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# GLASSY STATES IN DORMANT CORN EMBRYOS

ROBERT J. WILLIAMS *

*American Red Cross Holland R&D Laboratories, 15601 Crabbs Branch Way, Rockville, MD 20855 (U.S.A.)*

A. CARL LEOPOLD

*Boyce Thompson Institute, Cornell University, Ithaca, NY 14853 (U.S.A.)*

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## ABSTRACT

Corn embryos contain oils with two melting points and a strong tendency to form glasses at temperatures down to −95 °C. When these were extracted, water : sugar glasses can also be detected, with the water serving as a plasticizer. When the water content drops below about 12% (the water content required for seed storage stability) the transition temperature of these water glasses rises above ambient. These data are consistent with our hypothesis that the dormant state in these seeds is a glassy state.

## INTRODUCTION

When seeds are dried and enter a dormant (cryptobiotic) state, water removal imposes very large stresses. The enormous concentration of solutes leads to precipitation and the maintenance of bilayer organization of membranes is threatened. Yet seeds can germinate after long periods of storage under quite severe conditions. The moisture isotherms of orthodox seeds at low water contents consistently show a region of water 'binding' which should be absent if substantial crystallization had occurred during drying [1]. In this study, we provide evidence that seeds survive desiccation in a glassy state, and that the 'bound' water is a component of the glasses formed.

Vitrification has been proposed as the only means for long-term protection against freezing injury [2] and has been found to be a natural component of freeze protection in trees [3]. It would appear to offer several advantages to prolonging the storage life of a dry seed at temperatures above freezing as well: it would limit the tendency of the cell constituents to

* Author to whom correspondence should be addressed.

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crystallize, it would immobilize the cell constituents, interfering with their reactivity, and it would limit the loss of the last water remaining upon the drying of the seed. DSC signals show glass transitions as low as $-100\,^{\circ}C$ in storage lipids. Glass transitions are also seen in the non-lipid components of corn embryos and our data indicate that when seeds are stored at below 12% (w/w) water content, the embryos exist in a glassy state at ambient temperatures.

## EXPERIMENTAL

All the experiments were performed in a modified [4] Perkin–Elmer DSC-4 with TADS software and with the sample holder cooled in liquid nitrogen. Corn embryos were equilibrated to known relative humidities at $20 \pm 0.5\,^{\circ}C$ in desiccator jars over various saturated salt solutions. Specimens were cooled to $-130\,^{\circ}C$ at $20\,^{\circ}C\ min^{-1}$ and subsequently warmed at $20\,^{\circ}C\ min^{-1}$ from $-130\,^{\circ}C$ to $+100\,^{\circ}C$. After scanning, the sealed pans were punctured and the calorimeter was used as a drying oven at $100\,^{\circ}C$ for at least 2 h to determine the water content. Lipids were extracted by soaking for 24 h in chloroform : methanol (2 : 1).

## RESULTS

Scanning calorimetry characteristically yielded a set of thermal signatures such as those in Fig. 1, showing a glass transition at $-95\,^{\circ}C$, and an

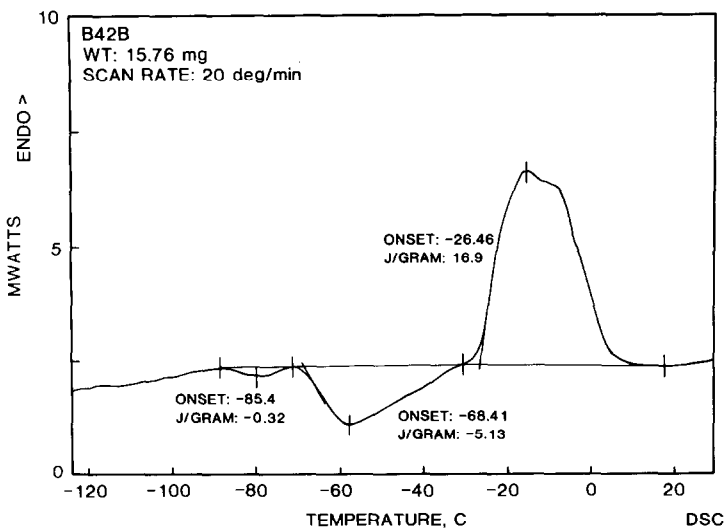


Fig. 1. DSC thermograms during heating of a corn embryo with a 7% water content. Two exothermic devitrification events are seen above the glass transition at about $-95\,^{\circ}C$. They are followed by a large melting endotherm above about $-30\,^{\circ}C$.

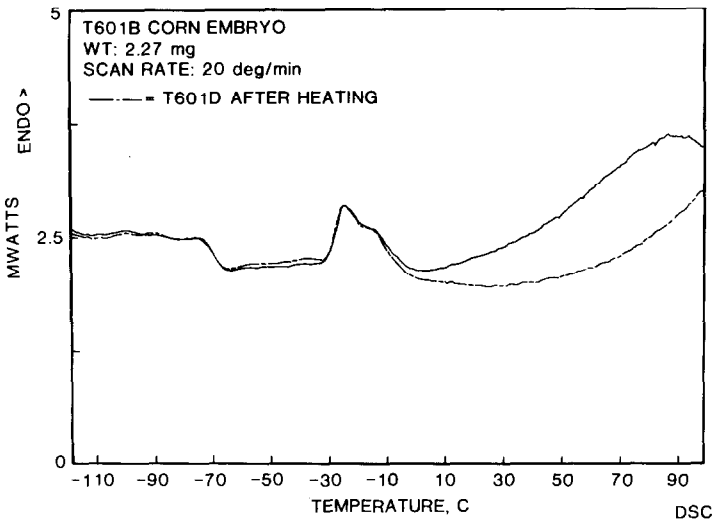


Fig. 2. The effect of drying on the DSC thermogram of corn embryos. An embryo (8.4% water content) was cooled at 20 °C $min^{-1}$ and warmed (solid line). The pan was punctured, the sample was dried for 2 h in the calorimeter at 100 °C, cooled at 20 °C $min^{-1}$ and rewarmed (dashed line). Except for the loss of heat capacity in the dried sample at temperatures above freezing, the two are similar, indicating that water is not responsible for these transitions.

apparent double melting peak at about −15 °C. The exothermic events in the region between −85 and −80 °C, and between −70 and −30 °C are most easily interpreted as devitrification events. After these measurements

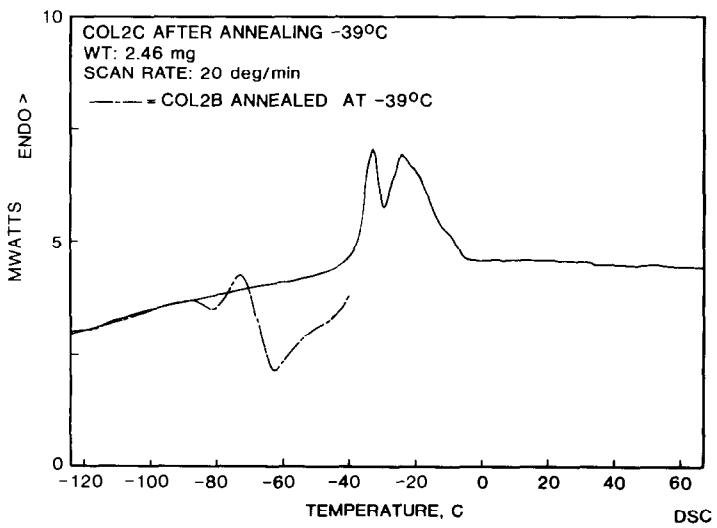


Fig. 3. DSC thermograms of the annealing (dashed line) and heating (solid line) of commercial corn oil, indicating that these storage lipids are responsible for the bulk of the thermal signal in corn embryos.

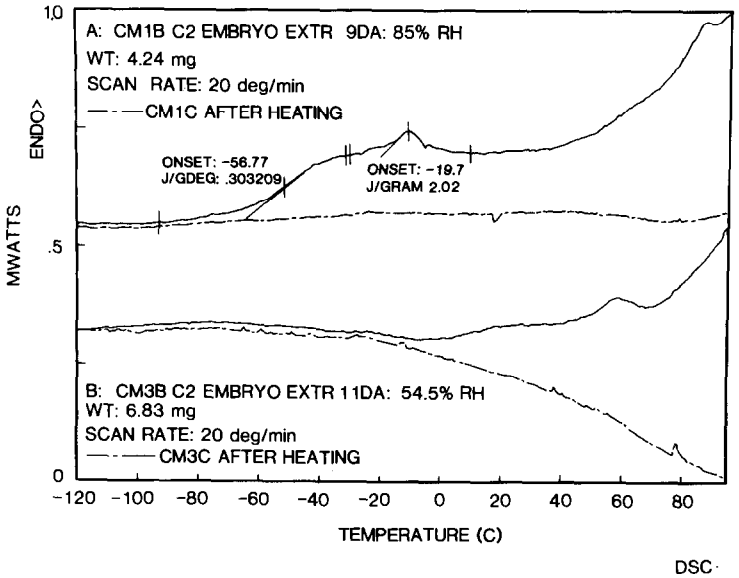


Fig. 4. DSC thermograms of defatted corn embryos. In part A (upper curves) the sample was equilibrated to a water content of 22.2%. It can be seen by comparing the thermogram of the embryo (solid curve) with that of the same embryo after drying (dashed curve) that a glass transition begins below −60 °C. An endotherm is present at just below 0 °C, indicating the presence of freezable water at this water content. Part B (lower curves) presents thermograms of an embryo equilibrated to a water content of 14%. The glass transition is elevated to a higher temperature and is less distinct.

were made, the embryo was planted in Perlite and found to be still germinable.

To establish whether or not these glass transitions involved water, comparisons were made of an embryo before and after drying (Fig. 2). It is clear during reheating (dashed line) that, except for the lowered heat capacity at temperatures above 0 °C in the dried specimen, the signal pattern was unchanged by the removal of water.

Suspecting that lipids are involved in producing these signals, DSC runs were made of commercial corn oil (Mazola); the annealing (dashed line) and warming (solid line) curves for the oil alone, shown in Fig. 3, resemble signals for whole embryos such as those presented in Fig. 1. Membrane lipids are present in too small an amount to be detectable by this instrument.

To determine whether the oils might mask other signals, embryos from which oils had been extracted were equilibrated at a series of relative humidities. Representative thermograms are given in Fig. 4, in which each sample is compared with itself after drying. An embryo with a 22.2% water content (4A) shows a glass transition beginning below −60 °C. An aqueous melt is also apparent at about −10 °C. An embryo at 14% water content (4B) shows a smaller glass transition signal which is elevated to about −10 °C.

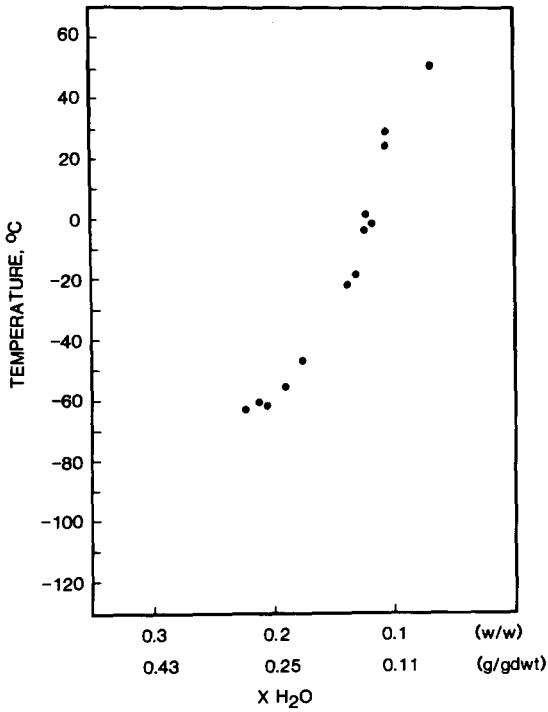


Fig. 5. The glass transition temperature as a function of the water content of corn embryos, derived from data including those in Fig. 4. This line closely parallels that of sucrose.

The temperature at which the principal glass transition begins as a function of the water content of the embryo is plotted in Fig. 5. The glass transition begins at increasing temperatures as the water content is lowered. The data in Fig. 5 closely parallel those reported for sucrose.

## DISCUSSION

It is evident that vitrification can occur in the corn embryo in both the lipidic and the non-lipid components. Judging from Fig. 5, at the water content usually associated with corn seeds in storage ($< 12\%$ water) the glassy state associated with non-lipid fractions exists even at ambient temperatures. Vitrification may provide real advantages in terms of the desiccation tolerance of seeds, greatly delaying solute crystallization and total dehydration. The inhibition of molecular mobility would restrict denaturation as well as chemical and biochemical reactions. Also, the unfreezability of the water component would contribute osmotic tolerance over a wide range of temperatures and humidities. The stability of glasses varies widely with temperature and composition and aqueous glasses are not especially stable. Thus, the processes we propose to be operating in seeds

cannot confer immortality, but can postpone the inevitable for a sufficient period of time to be significant to the life cycle of the organism.

The solutes which contribute to the non-lipid glassy state probably include the common sugars. Sucrose contributes 17% and raffinose 3% to the dry weight. Thus, 20% of the embryo dry weight is composed of solutes that readily vitrify. The lack of freezing or melting transitions for water in embryos drier than 20% water confirms the absence of freezable water in dry orthodox seeds. Finally, the existence of the glassy state in dry corn embryos suggests an important role for this state of highly restricted molecular mobility in the survival of the desiccated seed.

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