# Determination of the hydration window for cryopreservation of intermediate oily seeds

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**Introduction.** Cryopreservation is the only technique available for long-term conservation of genetic resources of non-orthodox seed species. In order to determine the limits of the hydration window for cryopreservation (HWC) of intermediate oily seeds, the effect of exposure to liquid nitrogen (LN) temperature on viability of seeds desiccated to various water contents was investigated in nine coffee species previously shown to display a high variability in seed desiccation tolerance [1].

**Results and Discussion (1).** Three groups of species could be distinguished based on seed survival after LN exposure (Table 1). In group 1 species, no seedling production could be obtained after LN exposure. In group 2 species, recovery was very low or nil after rapid cooling and only moderate after slow cooling (see Fig. 1 for *C. eugenioides*). In group 3 species, very high percentages of normal seedling development were observed after both rapid and slow cooling. When expressed in terms of water content, the higher limit of the HWC (Fig. 1) was highly variable since it ranged from 0.14 to 0.26 g $H_2O$ $g^{-1}$ dw (g/g) (Table 1). In group 3 species, the sensitivity to LN exposure was due to endosperm injury as shown by the development into normal seedlings of embryos extracted from seeds after thawing (see Fig. 2 for *C. stenophylla*).

**Table 1.** Desiccation sensitivity, as estimated by the water content at which 50% of the initial viability was reached, $WC_{50}$, higher limit of the hydration window for cryopreservation, *HL*, percentages of normal seedlings recovered after desiccation to *HL* and direct immersion into LN (rapid cooling) or by a precooling to -50°C at 1°C.min$^{-1}$ prior to immersion in LN (slow cooling), unfreezable water content, $WC_u$, and corresponding water activity, $a_w$, of seeds of nine coffee species classified in three groups according the percentages of normal seedling recovery after LN exposure (g/g = g $H_2O$ $g^{-1}$ dw; ND = not determined).

| Group | Species | $WC_{50}$ (g/g) | *HL* (g/g) | Normal seedlings (%) Rapid cooling | Normal seedlings (%) Slow cooling | $WC_u$ (g/g) | $a_w$ |
|---|---|---|---|---|---|---|---|
| 1 | *C. brevipes* | 0.20 | - | 0 | 0 | ND | ND |
| | *C. canephora* | 0.17 | - | 0 | 0 | 0.28 | 0.86 |
| | *C. liberica* | 0.29 | - | 0 | 0 | 0.26 | 0.85 |
| | *C. stenophylla* | 0.16 | - | 0 | 0 | ND | ND |
| 2 | *C. arabica* | 0.11 | 0.21 | 0 | 17 | 0.21 | 0.78 |
| | *C. eugenioides* | 0.11 | 0.26 | 8 | 19 | 0.26 | 0.86 |
| 3 | *C. pseudozanguebariae* | 0.06 | 0.14 | 73 | 68 | 0.14 | 0.75 |
| | *C. racemosa* | <0.14 | 0.23 | 67 | 73 | 0.24 | 0.83 |
| | *C. sessiliflora* | <0.14 | 0.19 | 81 | 76 | 0.18 | 0.79 |

**Results and Discussion (2).** With all species of groups 2 and 3, the higher limit of HWC corresponded also to the optimal hydration level for seed cryopreservation (see Fig. 1 for *C. eugenioides*). In those species, a highly significant correlation was found between the unfreezable water content, as determined from DSC analysis [2], and the higher limit of the HWC (Fig. 3). This result is consistent with the earlier study of Vertucci [3] with orthodox soybean seeds, and suggests that intracellular ice formation is lethal in lipid-rich seeds, independent of their level of desiccation sensitivity.

The unfreezable water content was negatively correlated with seed lipid content (Fig. 4). However, this relationship was not linear, even when expressed on a non-lipid dry weight basis, indicating that compounds other than lipids influence the unfreezable water content.

When expressed in terms of water activity, the interspecific variability for the higher limit of the HWC was very low and independent of seed lipid content (Table 1). This result, in combination with the fact that the higher limit of the HWC was always the optimal hydration level for seed cryopreservation, suggests that desiccating seeds under 75-85% RH at 25°C allows to reach directly the optimal water content for intermediate oily seed cryopreservation. This hydration level could be slightly above the optimal level for minimizing seed deterioration at LN temperature [4].

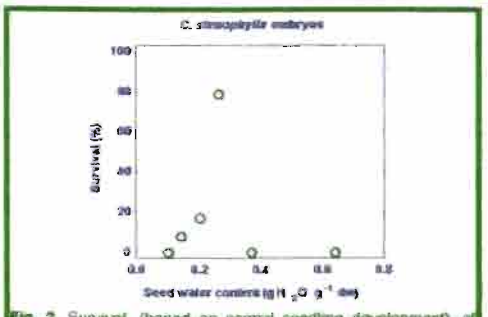


**Fig. 2.** Survival, (based on normal seedling development), of zygotic embryos extracted after thawing from *C. stenophylla* seeds desiccated to various water contents and frozen rapidly or slowly.

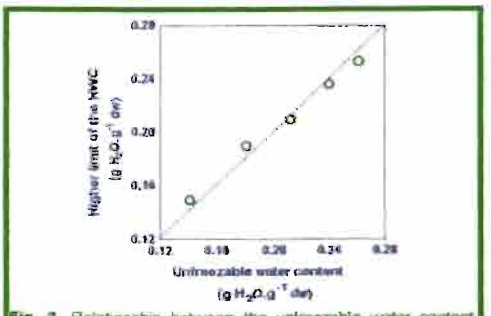


**Fig. 3.** Relationship between the unfreezable water content, $WC_u$, as determined from DSC analysis, and the higher limit of the HWC of seeds of the five species of groups 2 and 3, as determined in Table 1.

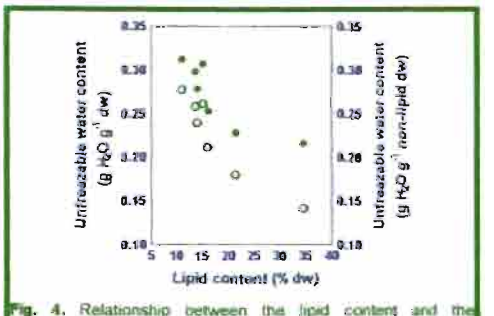


**Fig. 4.** Relationship between the lipid content and the unfreezable water content, expressed on a dry weight basis (●) or a non-lipid dry weight basis (○), of seeds of seven coffee species.

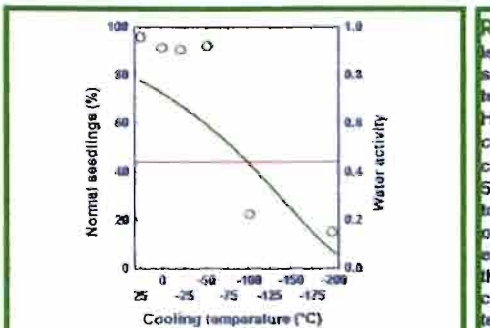


**Fig. 5.** Survival (○), water activity (—) and critical water activity for desiccation tolerance (---) of *C. arabica* seeds cooled to various temperatures after desiccation to 0.2 g $H_2O$ $g^{-1}$ dw. Seed water activity was calculated according to the van't Hoff isochore [4], with an enthalpy of sorption of -2 kJ.mol$^{-1}$ $H_2O$ at 0.2 g $H_2O$ $g^{-1}$ dw [5].

**Results and Discussion (3).** The lower limit of the HWC was always much higher than the critical hydration level for desiccation tolerance. Assuming that a single critical water activity determines the desiccation sensitivity of seeds of a given species, this result can be explained by the decrease in seed water activity with decreasing temperature. The relationship between the water activity, $a_w$, and the temperature, T (K), is described by the van't Hoff isochore [4]: $d(\ln(a_w)) / d(1/T) = \Delta H_{sorp(T)}/R$, where $\Delta H_{sorp(T)}$ is the enthalpy of sorption and R is the ideal gas constant. Assuming that $\Delta H_{sorp}$ is constant with temperature [4], the water activity of *C. arabica* seeds at 0.2 g/g was calculated for temperatures between +25 and -196°C using the value of $\Delta H_{sorp}$ at 0.2 g/g given by Eira *et al.* [5] (Fig. 5). When cooled to LN temperature, the water activity of seeds crossed the critical water activity for desiccation tolerance (0.45 [1]) at about -95°C. Interestingly, an abrupt decline in survival of *C. arabica* seeds at 0.2 g/g was observed between -50 and -100°C (Fig. 5). Therefore, the prediction of the existence of an hydration window for LN exposure in non-orthodox oily seeds should not be based on the comparison of the unfreezable water content with the critical water content for desiccation sensitivity, as measured at room temperature, but on the comparison of the critical water activity for desiccation damage, measured at room temperature, with the calculated water activity at LN temperature of seeds desiccated to their unfreezable water content.

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