccation down to 0.05–0.08 g H_2O g dw^{-1}, they cannot be considered orthodox since they remain cold-sensitive and desiccation does not increase their longevity (Van der Vossen, 1977; Couturon, 1980; Ellis *et al.*, 1990). These results led Ellis and co-workers (1990) to define a new category of seed storage behaviour, termed ' intermediate ', for seeds which do not fall in the orthodox or recalcitrant categories, as defined by Roberts (1973). Seeds of the two additional coffee species tested so far for their storage behaviour, *C. canephora* and *C. stenophylla*, also show intermediate characteristics (Couturon, 1980; Hong and Ellis, 1995).

Because of the seed storage behaviour of coffee species, genetic resources are conserved in field collections. However, significant problems in the maintenance of such field collections have been reported: (i) genetic erosion in some species due to their poor adaptation to the local environment and to attacks by pests and pathogens; (ii) important labour costs and large space requirements (Berthaud and Charrier, 1988). Thus, research for alternative methods to field conservation for coffee genetic resources became a priority. The establishment of an *in vitro* coffee core collection was initiated in 1991 at ORSTOM but, a few years later, the limits of this technique became apparent with the occurrence of some genotypic selection and intraspecific genetic drift (Dussert *et al.*, 1997). This stressed the importance of developing cryopreservation protocols as a complementary option to *in vitro* slow growth storage.

Cryopreservation of orthodox seeds routinely involves only partial desiccation prior to immersion in LN (for review see Stanwood, 1985). Recent studies have shown that seeds of some intermediate species

such as *Camellia sinensis, Carica papaya, C. liberica, Piper nigrum* and *Salix nigra,* which are sufficiently tolerant of desiccation, could be cryopreserved using the same procedure (Becwar *et al.,* 1983; Normah and Vengadasalam, 1992; Chaudhury and Chandel, 1994; Hu *et al.* 1994; Pence, 1995). However, the ability to withstand desiccation is not always sufficient for seeds to withstand LN exposure. For example, whatever their water content, seeds of *C. arabica* (Becwar *et al.,* 1983) and *Corylus avellana* (Normah *et al.,* 1994) did not survive after immersion in LN.

These differences in response to LN exposure obtained with *C. arabica* and *C. liberica* suggest that, within the genus *Coffea,* each species should be considered separately for setting up a cryopreservation protocol. Moreover, even though whole *C. arabica* seeds do not withstand direct immersion in LN, excised zygotic embryos have been successfully cryopreserved if desiccated to 0.20 g H_2O g dw^{-1} prior to immersion in LN (Abdelnour-Esquivel *et al.,* 1992). This suggests that the endosperm and the embryo of *C. arabica* seeds differ in their response to desiccation and freezing. Desiccation duration, freezing and thawing rates, should therefore be investigated simultaneously to define conditions under which both the endosperm and the embryo survive.

In the present work, the sensitivity to cryopreservation of seeds at various water contents was studied with four coffee species, *C. arabica, C. costatifructa, C. racemosa* and *C. sessiliflora,* and the effects of cooling and thawing rates were investigated for *C. arabica* seeds.

Materials and methods

Plant material

The geographical origin, seed dry weight and seed water content upon receipt of the four coffee species studied, *C. arabica* L., *C. costatifructa* Bridson, *C. racemosa* Lour. and *C. sessiliflora* Bridson, are presented in Table 1. Dry weight of *C. arabica* seeds (1278 mg) was about five times that of *C. costatifructa* (241 mg) and three times that of *C. racemosa* (402 mg) and *C. sessiliflora* (417 mg). *C. arabica* var. *typica* seeds were provided by CATIE, Turrialba, Costa Rica (harvested in October). For *C. costatifructa, C. racemosa* and *C. sessiliflora,* seed bulks were provided from the base collections in Côte d'Ivoire (IDEFOR-DCC, Divo and ORSTOM, Man), by harvesting seeds on randomly chosen trees (in February). Seeds were partially desiccated after harvest and water contents at receipt (Table 1) were lower than those of fresh seeds, i.e. 1.2–1.5 g H_2O g dw^{-1} (Couturon, 1980).

Desiccation and cryopreservation

After testa removal, seeds were desiccated over 80 g silica gel for 0–16 h in 1160-ml glass vessels (60 seeds per vessel). For each desiccation period, water content (expressed in g H_2O g dw^{-1}) was determined on 10 individual seeds. Dry weight was measured after 2 days of desiccation in an oven at 105°C.

Before cryopreservation, seeds were hermetically sealed in aluminium foil-polyethylene bags (50 seeds per bag). Rapid cooling (200°C min^{-1}) was achieved by plunging bags directly in LN. Slow cooling consisted of precooling seeds to –50°C at 2, 4 or 20°C min^{-1} prior to immersion in LN. Precooling was carried out using a programmable cooling apparatus (Minicool LC 40, L'Air Liquide, France). The effect of precooling was assessed by thawing seeds directly after the precooling step. Cryopreserved seeds were stored for one week at –196°C before thawing. Thawing was carried out either rapidly (mean rate of 420°C min^{-1} between –196°C and 0°C) by plunging bags in a 40°C water-bath for 2 min or slowly (mean rate of 70°C min^{-1} between –196°C and 0°C) by placing bags at room temperature (25°C) for 30 min. Cooling and thawing rates were measured separately using a K type

Table 1. Country of origin of the four coffee species studied, dry weight and water content of seeds at receipt. Results of one-way ANOVA: *F* and *P*

Species	Country of origin	Seed dry weight (g)	Seed water content (g H_2O g dw^{-1})
C. arabica	Ethiopia	1.278 [a]	0.4054 [a]
C. costatifructa	Tanzania	0.241 [b]	0.3377 [b]
C. racemosa	Mozambique	0.402 [c]	0.2976 [c]
C. sessiliflora	Kenya, Tanzania	0.417 [c]	0.2868 [c]
F		518.6	190.8
P		0.0000	0.0000

Means followed by the same letter were not significantly different at the 0.05 probability level as determined by the Newman and Keuls' test.

thermocouple embedded in one seed linked to a data logger (Model 50, Electronic Controls Design, USA).

Culture conditions

Both seeds and embryos were inoculated and cultured *in vitro* after freezing for survival assessment. Seeds were first washed with soap and tap water then disinfected by soaking in sodium hypochlorite (12%) for 15 min with continuous shaking on a rotary shaker, followed by 5 min under vacuum and 10 min again with shaking. Seeds were rinsed three times with sterile water before inoculation in test tubes (250 × 24 mm) sealed with Parafilm Ribbon on 15 × 20 mm cellulose plugs (Sorbarod system, Baumgartner Papiers SA, Switzerland) fully saturated with 3 ml sterile water. Cultures were kept in the dark at 27 ± 1°C in a room operating at 55 ± 2% relative humidity.

Before excision of embryos, disinfected seeds were immersed for two days in sterile water for rehydration. Excised embryos were inoculated on the germination medium (20 ml) defined by Bertrand-Desbrunais and Charrier (1989) in test tubes (250 × 24 mm) sealed with Parafilm Ribbon. Cultures were kept at 27 ± 1°C in a room operating at 55 ± 2% relative humidity. Cultures were maintained in the dark until the hypocotyl stood upright and were then transferred to light (Gro-Lux, Sylvana, 30 µmol m^{-2} s^{-1}, 12-h light/12-h dark photoperiod).

Survival assessment

Both germination and development of normal seedlings were estimated to assess seed survival. Emergence of hypocotyl and radicle was used as the criterion for germination rate after 3 months in culture while the development of the cotyledonary leaves was the criterion for normal seedling formation after 6 months in culture. Excised embryos were considered viable when they were vertical on the culture medium and the first pair of leaves was developed. In non-viable embryos, the necrosed (black and undeveloped) parts were noted individually.

Statistical analysis

One sample of 50 seeds was used in each treatment, except for the effect of the precooling rate on the survival rate of cryopreserved *C. arabica* seeds where 3 samples of 50 seeds were used. With the four species studied, the effect of desiccation was estimated by testing the correlation between seed water content and the percentage of seeds which developed into normal seedlings. Multiple comparisons of germination rates of *C. arabica* seeds cooled slowly and rapidly at various water contents and subsequent percentages of normal seedlings were achieved using Ryan's test (1960). The Pearson test was used for all other comparisons, except for the effect of precooling rate on the development of normal seedlings of *C. arabica* seeds exposed to LN where a one-way ANOVA and the Newman and Keuls' test were applied.

Results

Desiccation effect

The effect of desiccation duration on water content and development of normal seedlings of *C. arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora* seeds are presented in Figure 1. For all species, seed water content decreased from 0.28–0.37 g H_2O g dw^{-1} to 0.11–0.14 g H_2O g dw^{-1} throughout the 16-h desiccation period. For *C. arabica*, *C. racemosa* and *C. sessiliflora*, production of normal seedlings was highly correlated with seed water content throughout the desiccation period (respectively R^2=0.97, P=0.0164; R^2=0.98, P=0.0080; R^2=0.99, P=0.0048), whereas, for *C. costatifructa* seeds, no decrease in survival was noted above 0.19 g H_2O g dw^{-1} (4 h desiccation) and the percentage of normal seedlings was significantly correlated with water content within the 4- to 16-h interval only (R=0.94, P=0.0066). However, at lower water contents (0.11–0.14 g H_2O g dw^{-1}), the percentage of seeds which developed into normal seedlings was significantly higher for *C. arabica* (84% of initial percentage) than for the three other species (19–30%).

Effect of direct immersion into liquid nitrogen

Whatever their water content, *C. arabica* seeds never developed into normal seedlings after direct immersion in LN (Fig. 1). Maximum production of normal seedlings in *C. racemosa* and *C. sessiliflora* was obtained without desiccation. At 0.28 g H_2O g dw^{-1}, 52% (78% of the desiccation control) of *C. racemosa* seeds previously immersed in LN developed into normal seedlings, while only 12% at 0.20 g H_2O g dw^{-1} and no seedlings were produced at lower water contents. For *C. sessiliflora*, 14% (31% of the desiccation control) of seeds cryopreserved at 0.31 g H_2O g dw^{-1} developed into normal seedlings, 5% at 0.19 g H_2O g dw^{-1} and 0% at lower water contents. By contrast, development of normal seedlings was observed in cryopreserved seeds of *C. costatifructa* after 4 h of desiccation (0.19 g H_2O g dw^{-1}) only, when 13% of the seeds produced normal seedlings (26% of the desiccation control).

Comparison of slow and rapid cooling with C. arabica

When cooled rapidly (200°C min^{-1}) by direct immersion in LN, later emergence of hypocotyl and radicle was

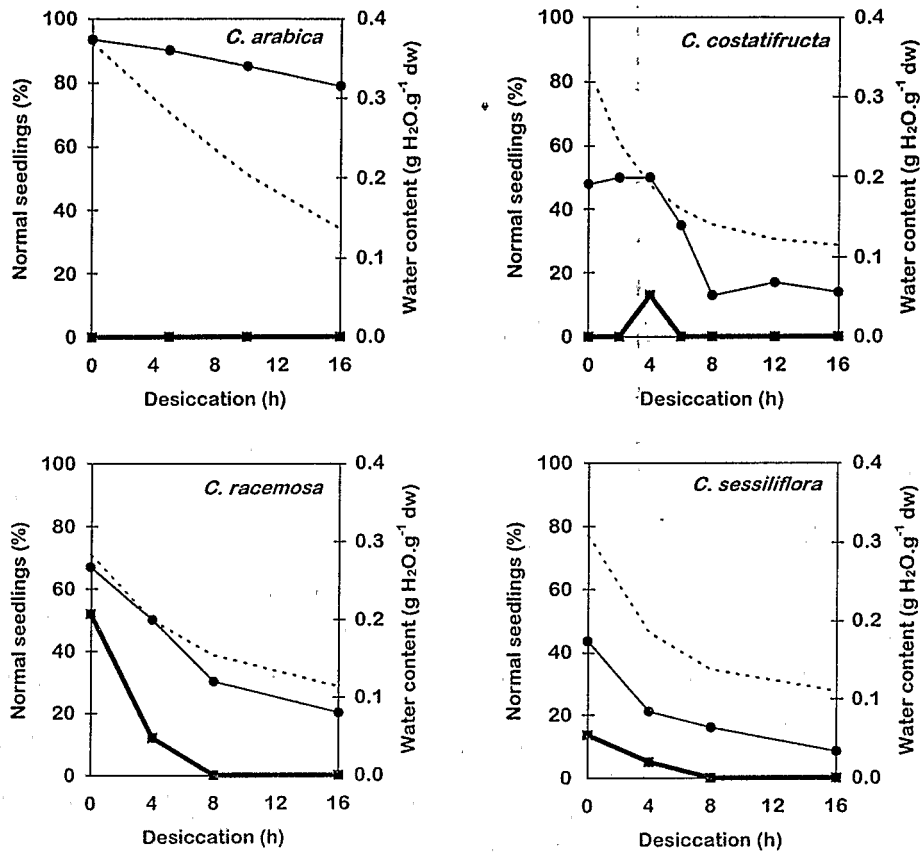

Figure 1. Effect of desiccation duration on water content (· · · ·) and development of normal seedlings (%) from control (—) and cryopreserved (——) seeds of *C. arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora*. Cryopreserved seeds were cooled to liquid nitrogen temperature at 200°C min^{-1} (rapid cooling).

observed in 53% of seeds at 0.20 g H_2O g dw^{-1}, while at other water contents, germination was very low or nil (Table 2). However, none of these germinated seeds produced normal seedlings and, after six months in culture, no further development had been noted. A germinated seed, arrested in its development after emergence of the radicle, and a normal seedling are shown on Figure 2. If seeds were precooled to –50°C at 4°C min^{-1} prior to immersion in LN (slow cooling), the rate of emergence of hypocotyl and radicle was about 4% at 0.28 g H_2O g dw^{-1}, 63% at 0.20 g H_2O g dw^{-1} and 35% at 0.14 g H_2O g dw^{-1}. Moreover, 12% of germinated seeds developed into normal seedlings after desiccation to 0.20 g H_2O g dw^{-1} only.

The different viability levels of embryos excised from seeds exposed to LN are shown in Figure 2: in embryos d and e, hypocotyl and radicle developed while the shoot apex was damaged. When seeds at 0.20 g H_2O g dw^{-1} were precooled to –50°C at 4°C min^{-1} prior to immersion in LN, 39% of excised embryos were fully viable and only 14% were completely non-viable. A total of 47% of embryos presented partial necrosis only, localized at the shoot apex and cotyledons (33%) or at the hypocotyl (14%), while the radicle was never damaged. Thirty percent of embryos excised from seeds immersed directly in LN were fully viable and 41% of them were completely necrosed. As with slow cooling, necrosis was never localized on the radicle in partly viable embryos.

Effect of precooling on C. arabica *seeds*

At 0.20 g H_2O g dw^{-1}, there was no significant difference in the germination rate and the rate of development of normal seedlings between seeds thawed immediately after the precooling step (prefrozen controls) and seeds immersed in LN after precooling. In both cases, emergence of hypocotyl and radicle was observed in 63–69% of seeds and 12–14% of them developed into normal seedlings.

Effect of thawing rate on C. arabica *seeds*

The thawing rate had no significant effect on the germination and the development of normal seedlings with seeds at 0.20 g H_2O g dw^{-1}, precooled down to

Table 2. Germination (%) of *C. arabica* seeds at various water contents cooled slowly or rapidly (200°C min^{-1}) and development of normal seedlings (%). Slow cooling consisted of precooling seeds to −50°C at 4°C min^{-1} prior to immersion in liquid nitrogen

Water content ($g H_2O g dw^{-1}$)	Cooling	Germination (%)	Normal seedlings (%)
0.37	Slow	0.0 [a]	0.0 [a]
	Rapid	0.0 [a]	0.0 [a]
0.28	Slow	4.4 [a]	0.0 [a]
	Rapid	0.0 [a]	0.0 [a]
0.20	Slow	63.2 [b]	12.2 [b]
	Rapid	53.1 [bc]	0.0 [a]
0.14	Slow	35.4 [c]	0.0 [a]
	Rapid	4.3 [a]	0.0 [a]

Values followed by the same letter were not significantly different at the 0.05 probability level as determined by Ryan's test.

−50°C at 4°C min^{-1} prior to immersion in LN. After both slow (70°C min^{-1}) and rapid (420°C min^{-1}) thawing, emergence of hypocotyl and radicle was observed in 55 and 70% of seeds, respectively, and 13% of them developed into normal seedlings.

Effect of precooling rate with C. arabica

Precooling rate had a highly significant effect on the production of normal seedlings from *C. arabica* seeds (0.2 g H_2O g dw^{-1} precooled to −50°C before immersion in LN. Seeds precooled at 20°C min^{-1} did not survive but 13.0% and 24.2% of *C. arabica* seeds developed into normal seedlings when precooled at 4°C min^{-1} and 2°C min^{-1}, respectively.

Discussion

In the range of water contents studied, desiccation tolerance of *C. arabica* seeds was higher than that of

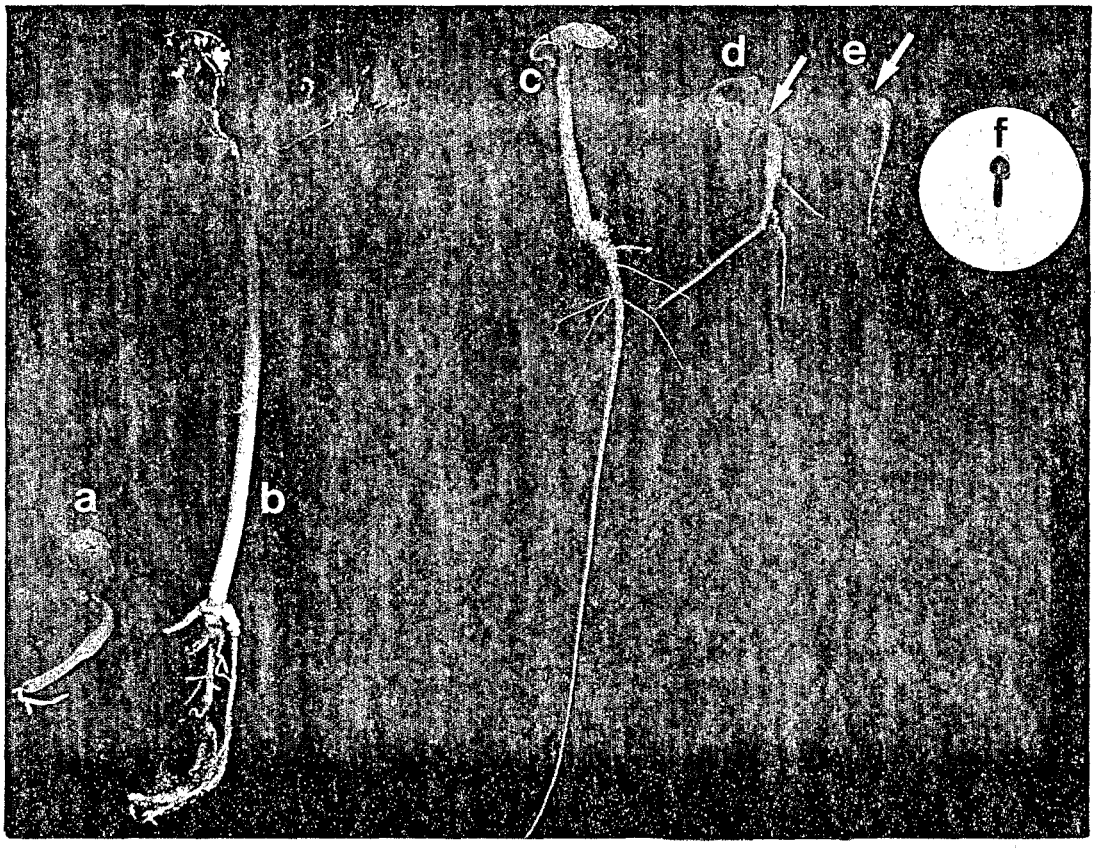

Figure 2. Survival assessment in cryopreserved *C. arabica* seeds after six months in culture: germinated seed arrested in its development after emergence of hypocotyl and radicle (a) and normal seedling (b) possessing well-developed cotyledonary leaves. Different stages, after 2 months in culture, of embryos excised from cryopreserved *C. arabica* seeds: viable embryo (c) with well-developed cotyledonary leaves and emergence of the first pair of leaves; partially viable embryos (d and e) showing a development of hypocotyl and radicle while shoot apex (d and e, arrows) and cotyledons (e) were necrosed ; completely non-viable embryo (f).

C. costatifructa, *C. racemosa* and *C. sessiliflora* seeds. This is consistent with the results of Ellis *et al.* (1990, 1991) who observed that desiccation damage occurred in *C. arabica* seeds below 0.05–0.08 g H_2O g dw^{-1}. Also, no decrease in survival was noted above 0.19 g H_2O g dw^{-1} for *C. costatifructa* seeds, while with *C. racemosa* and *C. sessiliflora*, desiccation sensitivity could be detected at 0.30 g H_2O g dw^{-1}. Hong and Ellis (1995) observed differences in desiccation sensitivity between *C. canephora* and *C. liberica*. Other genera are known to include species with different seed storage behaviours, for example, *Araucaria* (Tompsett, 1984), *Dipterocarpus* (Tompsett, 1987) and *Acer* (Dickie *et al.*, 1991).

Seeds of *C. costatifructa*, *C. racemosa* and *C. sessiliflora* could survive after immersion in LN, the percentage of seeds developing into normal seedlings after LN exposure being 26, 78 and 31% of the desiccation controls, respectively. Normah and Vengadasalam (1992) reported that *C. liberica* seeds withstood LN exposure if partially desiccated. Thus, it appears that, within the genus *Coffea*, four of the five species tested so far could be cryopreserved using rapid cooling to –196°C. In our experiments, the highest survival was observed at 0.28–0.31 g H_2O g dw^{-1} for *C. sessiliflora* and *C. racemosa* and at 0.20 g H_2O g dw^{-1} for *C. costatifructa*. In the case of *C. sessiliflora* and *C. racemosa*, since the highest survival was observed with seeds at the initial water content, higher water contents should be tested using fresh seeds (1.2–1.5 g H_2O g dw^{-1}). The low survival rates obtained with *C. costatifructa* and *C. sessiliflora*, compared with *C. racemosa*, could also be explained by the lower initial viability of seeds or by sub-optimal cooling and thawing rates.

In all cryopreservation experiments, with *C. arabica*, germination was not always followed by the development into normal seedlings. Hypocotyl and radicle emergence was observed in 53% of seeds at 0.20 g H_2O g dw^{-1} cooled rapidly, but no normal seedlings were formed. Thus, tests other than germination tests alone should be used for the development of a cryopreservation protocol. Studies with excised embryos helped to explain partly the difference between the germination value and the development of normal seedlings as, in many embryos, the shoot apex was destroyed whereas the hypocotyl and radicle could develop normally. In a recent review, Pence (1995) mentioned numerous species in which shoot and root meristems had different responses to desiccation and freezing. Chin *et al.* (1988) showed that in two palm species, the haustorium (distal part of the cotyledon) of cryopreserved embryos failed to develop while the shoot and the root appeared to develop normally. However, in our experiments, the greater resistance to low temperature of the radicle and hypocotyl was not sufficient to explain the non-development into seedlings of seeds at 0.20 g H_2O g dw^{-1} cooled rapidly in which 30% of the embryos were completely viable. This result could be explained by greater damage occurring to the endosperm than to the embryo during the freeze/thaw cycle so that the endosperm could not provide nutritional support for normal embryo development.

It was shown that slow precooling of *C. arabica* seeds had a beneficial effect on their survival and their capacity to develop normally. Rapid cooling (200°C min^{-1}) of seeds with high moisture content had a similar effect in lettuce (Roos and Stanwood, 1981), sesame (Stanwood, 1987), pea, soybean and sunflower (Vertucci, 1989). By contrast, at low moisture contents, survival was higher if seeds were cooled slowly (1°C min^{-1}) in sesame, soybean and sunflower, and the cooling rate had no effect in lettuce and pea. Moreover, Vertucci (1989) clearly showed that the detrimental effect of rapid cooling in soybean and sunflower seeds at low moisture contents was associated with glass transitions occurring in the lipid phase of seeds. Very little is known about specific seed lipid contents in the genus *Coffea* and further analysis should be carried out to observe if lipids are involved in the sensitivity of seeds to rapid freezing. However, in the case of sesame, soybean and sunflower, survival of seeds at low water contents was 30–90% of desiccation controls, even if cooled rapidly. With *C. arabica*, the situation seemed more drastic since, whatever their water content, seeds never produced normal seedlings after rapid cooling. The high sensitivity of *C. arabica* seeds to rapid freezing should thus be the consequence of multifactorial effects. However, the seed size hypothesis (Pence, 1995) does not explain the differences in sensitivity to rapid cooling within the genus *Coffea* since *C. liberica* seeds, which are larger than *C. arabica* seeds, withstood direct immersion in LN (Normah and Vengadasalam, 1992).

Acknowledgements

We are grateful to Dr A. Rival for photographs.

References

Abdelnour-Esquivel, A., Villalobos, V. and Engelmann, F. (1992) Cryopreservation of zygotic embryos of *Coffea* spp. *Cryo-Letters* 13, 297–302.

Becwar, M.R., Stanwood, P.C. and Lehonardt, K.W. (1983) Dehydration effects on freezing characteristics and survival in liquid nitrogen of desiccation-tolerant and desiccation-sensitive seeds. *Journal of the American Society for Horticultural Science* 108, 613–618.

Berthaud, J. and Charrier, A. (1988) Genetic resources of *Coffea*. pp 1–42 *in* Clarke, R.J. and Macrae, R. (Eds) *Coffee, Vol. 4, Agronomy*. London, Elsevier Applied Science.

Bertrand-Desbrunais, A. and Charrier, A. (1989) Conservation des ressources génétiques caféières en vitrothèque. pp 438–447 *in Proceedings of the 13th ASIC, Paipa, Colombia.*

Chaudhury, R. and Chandel, K.P.S. (1994) Germination studies and cryopreservation of seeds of black pepper (*Piper nigrum* L.) – a recalcitrant species. *Cryo-Letters* **15**, 145–150.

Chin, H.F., Krishnapillany, B. and Alang, Z.C. (1988) Cryopreservation of *Veitchia* and *Howea* palm embryos: non-development of the haustorium. *Cryo-Letters* **9**, 372–379.

Couturon, E. (1980) Le maintien de la viabilité des graines de caféiers par le contrôle de leur teneur en eau et de la température de stockage. *Café Cacao Thé* **1**, 27–32.

Dickie, J.B., May, K., Morris, S.V.A. and Titley, S.E. (1991) The effects of desiccation on seed survival in *Acer platanoides* L. and *Acer pseudoplatanus* L. *Seed Science Research* **1**, 149–162.

Dussert, S., Chabrillange, N., Anthony, F., Engelmann, F., Recalt, C. and Hamon, S. (1997) Variability in storage response within a coffee (*Coffea* spp.) core collection under slow growth conditions. *Plant Cell Reports* **16**, 343–348.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990) An intermediate category of seed storage behaviour ? I. Coffee. *Journal of Experimental Botany* **41**, 1167–1174.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1991) An intermediate category of seed storage behaviour ? II. Effects of provenance, immaturity, and imbibition on desiccation-tolerance in coffee. *Journal of Experimental Botany* **42**, 653–657.

Hu, J., Guo, C.G. and Shi, S.X. (1994) Partial drying and post-thaw preconditioning improve the survival and germination of cryopreserved seeds of tea (*Camellia sinensis*). *Plant Genetic Resources Newsletter* **98**, 25–28.

Hong, T.D. and Ellis, R.H. (1995) Interspecific variation in seed storage behaviour within two genera – *Coffea* and *Citrus*. *Seed Science and Technology* **23**, 165–181.

Normah, M.N. and Vengadasalam, M. (1992) Effects of moisture content on cryopreservation of *Coffea* and *Vigna* seeds and embryos. *Cryo-Letters* **13**, 199–208.

Normah, M.N., Reed, B.M. and Yu, X. (1994) Seed storage and cryoexposure behavior in hazelnut (*Corylus avellana* L. cv. Barcelona). *Cryo-Letters* **15**, 315–322.

Pence, V.C. (1995) Cryopreservation of recalcitrant seeds. pp 29–50 *in* Bajaj, Y.P.S. (Ed.) *Cryopreservation of plant germplasm I*. Berlin, Springer-Verlag.

Roberts, E.H. (1973) Predicting the storage life of seeds. *Seed Science and Technology* **1**, 499–514.

Roos, E.E. and Stanwood, P.C. (1981) Effects of low temperature, cooling rate, and moisture content on seed germination of lettuce. *Journal of the American Society for Horticultural Science* **106**, 30–34.

Ryan, T.A. (1960) Significance tests for multiple comparison of proportions, variances and other statistics. *Psychological Bulletin* **57**, 318–328.

Stanwood, P.C. (1985) Cryopreservation of seed germplasm for genetic conservation. pp 199–226 *in* Kartha, K.K. (Ed.) *Cryopreservation of plant cell and organs*. Boca Raton, CRC press.

Stanwood, P.C. (1987) Survival of sesame seeds at the temperature (–196°C) of liquid nitrogen. *Crop Science* **27**, 327–331.

Tompsett, P.B. (1984) Desiccation studies in relation to the storage of *Araucaria* seed. *Annals of Applied Biology* **105**, 581–586.

Tompsett, P.B. (1987) Desiccation and storage studies on *Dipterocarpus* seeds. *Annals of Applied Biology* **110**, 371–379.

Van der Vossen, H.A.M. (1977) Methods of preserving the viability of coffee seed in storage. *Kenya Coffee* **45**, 31–35.

Vertucci, C.W. (1989) Effects of cooling rate on seeds exposed to liquid nitrogen temperatures. *Plant Physiology* **90**, 1478–1485.

Received 27 January 1997, accepted after revision 20 October 1997

Volume 8(1) 1–76 March 1998 ISSN 0960–2585

SEED SCIENCE RESEARCH

CAB INTERNATIONAL