Differences in Rehydration of Three Desiccation-tolerant Angiosperm Species

HEATHER W. SHERWIN* and JILL M. FARRANT

Botany Department, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa

Received: 20 February 1996 Accepted: 5 June 1996

The rehydration characteristics of the desiccation-tolerant plants Craterostigma wilmsii and Myrothamnus flabellifolia (homoiochlorophyllous) and Xerophyta viscosa (poikilochlorophyllous) were studied to determine differences among them. A desiccation-sensitive plant (Pisum sativum) was used as a control. Recovery of water content, quantum efficiency ($F_v/F_m$), photosynthetic pigments and chloroplast ultrastructure were studied.

P. sativum did not recover after desiccation and considerable damage occurred during rehydration. The desiccation-tolerant plants appeared to differ in their responses to dehydration and rehydration. The small herbaceous C. wilmsii generally showed little damage in the dry state and recovered faster than the other tolerant species. M. flabellifolia took longer to recover than C. wilmsii probably due to the presence of a woody stem in which dehydration-induced xylem embolisms slowed the rate of recovery. The poikilochlorophyllous species X. viscosa took the longest to recover because it took longer to reconstitute the chloroplasts and the photosynthetic pigments. Quantum efficiency recovered in all species before water content and chlorophyll content recovered to control levels. The significance of these different responses to desiccation and recovery from desiccation is discussed.

© 1996 Annals of Botany Company

Key words: Desiccation-tolerant, $F_v/F_m$, homoiochlorophyllous, poikilochlorophyllous, chlorophyll, chloroplast, ultrastructure, Craterostigma wilmsii, Myrothamnus flabellifolia, Xerophyta viscosa, Pisum sativum.

INTRODUCTION

While the vegetative tissues of most angiosperm species cannot survive severe water stress there are a few species which do tolerate desiccation. These plants are commonly known as ‘resurrection’ plants, due to their unique ability to revive from an air-dried state (Gaff, 1971, 1989). Two types of desiccation-tolerant angiosperms have been recognized: those that lose chlorophyll on drying (poikilochlorophyllous) and those that retain chlorophyll (homoiochlorophyllous) (Gaff, 1977; Bewley, 1979; Tuba et al., 1993). To date there have been no studies reported which have compared the desiccation responses of these two types of plant.

It has been suggested that there are several challenges which have to be overcome if a plant tissue is to be truly tolerant of desiccation (Vertucci and Farrant, 1995). These include the ability to: (a) minimize mechanical damage associated with turgor loss; (b) maintain the integrity of macromolecules and membranes by the accumulation of water stress proteins (dehydrins) and compatible solutes; and (c) minimize toxin accumulation and free radical damage generated as metabolism becomes impaired. Upon rehydration, such plant tissues must be able to repair desiccation-induced damage. It has been proposed that different plants will rely on the processes of protection and repair to differing extents (Bewley and Oliver, 1992). Most of the studies on desiccation tolerance have focused on how plants survive in the dry state, while less work has been done on the processes occurring during rehydration. During rehydration the passage to a metabolically active state poses particular problems if metabolic ‘mayhem’ is to be avoided (Stewart, 1990). Too rapid rehydration has been shown to cause damage to dry seeds (Simon, 1974; Hoekstra and van der Wal, 1988). While rehydration causes its own set of challenges to the desiccation-tolerant plant, it is also the period when repair of physical damage and the restoration of metabolic activity must take place. Thus, in studying the phenomenon of desiccation tolerance, the study of the processes occurring on rehydration are important.

Studies on the rehydration of desiccation-tolerant mosses, ferns and angiosperms (reviewed in Bewley, 1979; Bewley and Krochko, 1982; Gaff, 1989) have shown that recovery is rapid and that, in general, it is quicker in homoiochlorophyllous than in poikilochlorophyllous plants. Ultrastructural studies on the former have shown that some damage does occur on drying but this is repaired on rehydration (Bewley, 1979; Schneider, et al., 1993; Sherwin, 1995). In Craterostigma nana, chlorophyll fluorescence parameters were almost fully recovered after 18 h of rehydration (Sherwin, 1995). In the poikilochlorophyllous types examined to date, there were extensive changes to chloroplast structure which can take up to 3 d after rehydration to return to normal (Hetherington, Hallam and Smillie, 1982; Gaff, 1989; Tuba et al., 1993). At least 2 d are required for normal fluorescence characteristics (Hetherington et al., 1982; Tuba et al., 1994). Studies on rehydration of excised leaves of the poikilochlorophyllous Xerophyta scabrida (Tuba et al., 1994) have shown that the leaves take approximately 24 h to rehydrate, after which chlorophyll

* For correspondence.

0305-7364/96/120703+08 $25.00/0 © 1996 Annals of Botany Company
production increases. In all species examined, carbon assimilation reached a maximum after chlorophyll production was complete and chloroplast ultrastructure regained a normal appearance.

Studies done to date have used either whole plants or excised leaf parts, making comparisons difficult. Furthermore, the rehydration studies reported have generally only used isolated leaves. Unpublished work from our laboratory has indicated that rehydration of isolated leaves does not result in the full recovery of photosynthetic activity.

The aim of this work was to compare the rehydration of whole plants of three different desiccation-tolerant plant species and compare those to the rehydration of a desiccation-sensitive plant, examining specifically the changes occurring to the photosynthetic apparatus. The three tolerant species chosen differed in form and in their physical and physiological response to desiccation. *Craterostigma wilmsii* Engl. (Scrophulariaceae) is a small herbaceous plant that curls its leaves tightly on drying and is homiochlorophyllous. *Myrothamnus flabellifolia* Welw. (Myrothamnaceae) is a woody shrub often reaching heights of 1 m. On dehydration, the leaves fold against the stem and chlorophyll is retained in the adaxial mesophyll layers. The outer, abaxial surfaces become dark brownish-red. *Xerophyta viscosa* Baker (Velloziaceae), a monocotyledonous plant, is poikilochlorophyllous; the leaves yellow and fold in half along the main rib on drying. The desiccation-sensitive plant *Pisum sativum* L. (Fabaceae) was used as a control to establish differences between the tolerant plants and one sensitive to desiccation.

**MATERIALS AND METHODS**

Plants which had been dried to $<5\%$ relative water contents (RWC) were allowed to rehydrate and their recovery was monitored. Measurements of electrolyte leakage were performed to assess membrane integrity and thus viability. Changes in pigment content were determined and chloroplast status was assessed in terms of quantum efficiency and subcellular organization.

**Plant material**

The desiccation-tolerant plants were collected from the Buffelskloof Nature Reserve near Lydenberg (Mpumalanga Province, South Africa). They were grown in a mixture of peat, river sand and potting soil and were maintained in a greenhouse under 30\% shadecloth with no supplementary lighting. To maintain the hardness of the plants, they were subject to regular cycles of desiccation and rewatering.

Seeds of *P. sativum* (cv. Greenfeast) were sown in trays of potting soil and the material was kept in a greenhouse under the same conditions as the other plants. Plants were dried after 3 weeks of growth.

**Dehydration and rehydration**

Whole plants of the four species were dried by withholding water and allowing the plant to dry out naturally. Once no more water was lost from the leaves (RWCs $<5\%$) the plants were left in the dry state exposed to natural sunlight for between 1 and 3 months. Rehydration was carried out by watering the plants using a spray to simulate rainfall. Plants were well watered on the first day and then the soil was kept damp for the remainder of the experiment. *P. sativum* leaves did not rehydrate upon soil watering. Rehydration was achieved by placing excised leaves directly into water.

At regular intervals during the rehydration process, the procedures outlined below were performed on leaf tissues. In the case of *C. wilmsii* only the middle leaves of the rosette were used and for *M. flabellifolia* and *X. viscosa* mature, fully expanded leaves were used. The two youngest fully expanded leaves were used in the case of *P. sativum*. Several separate rehydration experiments were conducted (four for *C. wilmsii*; three for *M. flabellifolia*; two for *X. viscosa*; and five for *P. sativum*) and in each case five leaf samples were taken for each of the experimental procedures.

**Electrolyte leakage**

Leakage measurements were performed on desiccated and fully rehydrated leaves of each species. As a control, the leakage of leaves which had not been desiccated and rehydrated was also monitored. The leaves (*M. flabellifolia*) or leaf pieces (*C. wilmsii, X. viscosa* and *P. sativum*) were placed directly into pure (distilled, de-ionised and filtered) water and electrolyte leakage was measured every minute for 40 min using a conductivity meter (Jenway 4070). The rate of leakage was calculated (the slope of line generated from the time course of leakage) and was corrected by leaf dry mass.

**Chlorophyll fluorescence**

A modulated portable fluorometer (OS-500: Opti-Sciences, USA) was used to calculate the quantum efficiency of leaves at various stages of hydration. The initial fluorescence, $F_o$, and maximum fluorescence, $F_m$, using a saturating light intensity of approximately 4 mmol photons m$^{-2}$ s$^{-1}$ and a duration of 1 s, were measured. $F_v$ was obtained by subtracting $F_o$ from $F_m$ and $F_v/F_m$ was calculated.

**Chlorophyll and carotenoid content**

Photosynthetic pigments were extracted in 100\% acetone and the absorbances were measured using a Schimadzu UV-2201 light/UV spectrophotometer (Schimadzu Scientific Instruments Inc., USA). Chlorophyll and carotenoid contents were calculated using the adjusted extinction coefficients according to Lichtenthaler (1987).

**Ultrastructure**

Leaf segments (approximately 5 mm$^2$) were excised randomly from fully turgid leaves (which had not undergone any dehydration) as well as leaves in the dry and partially rehydrated state (approximately 50\% RWC). These were fixed in 2.5\% glutaraldehyde in 0.1 M phosphate buffer
hydrate at all while the three desiccation-tolerant plants. The desiccation-sensitive

Figure 1 shows the time course of rehydration of the four species. The desiccation-sensitive *P. sativum* did not rehydrate at all while the three desiccation-tolerant plants regained full turgor. Leaves of *C. wilmsii* were fully hydrated by 48 h after rewatering while those of *M. flabellifolia* took 65 h and *X. viscosa* 92 h. Both *C. wilmsii* and *X. viscosa* rehydrated at relatively constant rates. The more rapid rehydration in *C. wilmsii* was presumably because it was smaller and is herbaceous. In *M. flabellifolia*, water uptake during the initial 12 h was slow, but thereafter the rate of rehydration increased and was similar to that of *C. wilmsii*. *M. flabellifolia* is a woody plant, which probably accounts for this pattern of water uptake. Once the xylem system became functional (presumably after 12 h) the rate of uptake increased.

There was no significant difference in the electrolyte leakage rate among the control, dry and rehydrated leaves of *C. wilmsii* (Fig. 2), suggesting that the integrity of the plasma membrane was maintained during both drying and rehydration of this species. Leakage in the dry state was greater in *X. viscosa* than in *M. flabellifolia*, but in both species leakage returned to control levels on rehydration. Thus drying appeared to cause some change in membrane configuration, but this was reversed and/or repaired on rehydration of those species. In contrast to the tolerant species, dry leaves of *P. sativum* showed a significant increase in electrolyte leakage compared to the control and there was a further substantial increase in leakage upon rehydration of these leaves (Fig. 2). Thus, the desiccation-induced membrane changes were not reversible on rehydration in this species.

The changes in quantum efficiency (*F*/Fm) with rehydration are shown in Fig. 3A. The desiccation-tolerant species all showed complete recovery of this variable, indicating that chloroplasts became fully functional upon rehydration. *C. wilmsii* recovered most rapidly, reaching control levels by 32 h. In *M. flabellifolia* and *X. viscosa* the rate of recovery was similar, but the latter took longer for the quantum efficiency to return to control levels (76 h in *X. viscosa* cf. 55 h in *M. flabellifolia*). In each case, *F*/Fm recovered to control levels before water content had reached maximum levels. *P. sativum* did not recover photosynthetic capacity (Fig. 3A).

Figure 3B shows the changes in chlorophyll content during rehydration. *X. viscosa* lost almost all chlorophyll on drying; very little was reconstituted within the first 24 h but thereafter chlorophyll content increased, reaching control levels after 120 h. *C. wilmsii* and *M. flabellifolia* lost 50 and 70% of leaf chlorophyll, respectively. The chlorophyll content of *M. flabellifolia* recovered rapidly and reached control levels after 24 h. The chlorophyll content of *C. wilmsii* recovered almost to control levels after 45 h. Thus *M. flabellifolia* recovered chlorophyll content to control levels before *F*/Fm had recovered fully. On the contrary, *C. wilmsii* and *X. viscosa* recovered *F*/Fm before their chlorophyll contents had fully recovered. As with the other variables *P. sativum* showed no recovery of the 75% of chlorophyll that it lost on drying.

The carotenoid content declined during dehydration of *M. flabellifolia*, *X. viscosa* and *P. sativum* whereas *C. wilmsii* retained its carotenoid levels (Fig. 3C). Carotenoid levels declined by 60% in *X. viscosa* and, as with chlorophyll production, there was a 24 h delay before recovery of those

**RESULTS**

**Fig. 1.** Increase in water content with time after rehydration. The water content is represented as a percentage of the control value. The plotted data points are means of at least ten measurements and the standard deviations of these means are represented by the vertical lines. (■) *C. wilmsii*, (□) *M. flabellifolia*, (▲) *X. viscosa* and (●) *P. sativum*.

**Fig. 2.** Changes in electrolyte leakage (as a percentage of the control) in the four species. Control (□) levels were taken from fully turgid leaves which had not been dehydrated or rehydrated. These were compared with levels of hydrated (◼) and tissue that had been rehydrated (■) to full water content. Differences between letters indicate significant differences (Anova and Duncan’s Multiple Range test, 95% confidence level) in leakage between treatments within and among species.
pigments commenced. Total recovery occurred after 96 h. In *M. flabellifolia*, the recovery of carotenoid levels was slower (72 h) than the recovery of chlorophyll (24 h). *P. satium* showed no recovery of carotenoids on rehydration.

Figures 4–7 show the chloroplast organization from control plants and those from dry and partially rehydrated leaves. The use of standard fixation techniques might have allowed some rehydration of the desiccated leaves. However, as all species were fixed in the same manner, comparisons between species are valid. A study using vapour fixation of dried leaves of *M. flabellifolia* (Goldsworthy and Drennan, 1991) gave very similar results to those observed in the present study.

Chloroplasts from control leaves of *C. wilmsii* were typical of hydrated, photosynthetically active tissues. Thylakoid membranes were clearly defined and some starch was present (Fig. 4A). In the dry state chloroplasts became rounded, the internal membranes were located peripherally and no starch was evident. The boundary membranes were intact and thylakoid stacks were still clearly visible, although...
there was a small degree of ‘blistering’ or separation of the appressed membranes (Fig. 4B). After 12 h of rehydration (60% RWC and 80% recovery of \( F_v/F_M \)), the chloroplasts resembled those in the dry state, although they were more oval than rounded (Fig. 4C). Upon further hydration the chloroplasts progressively assumed the shape and thylakoid distribution typical of control tissue (not shown). Lipophilic bodies were present at all stages of dehydration and rehydration.

Chloroplasts from control plants of \( M. \) flabellifolia were typically elongated with well defined thylakoid membranes and starch was present (Fig. 5A). The thylakoid membranes had a unique form of stacking (Fig. 5B) referred to as a ‘staircase’ arrangement (Wellburn and Wellburn, 1976). They suggested that such an arrangement allows a more effective presentation of photosynthetically active membranes to incoming radiation. This feature is not specific to resurrection species in general, as it has not been found in other resurrection species such as \( Craterostigma \) spp. or \( Xerophyta \) spp. (Tuba et al., 1993; Sherwin, 1995). In the dry state the chloroplasts appeared ovate, the stroma stained darkly and, while the thylakoids retained their staircase arrangement, they had a blistered appearance. There was no starch present (Fig. 5C). After 12 h of rehydration (20% RWC and 40% recovery of \( F_v/F_M \)) the appearance of the chloroplasts was very unusual. They had become rounded and the appressed membranes of the thylakoids appeared to have pulled apart (Fig. 5D). This ‘honeycomb’ appearance of the thylakoids was lost between 18 and 24 h of rehydration. After 24 h (60% RWC and 60% recovery of \( F_v/F_M \)) the staircase arrangement of the thylakoids was evident, although electron transparent regions were still present within the stroma (Fig. 5E).

Like \( C. \) wilmsii and \( M. \) flabellifolia, chloroplasts from the control plants of \( X. \) viscosa were elongated and contained starch. There were few granal layers in this species (Fig. 6A). In the dry state the chloroplasts were rounded and while the outer membranes remained continuous, the internal membranes lost their original conformation and appeared vesiculated (Fig. 6B). An unusual double-membraned structure became evident in virtually all chloroplasts examined (arrowed, Fig. 6B). The origin and function of this structure is unknown, but may have resulted from invagination and vesiculation of the outer boundary membrane. At this stage, the plant had lost more than 90% of its chlorophyll. After 24 h of rehydration (60% RWC) the internal membranes were less vesiculated and the circular membranous structure evident in the dry state was not present (Fig. 6C). At this stage less than 10% of the chlorophyll had been recovered yet there was a 60% recovery of \( F_v/F_M \). After 48 h of rehydration (80% RWC, 50% chlorophyll recovery and 90% recovery of \( F_v/F_M \)) the
thylakoid membranes had reassembled (Fig. 6D). Strands of rough endoplasmic reticulum (RER) and/or polysomes were present in close association with the boundary membranes of the chloroplasts (Fig. 6A–D). As in C. wilmsii, lipophilic bodies were present at all stages.

Figure 7 shows details of chloroplasts of *P. sativum* in leaves at full turgor (Fig. 7A), in the dry state (Fig. 7B) and after rehydration of individual leaves (Fig. 7C). In the dry state the chloroplasts became rounded and the thylakoids, while clearly visible and still arranged in stacks, were blistered. After rehydration it was difficult to distinguish the chloroplasts. Although in some instances thylakoid stacks were apparent, there was a general disruption of chloroplast membranes.

**DISCUSSION**

The results from this study show the unusual ability of three different resurrection plants to survive desiccation. It is also apparent that while these plants presumably undergo similar stresses on dehydration, there are differences in the way in which they cope with these stresses and in the nature of recovery during rehydration.

The nature of the damage associated with desiccation is typified in the response of *P. sativum* to dehydration and rehydration. The pattern of electrolyte leakage suggests that there is irreversible damage to the plasmalemma and, while organelles such as chloroplasts appear to retain some degree of integrity on drying, there is total dissolution on rehydration. Photosynthetic pigments were lost and not recovered. Thus there appears to be no mechanism of subcellular protection operating in this species and, furthermore, there is an inability to repair the damage induced by desiccation (presumably because the repair enzymes are damaged). Thus, on drying, metabolism such as photosynthesis is disrupted (rather than interrupted) by desiccation and this cannot be restored on rehydration.

Among the desiccation-tolerant species there were differences in the rate of recovery of water content and in photochemical activity. Recovery was generally more rapid in the homoiochlorophyllous species than in the poikilochlorophyllous *X. viscosa*, but there was also a difference in the rate of recovery between the homoiochlorophyllous types. The rate of recovery appears to depend on plant morphology and the extent of subcellular repair/reconstitution required for the resumption of metabolism.

*C. wilmsii*, being a small herbaceous plant, recovered water content very rapidly and this facilitated a rapid recovery of metabolism. Photochemical activity was restored before the plant was fully hydrated and before chlorophyll content reached control levels, suggesting that maximal
levels of these parameters are not required for full photosynthetic activity. Carotenoid levels were maintained, however, and it is possible that these were sufficient for maximal levels of photosynthesis. The rapid recovery is presumably also facilitated by the apparent ability of this plant to maintain integrity of the plasmalemma and chloroplast organization during dehydration and rehydration. Thus metabolism can resume without the delays caused by reconstituting these organelles. These results suggest that a mechanism of subcellular protection may operate during desiccation. Dehydrin proteins have been reported to be induced by drying in Craterostigma plantagineum (Piatkowski et al., 1990) and Craterostigma nanum (Sherwin, 1995). The presence of such proteins in C. wilmsii may contribute towards subcellular stabilization during dehydration.

The rehydration of leaves of M. flabellifolia was slower than in C. wilmsii, possibly because of the time to recover hydraulic conductance in the xylem vessels in the former. Embolisms in the xylem vessels could initially have posed a significant resistance to water uptake. Thus, photochemical activity was recovered over a longer period although, like C. wilmsii, this occurred before the plant was fully rehydrated. There was a delay in the resumption of photochemical activity during initial rehydration despite recovery of chlorophyll content. This is possibly due to the lack of continuity between adjacent thylakoid membranes and their reconstitution appears to be necessary for full recovery of photosynthetic activity. Increased levels of electrolyte leakage occurred upon desiccation, suggesting that some reconstitution of plasma membranes is required during rehydration. Thus repair/reconstitution of subcellular organization appears to play a more significant role in the recovery of this species compared to C. wilmsii. However, considering the degree of subcellular integrity maintained and the relatively rapid recovery of this plant on rehydration, there must also be some mechanism of protection. Drennan et al. (1993) reported an increase in the levels of trehalose and Bianchi et al. (1993) increases in the levels of sucrose, arbutin and glucopyranosyl-β-glycerol on desiccation of this species, which may contribute to subcellular stabilization. There is to date no information on the presence of dehydrins in M. flabellifolia.

Recovery of turgor and photochemical activity was slowest in X. viscosa. While slow water uptake must delay metabolic recovery, this species showed the greatest degree of membrane leakage and almost total loss of photosynthetic apparatus. The greater degree of subcellular reorganization required on rehydration of this species could account for the longer time taken for its recovery compared to the homoiochlorophyllus types. There is no information on the nature of subcellular protection in this species. In the present study, X. viscosa recovered photochemical activity before chlorophyll levels reached their pre-desiccation levels, and before full water content was attained. Tuba et al. (1994) showed that the opposite is true of X. scabrida. Those authors rehydrated isolated leaf tissues in their study, which may account for the discrepancy in results between the studies.

We suggest that recovery rate is influenced by plant form (which influences the rate of hydration) and also the extent of reconstitution of subcellular organization that is required. We further suggest that the degree of reconstitution required for the photosynthetic apparatus depends on the strategies adopted to prevent light-related desiccation stresses. Removal of water from plant tissues ultimately results in interruption of metabolic pathways. One consequence of this in chlorophyllous tissue is that the energy generated from light absorbed by a chlorophyll molecule cannot be dissipated via the normal metabolic pathways. The energized electrons (which may in turn generate free radical species) can cause considerable subcellular damage. Poikilochlorophyllous plants like X. viscosa avoid such stress by losing
chlorophyll and dismantling the thylakoid membranes during desiccation. On rehydration the photosynthetic apparatus has to be reconstituted and this delays recovery of carbon metabolism. Poikilo-chlorophyllous plants retain most of the photosynthetic apparatus, but prevent light-chlorophyll interactions, for example by leaf curling in C. wilmsii and folding in M. flabellifolia. Recovery of photosynthesis can be more rapid in these species relative to poikilo-chlorophyllous types.

**ACKNOWLEDGEMENTS**

Thanks to John and Sandie Burrows of the Buffelskloof Nature Reserve for help in collecting the plant material. Heather Sherwin acknowledges the Foundation for Research Development (FRD) for a post-doctoral bursary. The project was funded by an FRD grant awarded to Jill Farrant.

**LITERATURE CITED**


