BIOLOGIA PLANTARUM 53 (2): 373-377, 2009

BRIEF COMMUNICATION

# Ultrastructural responses of the desiccation tolerant plants *Xerophyta viscosa* and *X. retinervis* to dehydration and rehydration

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## Abstract

This paper compares the changes in water content, chlorophyll *a* fluorescence and leaf ultrastructure during dehydration and rehydration in two desiccation tolerant plants *Xerophyta viscosa* and *X. retinervis*. Both species showed decreasing quantum efficiency of photosystem 2 ($F_v/F_m$) with decreasing water content. Extreme water loss observed after 25 d of dehydration resulted in considerable damage of leaf tissue ultrastructure. After rehydration, both species need several days to reconstitute their photosynthetic machinery.

*Additional key words*: drought tolerance, poikilochlorophyllous species, quantum efficiency, relative water content, resurrection plant, transmission electron microscopy.

Water is a limiting factor in many environments, and some higher plant species are well adapted to arid habitats. Resurrection plants are able to shut down metabolism and dismantle cell structure during times of drought and then restart living processes when water becomes available. Resurrection plants are found in tropical and sub-tropical areas, and to a lesser extent in temperate zones. The habitats of these plants are characterized by lengthy periods of drought, where rainfall is extremely sporadic (Porembski and Barthlott 2000). Studies have revealed that to survive in dry habitats, resurrection plants need to overcome a number of stresses caused by dehydration, such as oxidative stress, destabilization or loss of membrane integrity, and mechanical stress (Vicré *et al*. 2004). Different resurrection plants may utilize various physiological and biochemical mechanisms to adapt to desiccation and to regain normal metabolic status upon rehydration (Bewley and Krochko 1982, Navari-Izzo and Rascio 1999).

Generally, two types of desiccation tolerant plants have been recognized based on chlorophyll quantities during desiccation. One group of plants is poikilochlorophyllous, those that lose their chlorophyll on drying, and the other is homoiochlorophyllous, those that retain chlorophyll (Gaff 1977, Tuba *et al*. 1993). Studies by Bernacchia *et al*. (1996) on the homoiochlorophyllous resurrection species *Craterostigma plantagineum* revealed that fully mature leaves can lose up to 95 % of their water content and then, upon rewatering, the leaves are rehydrated and become fully photosynthetically active within 24 h. A number of morphological modifications associated with dehydration have been observed as adaptations in resurrection plants to minimize the damage caused by excess radiation and free radical stress in dry tissues (Tuba *et al*. 1996). Previous studies on the poikilochlorophyllous species *Xerophyta scabrida* (Tuba *et al*. 1994, 1996), *X. viscosa* (Mundree and Farrant 2000, Whittaker 2001) and *X. humilis* (Dace *et al*. 1998, Farrant *et al*. 1999, Farrant 2000, Farrant *et al*. 2003) showed that these plants lose their photosynthetic apparatus during drying, however, it is resynthesized following rehydration. A study by Ingle *et al*. (2007) on proteomic analysis of *X. viscosa* showed that a number of dehydration responsive proteins may play a role in desiccation tolerance, including antioxidants, RNA binding proteins and proteins involved in photosynthesis. Also, it

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*Received* 27 May 2008, *accepted* 15 April 2009.

*Abbreviations*: $F_v/F_m$ - variable to maximum fluorescence ratio; RWC - relative water content; TEM - transmission electron microscopy.

*Acknowledgements*: The authors gratefully acknowledge the National Research Foundation for financial support. Our thanks to Prof. Gonasageran Naidoo for plant collection and suggesting the line of research. The authors also thank J.W. Smith, P. Maartens, S. Eggers, F. Khan, P. Ellapen, T. Hiralal and A. Rajh for technical assistance.

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has been proposed that different plants rely on the protection and repair to different extents (Bewley and Oliver 1992). The present study aims to further investigate the ultracellular responses of *X. viscosa* and, for the first time, *X. retinervis* to dehydration and rehydration. A comparison of cellular ultrastructure and photosynthetic responses during dehydration and rehydration were made to explore their potential roles in the resurrection phenomenon of these two species.

Plants of *Xerophyta viscosa* Baker were collected from the Weza State Forest Reserve and *Xerophyta retinervis* Baker from Paris Dam, Vryheid. They were grown in a mixture of sand and potting soil and maintained in a glasshouse at the University of KwaZulu-Natal, Durban, South Africa. Slow drying of whole plants of *X. viscosa* and *X. retinervis* was achieved by withholding water from the substrate. Rehydration was carried out by watering the plants using a spray to simulate rainfall. Rehydration was initiated immediately after the plant leaves dried to a brown colour (relative water content, RWC, 37.88 % in *X. viscosa* and 36.25 % in *X. retinervis*). Experiments were performed in triplicate for each sampling date (three different plants were sampled). The different sampling stages are depicted as $D_1$ (control - 0 d), $D_2$ (15 d dehydration), $D_3$ (25 d dehydration), $D_4$ (5 d after rehydration) and $D_5$ (10 d after rehydration.

For determination of RWC and chlorophyll *a* fluorescence, five measurements were taken from mature fully expanded leaves of both species during every sampling stage. Relative water content was measured by gravimetric method according to Sherwin and Farrant (1996). The quantum efficiency of photosystem 2 was determined as variable to maximum fluorescence ratio ($F_v/F_m$) of leaves with a field portable pulse amplitude modulated fluorometer (*PAM-2100*, *Walz*, Effeltrich, Germany). All measurements were taken on the lamina, midway between the base and tip of young fully expanded leaves. For transmission electron microscopy (TEM), leaf segments of 2 $mm^2$ were excised and fixed overnight at 4 °C in 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Approximately 10 samples from 5 leaves were examined. Leaf samples were dehydrated in ethanol and embedded in low viscosity resin. Ultrathin sections were cut using a *Reichert ultracut-S* ultramicrotome and collected on square mesh copper grids. Sections were post stained with 2 % aqueous uranyl acetate followed by lead citrate and viewed with a *Jeol 1010 TEM* microscope at 60 kV.

In control plants, leaves were green and fully expanded. As the plant dried ($D_3$ stage), the leaves showed remarkable longitudinal zigzag folding and turned brown. The markedly decreased RWC in *X. retinervis* was found only in $D_3$ stage, while in *X. viscosa* in $D_2$ and $D_3$ stages. With the reduction of water content, the quantum efficiency ($F_v/F_m$) also showed decreasing patterns in both the species (Table 1). Upon rehydration, both species showed recovery of $F_v/F_m$, indicating that chloroplasts again became functional.

Chloroplasts in mesophyll cells of control plants occurred in the cell periphery and occupied most of the protoplasmic volume (Fig. 1*A*,*B*). Most of the cells contained a single large vacuole, ellipsoidal chloroplasts with well organized thylakoid membranes, dispersed plastoglobuli and starch. Mitochondria possessed well developed cristae, typical of metabolically active tissue. The palisade parenchyma comprised several layers of cells and had limited intercellular spaces. Vacuoles and mitochondria were visible in mesophyll cells in both hydrated and dehydrated plants. However, marked changes in cellular ultrastructure were observed at relative water contents between 76.25 to 37.88 % in *X. viscosa* and 95.17 to 36.25 % in *X. retinervis* (Fig. 1*E*,*F*). During the initial phase of progressive dehydration ($D_2$ stage) the mesophyll contains intercellular air spaces and mesophyll cells contained large vacuoles (Fig. 1*C*,*D*). More severe damage to the leaf subcellular structure was observed in plants of $D_3$ stage when changes in the chloroplast shape and structure were observed (Fig. 1*E*,*F*). The plasmodesmata were visible in mesophyll cells in both hydrated and dehydrated plants (not shown). The thick cell wall was present in both species during dehydration and rehydration, but the cells showed the highest plasma membrane damage at the $D_3$ stage (although plasmalemma remained appressed to the cell wall and the outer membranes of the organelles were intact) (Fig. 1*G*,*H*). However upon rehydration ($D_4$ and $D_5$ stages) tissues reorganized their photosynthetic apparatus (Fig. 1*I*,*J*).

Table 1. Changes in relative water content and quantum efficiency of PS 2 ($F_v/F_m$) in leaves during dehydration and rehydration in *Xerophyta viscosa* and *X. retinervis* ($D_1$ - control, $D_2$ - 15 d dehydration, $D_3$ - 25 d dehydration; $D_4$ - 5 d rehydration, $D_5$ - 10 d rehydration).

| | *X. viscosa* | | *X. retinervis* | |
|---|---|---|---|---|
| | RWC [%] | $F_v/F_m$ | RWC [%] | $F_v/F_m$ |
| $D_1$ | 91.70 | 0.789 | 97.97 | 0.779 |
| $D_2$ | 76.25 | 0.766 | 95.17 | 0.760 |
| $D_3$ | 37.88 | 0.092 | 36.25 | 0.285 |
| $D_4$ | 93.20 | 0.779 | 95.68 | 0.641 |
| $D_5$ | 89.48 | 0.718 | 93.02 | 0.700 |

It is well known that, unlike other plants, desiccation tolerant species are capable of surviving fairly prolonged dry periods. To survive during these periods, plants use a complex strategy that operates at the morphological, physiological and ultrastructural levels. Leaf tissues generally lose all free water, and appear ‘dead’. These tissues rehydrate in the presence of available water. This remarkable ability to survive extreme dehydration in the vegetative tissues has so far only been observed in approximately 100 angiosperm species (Gaff 1997).

In the present study, water stressed plants showed remarkable longitudinal zigzag folding of leaves. This

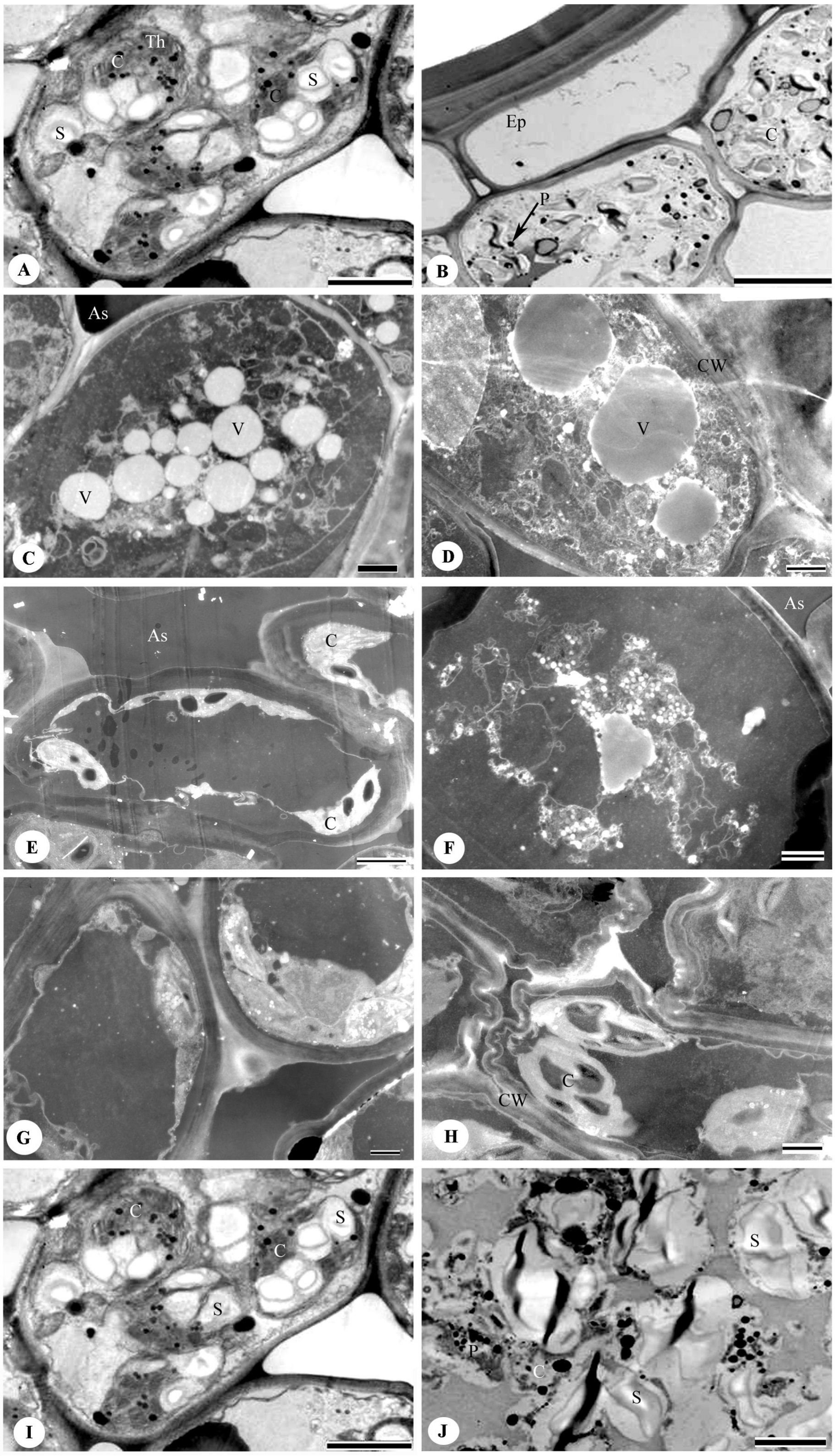

Fig. 1. Subcellular organization of mesophyll cells of *X. viscosa* (*A*,*C*,*G*,*E*,*I*) and *X. retinervis* (*B*,*D*,*F*,*H*,*J*) at different stages of dehydration and rehydration: D$_1$ - *A*,*B*; D$_2$ - *C,D*; D$_3$ - *E*,*F*; D$_4$ - *G*,*H*; D$_5$ - *I*,*J*. *Bar* = 10 µm (*A*,*B*,*I*,*J*,); 1 µm (*C*,*D*,*E*.*F*,*G*,*H*). As - air space, C - chloroplast, CW - cell wall, Ep - epidermis, P - plastoglobuli, S - starch, Th - thykaloid membrane, V - vacuole.

was probably due to alteration of the reinforced and non reinforced parallel cellulose bands in the leaf tissue of both species. These findings are consistent with the results of Rosetto and Dolder (1996) who reported such folding in *Nanuza blicata*. Similar contraction of mesophyll tissue between parallel veins of *X. scabrida* occurred during dehydration and resulted in a decrease in exposed leaf area, which in turn minimized water loss (Tuba *et al*. 1996). According to Sherwin and Farrant (1998) the drop in the chlorophyll content in the leaves of *X. viscosa* is accompanied by the accumulation of anthocyanins. The process of dismantling of the photosynthetic apparatus and the synthesis of anthocyanin are thought to be linked to the protection of plants against UV radiation and damage as a result of oxygen free radical generation (Sherwin and Farrant 1998). *X. viscosa* and *X. retinervis* lose chlorophyll and dismantle the internal (membrane) structure of chloroplasts during desiccation, turning the leaves brown. Similar findings have been reported for *X. scabrida* by Tuba *et al*. (1993).

In the poikilochlorophyllous resurrection plants, the main reason for the drop in $F_v/F_m$ during dehydration is the dismantling of chloroplasts, which may be necessary to prevent excess light absorption and photooxidative damage when they dry (Dace *et al*. 1998, Sherwin and Farrant 1998, Farrant 2000). These plants, however, have to reconstitute thylakoid membranes and resynthesize chlorophyll on rehydration, and may require a large repertoire of post desiccation recovery mechanisms (Sherwin and Farrant 1996).

In plants that are not drought tolerant, extreme water loss results in irreversible 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´s integrity (Dace *et al*. 1998). Metabolites and ions become concentrated, and membrane and macromolecular structure become impaired as water becomes scarce and hydration shells are removed (Vertucci and Farrant 1995). In contrast, our observations of drought resistant leaves of *X. viscosa* and *X. retinervis* showed that the plasmalemma remained appressed to the cell wall and the outer membranes of the organelles were intact. Similar results were reported for other poikilochloro-phyllous resurrection plants (Sherwin and Farrant 1996, 1998, Dace *et al*. 1998).

On rewatering, *X viscosa* and *X. retinervis* started reconstruction of thylakoids when the tissue reached full hydration. The recovery of photosynthetic activity is longer in poikilochlorophyllous plants than in homoiochlorophyllous plants (Tuba *et al*. 1996). The formation of many vacuoles during dehydration was consistent with the reports of Farrant (2000) who indicated that bundle sheath cells maintain cell volume by multiple (small) vacuole formation in *X. viscosa* and *X. humilis*. Also, compatible solutes such as proline, sugars, organic acids, phenols are accumulated (Van der Willigen *et al*. 2002) to maintain cell pressure potential.

Farrant (2000) reported that *X. viscosa* disassembles the chloroplast and chlorophyll on drying. This study confirms these findings and also reports for the first time on the poikilochlorophyllous nature of *X. retinervis*. After rewatering, plants achieved similar values of quantum efficiency as in control plants and dried tissue regained the subcellular organization typical of the hydrated state.

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