Use of metabolic inhibitors to elucidate mechanisms of recovery from desiccation stress in the resurrection plant \textit{Xerophyta humilis}

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\textbf{Abstract}

\textit{Xerophyta humilis} (Bak.) Dur. and Schinz is a poikilochlorophyllous resurrection plant in that it is tolerant of considerable water loss (< 5% relative water content [RWC]) and thylakoid membranes are dismantled and chlorophyll is lost during dehydration. In this paper we examined the processes associated with recovery from desiccation upon rehydration. Dried leaf explants were rehydrated in water (control) or in solutions of actinomycin-D or cyclohexamide in order to determine to what extent initial recovery was dependant on \textit{de novo} transcription and translation respectively. Our results suggest that considerable protection of subcellular organisation and components of metabolism occurs during drying such that the initial recovery of metabolism on rehydration is virtually independent of \textit{de novo} transcription of nuclear genes. However recovery does require the synthesis of new proteins. The plasmalemma remains intact and macromolecular synthesis is not required for maintenance of its integrity. Messenger RNA’s for chlorophyll biosynthesis appear to be stored in a stable form in the dried leaves and are translated on rehydration. Similarly most of the mRNA’s necessary for recovery of electron transport in the chloroplast (as determined by measuring the quantum efficiency of photosystem II [$F_v/F_M$] using chlorophyll fluorescence) appear to be stably present in the dried leaves. However, for total recovery of $F_v/F_M$ new genomic transcription is necessary.

\textbf{Introduction}

The phenomenon of desiccation tolerance is widespread in nature, being found among the simpler plants such as mosses and ferns, the seeds and pollen of higher plants and a few invertebrate animal systems such as brine shrimp eggs. It is relatively uncommon in the vegetative tissue of higher plants and is known only in a few taxonomically diverse angiosperms. The monocotyledonous plant \textit{Xerophyta humilis} (Bak.) Dur. and Schinz is an example of such a desiccation tolerant, or resurrection, plant [3].

Extreme water loss results in considerable damage at the subcellular level in sensitive tissues. Mechanical stress occurs when water loss from the vacuole and cytoplasm places tension on the plasmalemma and ultimately disrupts this membrane. Metabolites and ions become concentrated and membrane and macromolecular structures become impaired as water becomes scarce and hydration shells are removed (reviewed by Vertucci and Farrant [14]). Light-chlorophyll interactions cause damage to the photosynthethic apparatus [11]. Desiccation-related damage results not only from processes occurring during drying, but also from the inrush of water into dry cells on rehydration [12]. As many of the techniques used to examine the nature of damage inevitably cause tissue hydration, it has been difficult to distinguish between desiccation and rehydration induced damage.

Theories on the mechanisms by which resurrection plants tolerate dehydration have mostly been derived from observations of the cellular processes which
occur during drying of the plant (previously reviewed [6]). These include the accumulation of non-reducing sugars which are suggested to protect membranes in the dry state and/or to promote cytoplasmic vitrification [7, 15]. Similarly, the accumulation of metabolites (collectively called compatible solutes) such as proline and glycine betaine could facilitate glass formation and osmotic adjustment [2]. Changes occur in the protein complement, including the synthesis of LEA-like (dehydrin) and heat shock proteins, as well as other proteins of as yet unknown function, many of which are regulated by abscisic acid (ABA) [6]. Poikilochlorophyllous plants lose chlorophyll and dismantine thylakoid membranes [10, 13] and it has been suggested that this prevents photooxidative damage when dry [11].

The processes associated with tissue recovery on rehydration have been less extensively studied. It is not known whether protective systems set in place during drying are sufficient for physiological recovery after desiccation, or whether a new genetic programme must be implemented on rehydration. It has been suggested that in lower orders recovery is largely based on the ability to repair desiccation and/or rehydration associated damage. Furthermore, in species such as the moss Tortula ruralis recovery does not appear to require a new programme of gene expression but rather an upregulation, during rehydration, of certain mRNA’s [1, 9]. The situation may be more complex in desiccation tolerant angiosperms. Sherwin and Farrant [10] have shown that Craterostigma wilmsii retained chlorophyll and ultrastructural integrity on drying and have suggested that this species would rely more on protection during dehydration rather than repair on rehydration. Poikilochlorophyllous plants, however, have to reconstitute thylakoid membranes [5] and resynthesise chlorophyll on rehydration and might require a larger repertoire of post-desiccation recovery mechanisms [10].

X. humilis is a small poikilochlorophyllous resurrection plant. The aim of this study was to determine to what extent initial recovery from desiccation is dependent on de novo transcription and translation. Leaves from desiccated plants were rehydrated in the presence or absence of inhibitors of transcription (actinomycin-D) and translation (cyclohexamide) and the effect of these on ultrastructural organisation and recovery of photosynthetic apparatus was followed.

Materials and methods

Plant material and inhibitor application

Whole plants of X. humilis (Velloziaceae) collected in the Pilansberg Nature Reserve, Northwest Province South Africa (25°40’S, 28°32’E) were maintained as previously described for other resurrection plants [10]. Plants were dried in soil (to ca 5% RWC) by withholding water for at least 7 days. Actinomycin-D is excluded by roots but not by leaves of this plant. Thus in order to assess the effect of inhibition of transcription on the ability to recover from desiccation, studies had to be performed on leaf explants. Dried leaves were cut into 5 mm sections for further study. Similar explants were taken from fully hydrated plants to determine the turnover time of regular “housekeeping” metabolism.

Explants were placed for 48 hr in 50 µl of water or solutions of actinomycin-D or cycloheximide at 20 µg ml⁻¹. This concentration was previously determined to be sufficient to inhibit incorporation of radioactive precursors into RNA and protein respectively (data not shown). The relative water contents (RWC), the quantum efficiency of photosystem II (Fv/FM) and electrolyte leakage of explants were determined at regular intervals during the 48 hour period. Chlorophyll content and ultrastructural status was determined for dried and rehydrated explants and for hydrated (“housekeeping” control) explants after 48 h in inhibitor solutions. Five or six explants were used for each determination and the entire procedure was performed twice.

Relative water contents (RWC)

RWC was measured using the standard formula: RWC = water content / water content at full turgor; and was expressed as a percentage. Water content was determined gravimetrically by oven drying at 70 °C for 48 h.

Electrolyte leakage

Membrane integrity was assessed by immersing the explants in 2.5 ml Milli-Q (Millipore) ultra-pure water and monitoring the change in conductivity at regular intervals for 40 mins using a Jenway 4070 conductivity meter. The leakage rate was corrected for leaf dry mass. Total maximum leakage was determined on explants which had been killed by autoclaving. Leakage values are expressed as a percentage of the maximum.