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Sugar ratios, glutathione redox status and phenols in the resurrection species *Haberlea rhodopensis* and the closely related non-resurrection species *Chirita eberhardtii*

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ABSTRACT

Because of their unique tolerance to desiccation, the so-called resurrection plants can be considered as excellent models for extensive research on plant reactions to environmental stresses. The vegetative tissues of these species are able to withstand long dry periods and to recover very rapidly upon re-watering. This study follows the dynamics of key components involved in leaf tissue antioxidant systems under desiccation in the resurrection plant *Haberlea rhodopensis* and the related non-resurrection species *Chirita eberhardtii*. In *H. rhodopensis* these parameters were also followed during recovery after full drying. A well-defined test system was developed to characterise the different responses of the two species under drought stress. Results show that levels of H_2O_2 decreased significantly both in *H. rhodopensis* and *C. eberhardtii*, but that accumulation of malondialdehyde was much more pronounced in the desiccation-tolerant *H. rhodopensis* than in the non-resurrection *C. eberhardtii*. A putative protective role could be attributed to accumulation of total phenols in *H. rhodopensis* during the late stages of drying. The total glutathione concentration and GSSG/GSH ratio increased upon complete dehydration of *H. rhodopensis*. Our data on soluble sugars suggest that sugar ratios might be important for plant desiccation tolerance. An array of different adaptations could thus be responsible for the resurrection phenotype of *H. rhodopensis*.

INTRODUCTION

It is expected that a systems-biology approach will lead to a significantly improved understanding of the mechanisms associated with plant desiccation tolerance (Moore *et al.* 2009). In this respect, the so-called resurrection plants can be considered as excellent model systems (Gaff 1971; Oliver *et al.* 2000; Proctor & Tuba 2002; Toldi *et al.* 2006). These species belong to different families, live in different habitats and are exposed to a wide range of environmental challenges. However, they share one common feature – their vegetative tissues are able to withstand long periods of complete desiccation and yet can recover very rapidly upon re-watering (Oliver 1996; Scott 2000; Farrant *et al.* 2003; Bartels 2005; Toldi *et al.* 2009). Acquisition of desiccation tolerance is a complex process that is probably due to an elaborate set of specific adaptations. It has been postulated that different resurrection species may use a mixture of different protective metabolites to allow complete dehydration tolerance (Kranner *et al.* 2002; Kranner & Birtic 2005). In addition, resurrection plants are expected to have superior free radical-scavenging mechanisms, both in their cytoplasm and in vacuoles, to cope with the severe stresses. It has recently been suggested that vacuolar compounds such as fructans might be involved in vacuolar antioxidant mechanisms (Van den Ende & Valluru 2009).

Bulgaria is among the few countries in Europe where resurrection plants grow in the wild. *Haberlea rhodopensis* Friv and *Ramonda serbica* Pancic belong to the Gesneriaceae. Both species prefer shady slopes and limestone rocks and are found at a wide range of altitudes in mountain areas. *H. rhodopensis* was among the first plants recognised as genuine resurrection plants (Ganchev 1950). It is considered a homoiochlorophyllous species, in that it can preserve most of its chlorophyll during dehydration. Photosynthesis and transpiration during desiccation and recovery have been extensively studied in *H. rhodopensis* (Markovska *et al.* 1994, 1995; Peeva & Maslenkova 2004; Georgieva *et al.* 2005, 2007, 2008; Peeva & Cornic 2009). Lipid and sterol changes in leaves (Stefanov *et al.* 1992) and some aspects of compatible solute

accumulation during drying and recovery have also been analysed (Müller *et al.* 1997). Recently, the dynamics of changes in activity of the enzymes superoxide dismutase and peroxidase during dehydration and subsequent rehydration have been evaluated in this species (Yahubyan *et al.* 2009).

Experiments with resurrection plants are generally performed on plants taken from the wild with or without some adaptation to controlled growth conditions. The same is true for *H. rhodopensis*, even though the species is endemic and scarce in a few regions of Bulgaria, where plants are of unknown age, stage of growth or development. Here, for the first time, we use *in vitro* plants, enabling us to work under controlled conditions and with uniform material of known size and stage of growth (Djilianov *et al.* 2005).

Although various parameters of the antioxidant systems have been previously followed in a number of resurrection species, we describe here the first comprehensive study that includes analysis of sugars, malondialdehyde, glutathione, phenols and hydrogen peroxide. Moreover, the development of an *in vitro* propagation system allowed a direct comparison (at exactly the same physiological growth state) of a resurrection species (*H. rhodopensis*) with a closely related non-resurrection species (*Chirita eberhardtii* Pellegrin), both in the subfamily Cyrtandroideae, tribe Didymocarpeae, in the Gesneriaceae (Burtt & Wiehler 1995; Mayer *et al.* 2003).

MATERIALS AND METHODS

Plant material

In all experiments, *in vitro*-generated *Haberlea rhodopensis* and *Chirita eberhardtii* plants were used. The *in vitro* propagation system for *H. rhodopensis* has been described earlier (Djilianov *et al.* 2005). Seeds of *Chirita eberhardtii* were kindly provided by The Botanical Garden, Bulgarian Academy of Sciences, Sofia. They were surface sterilised with 70% (v/w) ethanol for 1 min and with a standard bleach solution for 10 min. Seeds were then rinsed three times with sterile water and germinated on basal woody plant medium (WPM) (Lloyd & McCown 1980) plus 0.5% filter sterilised GA_3 at pH 5.8. The plantlets were then transferred on WPM for further growth and rooting and routinely propagated by cuttings every 1.5–2 months. All *in vitro* plants (*H. rhodopensis* and *C. eberhardtii)* were cultured in a plant growth chamber at 16 h/8 h light/dark photoperiod, 22 °C, 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and about 50% air humidity. Well-developed and rooted 1.5- to 2-month-old plants were used in this study.

Dehydration and rehydration procedure

Well-developed plantlets were removed from culture vessels and left to dry to full air-dry stage in a culture room under controlled conditions (22–25 °C and 50% relative humidity in shade). Rehydration was carried out on cotton beds by watering the dried plantlets under the same conditions. Control plants were kept *in vitro* throughout the experiment. To follow the reactions to oxidative stress and changes in soluble sugar content, leaf samples were cut from treated plantlets at various stages of dehydration or rehydration, according to their relative water content (RWC) and immediately frozen in liquid nitrogen. They were kept at −70 °C for further analyses (hydrogen peroxide, H_2O_2; malondialdehyde, MDA; glutathione; total phenols) or freeze dried (soluble sugars determination).

Relative water content (RWC)

RWC was determined at each sampling interval. Leaves were detached from the plantlets and their initial weight (FW) recorded before immersion in Milli-Q water for 24 h at 22 °C in the dark. The weight at full turgor (FTW) was recorded and leaf samples subsequently dried at 80 °C for 48 h, and the dry weight (DW) recorded. The RWC was calculated using the formula (Barr & Weatherley 1962):

$$RWC\% = (FW - DW/FTW - DW) \times 100$$

Soluble sugar determination

About 150 mg of dried *H. rhodopensis* leaf material and 20 mg of *C. eberhardtii* were added to a glass tube. Twenty (*H. rhodopensis*) or 40 (*C. eberhardtii*) volumes of distilled water were then added and the mixture incubated at 90 °C for 20 min. The samples were then crushed with a mortar and pestle until a fine homogenate was obtained. The homogenate was centrifuged at 3000*g* for 5 min. A 200-µl sample of the supernatant was passed then through a 0.5-ml bed volume of Dowex®-50 H^+ and a 0.5-ml bed volume of Dowex®-1-acetate (both 100–200 mesh) (Acros Organics, Morris Plains, NJ, USA). The resins were rinsed six times with 200 µl distilled water. The combined eluate was diluted five fold with Milli Q water and centrifuged at 3000*g* for 5 min. From this fraction, 25 µl were analysed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described (Vergauwen *et al.* 2000). Quantification was performed on the peak areas using the external standards method for glucose (Glc), fructose (Fru), sucrose (Suc), raffinose (Raf) and stachyose (Sta) (Shiomi *et al.* 1991; Vergauwen *et al.* 2000).

Total phenols

Leaf material was homogenized in 0.1% w/v trichloroacetic acid (TCA). Total phenols were extracted and determined with Folin-Ciocalteau reagent supplemented with sodium carbonate, Na_2CO_3. The absorbance at 725 nm was recorded according to the method of Swain & Goldstein (1964) with minor modifications. Caffeic acid was used as a reference.

Hydrogen peroxide determination and estimation of lipid peroxidation

Leaf samples (about 200 mg) were homogenised in 1% (w/v) cold TCA and the homogenate then centrifuged at 15,000*g* for 25 min. The supernatant obtained was used for determination of H_2O_2 and lipid peroxidation levels. Hydrogen peroxide was measured spectrophotometrically after reaction with potassium iodide (KI; Alexieva *et al.* 2001). The reaction was developed for 1 h in the dark and the absorbance was measured at 353 nm using a microplate apparatus (Multiscan Spectrum; Thermo Electron Corp., Waltham, MA, USA). The

amount of H_2O_2 was calculated and presented as absorption units per gram dry weight. Lipid peroxidation was measured as the amount of MDA present. MDA is a product of unsaturated fatty acid peroxidation and was determined using the thiobarbituric acid reaction (Kramer *et al.* 1991) and presented as absorption units per gram dry weight.

Quantification of glutathione

Endogenous glutathione (GSH) levels were determined according to the enzymatic recycling procedure of Gronwald *et al.* (1987). Briefly, 300 mg of leaves were homogenised in 3 ml of 5% (w/v) TCA and centrifuged at 15,000*g* for 15 min in order to sediment insoluble material. A 1-ml aliquot of the supernatant was neutralised with 1 ml 0.5 M potassium phosphate buffer (pH 7.5). The standard incubation mixture for the quantification of total glutathione (GSH + GSSG) consisted of sodium phosphate buffer (pH 7.5) containing diaminoethanetetraacetic acid (EDTA), 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), 1 unit of glutathione reductase type III and neutralised plant extract. The change in absorption at 412 nm was followed. Oxidised glutathione (GSSG) was determined using a similar procedure after trapping the reduced form of GSH with *N*-ethylmaleimide. The amounts of total glutathione and GSSG were obtained from standard curves prepared with known concentrations of GSH and GSSG. GSH was calculated by subtracting the amount of GSSG from that of total glutathione.

Statistics

All experiments were repeated at least three times. Samples for analyses were collected from at least three to four independent plantlets at every point of the desiccation–rehydration procedure and controls. Differences between control and stressed (recovered) means were analysed with ANOVA tests.

RESULTS AND DISCUSSION

Comparison of RWC dynamics in *Haberlea rhodopensis* and *Chirita eberhardtii*

Being able to regenerate and routinely propagate *H. rhodopensis in vitro*, we focused our efforts on the development of an efficient micropropagation protocol for its close relative and non-resurrection species, *C. eberhardtii*. We were then able to establish procedures to study the response of both species to drying and rehydration under standardised, well-defined conditions.

The *C. eberhardtii* (Fig. 1) and *H. rhodopensis* (Fig. 2) plants had similar RWC (91–93%) at the start of the dehydration when removed from the culture vessels and exposed to air drying. *C. eberhardtii* plantlets started to dry out within 1 h, decreasing RWC to ~72% (Fig. 1); after 3 h of drying, RWC of leaves from *C. eberhardtii* reached ~45%; between 3 and 24 h, leaves resisted further water loss (points 45a, 45b in Figs 3–7B); finally leaves reached ~35% RWC at 48 h, which was the lowest RWC at which *C. eberhardtii* plantlets were still able to recover following re-watering. After this point, further water loss occurred to ~20% RWC at 72 h and ~12% at 168 h; since there was no recovery of these samples upon re-watering, plantlets were considered dead and no further analyses were carried out.

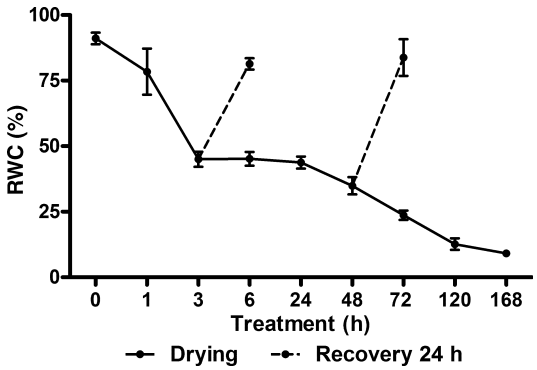

Fig. 1. Dynamics of RWC of *Chirita eberhartii*. The RWC of *Chirita eberhardtii* was followed during the drying process. See Table S1 for the link between sample timing and RWC content. Recovery (24 h) was possible till RWC 33%; further desiccation led to irreversible damage.

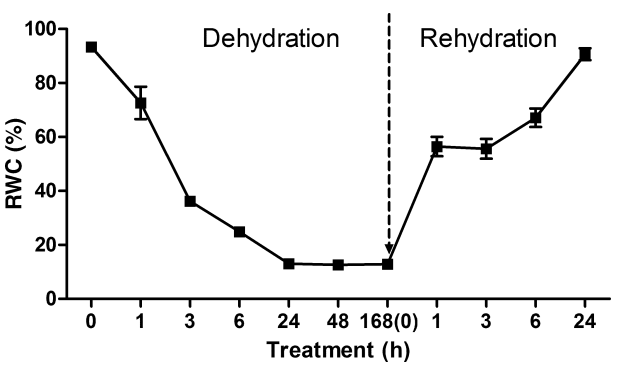

Fig. 2. Dynamics of RWC of *Haberlea rhodopensis*. The RWC of *H. rhodopensis* was followed during drying and the resurrection process. See Table S2 for the link between sample timing and RWC content. Recovery (24 h) was performed with samples dried for 168 h.

Similar to *C. eberhardtii*, the RWC of *H. rhodopensis* plantlets decreased to ~72% after 1 h of drying (Fig. 2); after 3 h of rapid water loss RWC was already ~35%; by 6 h it decreased to ~25%; while the lowest RWC (~12%) was reached at 24 h, and additional drying did not further reduce the plantlet RWC (Fig. 2). Rehydration experiments were performed on *H. rhodopensis* plantlets that had been kept desiccated (~12% RWC) for 168 h. Recovery occurred almost immediately, and 1 h after re-watering the RWC reached ~56%, this was maintained for 3 h after rehydration (Fig. 2); recovery continued and reached about two-thirds of the control RWC, and at 24 h, reached control levels.

It is accepted that the air drying method applied in our experiments is harsher than that in nature or in tests with potted plants. Drying rate was shown to be important, or even crucial for survival and recovery after long periods of continuous drying, when treated plants were taken from the wild (Farrant *et al.* 1999). However, having plants of known origin and uniform age, grown under controlled conditions and being able to apply a repeatable procedure for drying

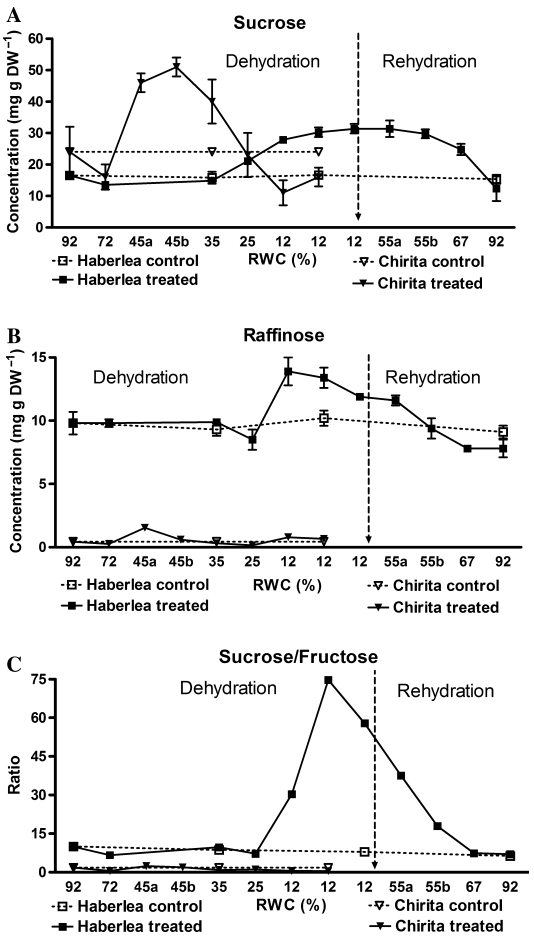

Fig. 3. Major stress response-related sugars in *Haberlea rhodopensis* and *Chirita eberhardtii*. Sucrose (A), Raffinose (B) and Suc/Fru ratio (C) dynamics during dehydration and recovery of *H. rhodopensis* and dehydration of *C. eberhardtii*. Vertical bars indicate SE for n = 3.

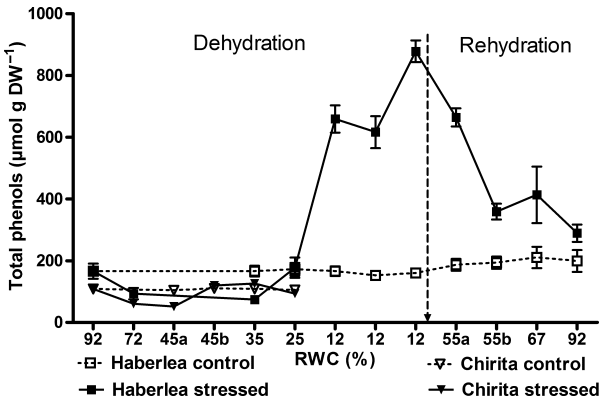

Fig. 4. Total phenols in *Haberlea rhodopensis* and *Chirita eberhardtii*. Levels of total phenols during dehydration and recovery of *H. rhodopensis* and dehydration of *C. eberhardtii*. Statistical significance ($P < 0.01$) was found for all points (except RWC 25% in *H. rhodopensis* and RWC 72% and 45% in *C. eberhardtii*).

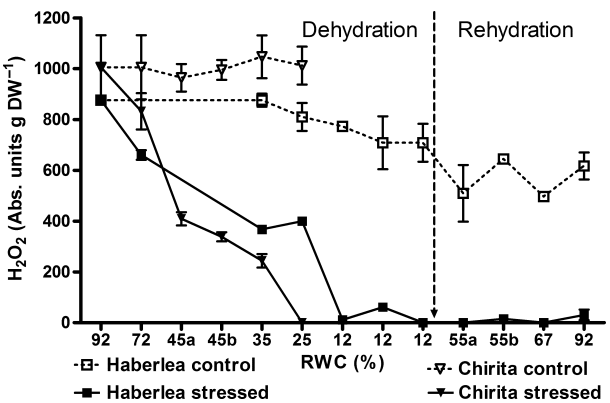

Fig. 5. Hydrogen peroxide levels in *Haberlea rhodopensis* and *Chirita eberhardtii*. H_2O_2 levels during dehydration and recovery of *H. rhodopensis* and dehydration of *C. eberhardtii*. Statistical significance ($P < 0.01$) was found for all points (except RWC 72% in *C. eberhardtii*).

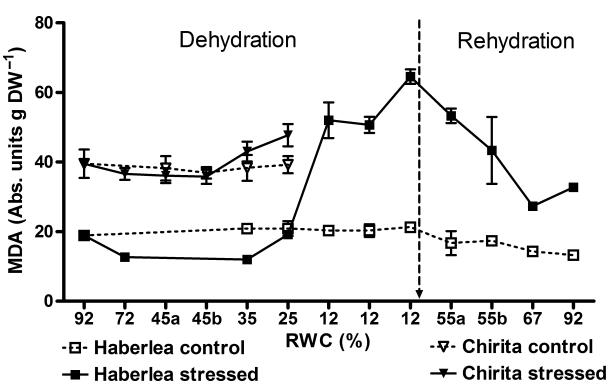

Fig. 6. MDA levels in *Haberlea rhodopensis* and *Chirita eberhardtii*. MDA levels during dehydration and recovery of *H. rhodopensis* and dehydration of *C. eberhardtii*. Statistical significance ($P < 0.01$) was found for all points (except RWC 25% in *C. eberhardtii*).

and recovery is a further advantage. Moreover, we found quite clear differences in the reaction of resurrection and non-resurrection species. The patterns of leaf RWC kinetics at dehydration and recovery were similar to those reported by other groups for such plants (*e.g.* Schwab *et al.* 1989; Deng *et al.* 2003; Georgieva *et al.* 2005). *H. rhodopensis* dried out quickly, reaching the highest dry state after 24 h, while *C. eberhardtii* dried more slowly, reaching this point after ~120 h. Although *C. eberhardtii* plants were able to recover after losing half to two-thirds of their water content (Fig. 1), further desiccation was irreversible, thus confirming their non-resurrection status. In contrast, *in vitro*-developed *H. rhodopensis* showed a typical resurrection capacity, and after 1 week at the fully dry stage, all plants fully recover upon re-watering (Fig. 2). This trait is confirmed to be stable when such plants are potted and exposed to soil drying under controlled or natural conditions (Petrova *et al.* 2010).

Soluble sugars

Mechanical stabilisation by means of compatible solutes (sugars, proline, *etc.*) has often been reported in a wide array of resurrection plants (Farrant 2000; Vander Willigen *et al.* 2003). It is also widely accepted that soluble sugars are involved in signalling processes and plant stress tolerance (Couée *et al.* 2006; Solfanelli *et al.* 2006; Ramel *et al.* 2009).

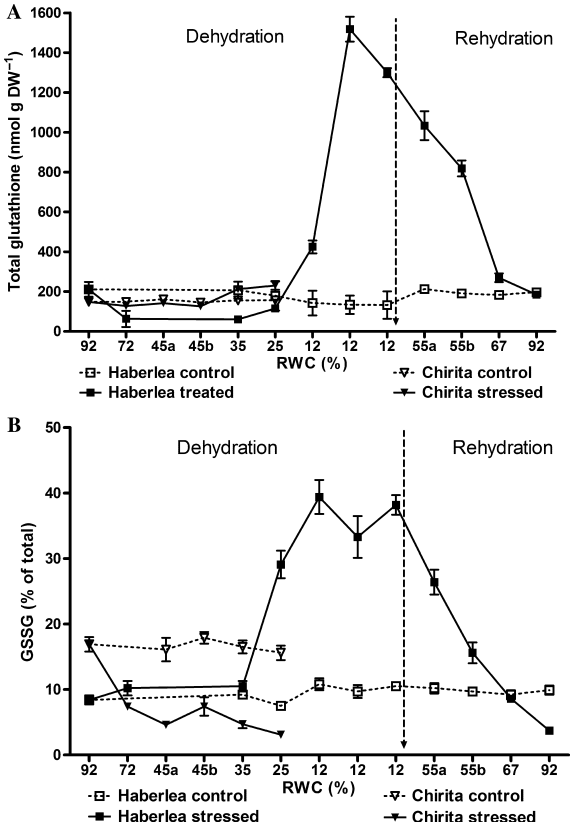

Fig. 7. Total and oxidised glutathione levels in *Haberlea rhodopensis* and *Chirita eberhardtii*. Total (A) and oxidised (B) glutathione levels during dehydration and recovery of *H. rhodopensis* and dehydration of *C. eberhardtii*. Statistical significance ($P < 0.01$) was found for all points (except RWC 25% and 92% in *H. rhodopensis* and RWC 35% and 25% in *C. eberhardtii*).

Recently, cross-talk between sugar signalling and reactive oxygen species (ROS) signalling pathways has been highlighted (Bolouri-Moghaddam *et al.* 2010 and references therein). Next to their function as membrane stabilisers, soluble sugars can act as ROS scavengers *in vitro* and *in planta* (Morelli *et al.* 2003; Nishizawa *et al.* 2008; Stoyanova *et al.* 2010). In particular, increases in Suc and Raf levels are often detected during the dehydration of resurrection plants (Ghasempour *et al.* 1998). Therefore, the dynamics of soluble sugars during drying (in *H. rhodopensis* and *C. eberhardtii*) and recovery (in *H. rhodopensis*) are of particular interest. A complete list of the dynamics of the water-soluble carbohydrates in *H. rhodopensis* and *C. eberhardtii* during dehydration and rehydration is tabulated in Tables S1 and S2, respectively.

The most prominent differences between the two species are described. First, *C. eberhardtii* shows a temporal accumulation of Suc during the earlier stages of dehydration (Fig. 3 A), which is not observed in the case of *H. rhodopensis*. In the latter species, a two-fold increase in Suc is only observed during the terminal stages of dehydration, and decreases again to the initial level during rehydration. Second, under control conditions, the Raf levels are about 20 times higher in *H. rhodopensis* compared to *C. eberhardtii* (Fig. 3B). As observed for Suc, a similar two-fold Raf increase is observed during the final stages of *H. rhodopensis* dehydration. This Raf increase was not observed by Müller *et al.* (1997), but they used a different treatment. Like Suc, Raf levels decrease again during rehydration. Third, the initial Suc/Fru ratio is much higher in *H. rhodopensis* than in *C. eberhardtii*, and this ratio peaks at the end of the dehydration (Fig. 3C), when the free Fru levels become extremely low in *H. rhodopensis* (Table S2). These important differences between the two species might be interpreted in terms of differences in sugar signalling (Kato-Noguchi *et al.* 2005; Smeekens *et al.* 2010). Indeed, it has been suggested that the Suc/Hexose ratio is an important parameter for sugar signalling under stress (Weber *et al.* 1995). As reported previously for the desiccation-tolerant species *Sporobolus stapfianus* and *Xerophyta viscosa*, the low Fru levels might be associated with strongly increased hexokinase activity (Whittaker *et al.* 2001), which is needed to generate fructose-6-phosphate (Fru-6-P) for Suc synthesis by sucrose phosphate synthase (SPS). Both SPS activity and protein levels increased significantly during drying of the C_4 resurrection plant, *Sporobolus stapfianus* (Whittaker *et al.* 2007).

We propose here that the constitutively high Suc and Raf concentration in *H. rhodopensis* (Fig. 3A and B) might represent one important factor for establishing the resurrection phenotype in this species. In other words, maintaining Suc and Raf levels at a constant high level appears to be a specific adaptation to be able to survive a very rapid dehydration such as that applied in our experiments. The initial Suc and Raf levels in *H. rhodopensis* (about 16 and 10 mg·gDW^{-1}) are higher than those observed in another resurrection species, *X. viscosa* (about 6 mg·gDW^{-1} for both sugars; Peters *et al.* 2007). Peters *et al.* (2007) suggested that the presence of another sugar (such as Raf) together with Suc might be important to prevent Suc crystallisation during the dehydration process. This suggestion fits well with the fact that Raf accumulates significantly in *H. rhodopensis* but not in *C. eberhardtii*. The Suc/Raf ratio does not change extensively during the dehydration and rehydration period, neither in our experiments, nor in those of Peters *et al.* (2007). Therefore, we propose here that the initial Suc/Raf ratio in leaves prior to desiccation might be one important parameter, among others, in Suc-accumulating resurrection plant species. The higher the absolute Raf and Suc levels, perhaps the higher the chance of surviving rapid desiccation. Accordingly, in protoplasts isolated from desiccation-tolerant pea embryos (Xiao & Koster 2001), mixtures of Raf and Suc could provide optimal protection during drying. Transmission electron microscopy revealed that dried desiccation-tolerant plants had intact membranes, while those of sensitive tissues had disrupted membranes. Combinations of other types of sugars might be very effective too (Amiard *et al.* 2003; Hincha *et al.* 2007; Farrant *et al.* 2009). Besides their capacity to stabilise membranes, such sugars might also assist in ROS scavenging mechanisms, perhaps in coordination with phenolic compounds (Yamasaki & Grace 1998; Van den Ende & Valluru 2009). It is known from the literature that particular proteins that are normally expressed only in the seeds of other plants can also contribute to the resurrection phenotype (Mowla *et al.* 2002; Illing *et al.* 2005; Mulako *et al.* 2008).

Total phenols

The total phenol content of *C. eberhardtii* controls was lower than that of untreated *H. rhodopensis* plantlets (Fig. 4). Dur-

ing the initial stages of dehydration, phenols of stressed *C. eberhardtii* were two-fold lower than in untreated plants. Later however, phenol concentrations increased to control levels, and remained unchanged until the end of the drying period. Similarly, in *H. rhodopensis* during the early stages of dehydration total phenols were almost two-fold lower than in untreated plants. Along with the increase in Suc (after day 30), a gradual increase in total phenols was observed of up to eight-fold more than in untreated plants (Fig. 4). Upon re-watering, a gradual decrease of total phenols occurred.

Although different polyphenol types occur in many subcellular compartments in plants (Zhao & Dixon 2010), they are most often observed in vacuoles, especially under stress (Moore *et al.* 2005). The antioxidant potential of total phenols in plants under stress is well described (Abreu & Mazzafera 2005; Kranner & Birtic 2005; Ksouri *et al.* 2007). For instance, 3,4,5 tri-*O*-galloylquinic is the major polyphenol in the resurrection species *Myrothamnus flabellifolius*. In this species, total phenols were found in very high concentrations: up to half of the dry mass in hydrated leaves and to about three-quarters of the dry mass of desiccated leaves (Moore *et al.* 2005). In the same study it was shown that polyphenols are present in the leaf pallisade and spongy mesophyll cell vacuoles. In hydrated leaves, the polyphenols occupied only a small proportion of the vacuole, whereas in dry leaves they filled the entire vacuolar space. These phenols protect the membranes against desiccation and also against free radical-induced damage. In *H. rhodopensis*, total phenols reached about 15–20% of plant total dry weight at desiccation. In contrast, the phenolic acids and enzymes involved in phenol metabolism of *Ramonda serbica* decreased under desiccation but increased upon rehydration (Sgherri *et al.* 2004; Veljovic-Jovanovic *et al.* 2008). Interestingly, the pattern and magnitude of total phenols accumulation in *H. rhodopensis* is similar to that of *M. flabellifolius* and opposite to that of the related species *R. serbica*. The accumulation of anthocyanins and other phenolic compounds under stress (Grace & Logan 2000; André *et al.* 2009) is specifically mediated through Suc-specific signalling pathways (Couée *et al.* 2006; Solfanelli *et al.* 2006). Synergistic effects between sugar metabolism and phenolic compounds under abiotic stress have been suggested (Van den Ende & Valluru 2009).

Hydrogen peroxide

Under control conditions, H_2O_2 levels in *C. eberhardtii* and *H. rhodopensis* remained essentially unchanged (Fig. 5). At the start of desiccation stress, H_2O_2 levels in *C. eberhardtii* were similar to the controls but, unexpectedly, further dehydration led to a significant decrease (three- to four-fold), and at the point at which dehydration became irreversible (RWC 25%), H_2O_2 was already undetectable. In comparison, dehydration of *H. rhodopensis* led to an immediate and significant decrease in H_2O_2. After 1 h of desiccation, H_2O_2 levels were ~30% lower than in controls (Fig. 5). This decline continued steadily with further desiccation, reaching values close to the detection limit. Following rehydration, H_2O_2 levels remained very low.

Under abiotic stress conditions, H_2O_2 functions in a concentration-dependent manner. At low concentrations it acts as a signalling molecule while at higher concentrations it is phytotoxic (Dat *et al.* 2000). For a long time, the endogenous levels of H_2O_2 were considered to be indicative of the level of stress in a tissue (Pirie & Mullins 1976). However, very recently this point of view changed (Cheeseman 2009).

The observations confirm other recent findings that the endogenous H_2O_2 concentration does not function as a stress indicator in *H. rhodopensis* and *C. eberhardti,* although the reason for the unexpected decrease in total H_2O_2 levels under stress is unresolved. It could be that the H_2O_2 production under stress is consumed by increasing catalase activity in *H. rhodopensis* (Mihailova *et al.* 2009); alternatively, H_2O_2 might be used for the massive biosynthesis of phenolic compounds. Typically, Class III type peroxidases (De Gara 2004; Passardi *et al.* 2004; Dunand *et al.* 2007) located on the inner side of the tonoplast use H_2O_2 to oxidise phenolic compounds and other secondary metabolites.

Malondialdehyde

MDA is as an end product of unsaturated lipid peroxidation and is widely used as an indicator of oxidative stress. MDA levels of control *C. eberhardtii* were twice as high as those of control *H. rhodopensis* (Fig. 6). MDA levels of controls of both species remained steady throughout the whole experiment. While the initial stages of drying did not influence MDA in *C. eberhardtii,* further desiccation (beyond the point of possible recovery) increased MDA levels by ~20%. Before reaching 25% RWC, the MDA levels of dehydrated *H. rhodopensis* were lower than those of the controls (Fig. 6). At this point, MDA levels of the stressed plants started to increase sharply, in concert with increasing sugar levels and total phenols. At full dryness, MDA was three to ten times higher than in controls. During the first stages of rehydration, the levels remained high but a gradual decrease then started. At full recovery, MDA remained about twice as high as in the controls.

The contrast between the reduced level of H_2O_2 (previous section) and the putative increased membrane damage (as judged from increasing MDA levels) in *H. rhodopensis* could be considered as a paradox. Possibly, classical cytosolic antioxidant mechanisms can detoxify H_2O_2 (Noctor & Foyer 1998). Additionally, the intrinsic hydroxylic cycle of peroxidases involved in the massive production of phenolic compounds could partially explain the phenomenon. Indeed, in the vicinity of membranes, peroxidases (POXs) not only use H_2O_2 for oxidation processes, but at the same time produce highly reactive OH· radicals that are involved as initiators of membrane peroxidation processes. It could be speculated, therefore, that sugars and phenols probably acting in concert, could work together to scavenge most of these OH· radicals produced in the vicinity of membranes. The protective effect provided by (a specific mixture of) sugars and phenols, perhaps combined with specific proteins that are usually observed in other resurrection species (see above), might contribute to overall membrane stability of resurrection plants, which seem able to survive complete dehydration despite the increasing levels of MDA generated during this process.

Glutathione

Glutathione is the most abundant low-molecular weight thiol compound present in cells. It constitutes a redox buffer that

keeps the intracellular environment reduced (Rouhier *et al.* 2008). The levels of total glutathione and GSH, and the ratio of reduced to oxidised form (GSH/GSSG; usually 9–10:1) are considered universal stress markers (Tausz *et al.* 2004; Foyer & Noctor 2005; Ivanov & Kerchev 2007). Numerous studies have attributed to glutathione the role of 'classical' antioxidant in resurrection plants (Navari-lzzo *et al.* 1997; Kranner 2002; Kranner *et al.* 2002; Kranner & Birtic 2005). In nearly all desiccation-tolerant plants examined, glutathione has been found to be involved in the dehydration stress responses (Toldi *et al.* 2009) and the genes controlling glutathione and its reduced form (GSH) are among the most sensitive to dehydration (Jiang *et al.* 2007). Similar patterns and an even greater increase of GSH content was found in *Boea hygroscopica* (Navari-lzzo *et al.* 1997).

The levels of total glutathione (GSH + GSSG) during the early stages of dehydration of *C. eberhardtii* varied insignificantly and were comparable to those of controls up to a RWC of 45% (Fig. 7A). Further drying led to a gradual increase, reaching 30% higher levels at a RWC 25%. *H. rhodopensis* responded to the first stages of drying with a significant reduction (about three-fold lower than controls) of GSH/total glutathione content. When RWC reached 25%, however, total glutathione content increased significantly and reached levels ten-fold higher than in control plants (Fig. 7A). During rehydration, GSH content decreased significantly to control levels at full recovery.

Oxidised glutathione (GSSG, presented as a percentage of total glutathione) of stressed *C. eberhardtii* samples was threefold lower than in controls during the whole experiment (Fig 7 B). At the start of dehydration, *H. rhodopensis* samples maintained GSSG levels similar to the controls, but at 35% RWC a drastic increase started (Fig. 7B). Further desiccation led to a GSSG content that was three times higher than in controls, reaching 40% of the total glutathione pool at full dehydration. At recovery, GSSG levels decreased rapidly to control levels.

In *H. rhodopensis*, the increase in GSH content started later than in *C. eberhardtii* but reached levels about ten times higher than in controls at full desiccation. Acting in concert with increasing sugar and phenol levels during desiccation, this prominent increase in total glutathione and the shift towards GSSG were not unexpected. Indeed, electrons are needed for regeneration of the sugar and phenolic compound radicals generated during the OH· scavenging processes, and these electrons need to be derived from GSH. During complete dehydration, photosynthesis and the accompanying production of NADPH are blocked, hampering reduction of GSSG and leading to a higher overall GSSG/GSH ratio. Alternatively, it can be speculated that the increased GSSG/GSH ratio in *H. rhodopensis* might act as a trigger to further activate antioxidant defence mechanisms, but this suggestion requires further research. Typically, H_2O_2 signalling pathways are involved in such processes. However, we failed to detect temporal H_2O_2 increases in *H. rhodopensis*. Therefore, we remain open to the possibility of putative alternative signalling pathways involved in antioxidative defence in resurrection plants.

CONCLUSIONS

We compared the reaction to desiccation of *Haberlea rhodopensis* and *Chirita eberhardtii* using plant material of known origin, grown and treated under similar controlled conditions. Based on the parameters examined in our studies we developed a hypothetical model including factors that possibly contribute to the resurrection behaviour of *H. rhodopensis*. *H. rhodopensis* and *C. eberhardtii* react in completely different ways. Some parameters involved in the *H. rhodopensis* resurrection phenotype (and behaving different in the non-resurrection phenotype) are summarised in Fig. 8. Some of the compounds analysed (H_2O_2, MDA, GSSG/GSH ratio) might be a consequence of stress rather than acting as triggers in the stress response. In our case, the main challenge of desiccation appears to be faced by coordinated changes in soluble sugars, total phenol content and total glutathione. To survive desiccation and the accompanying oxidative stress, *H. rhodopensis* activates a number of defence mechanisms. The comparison with the drought-sensitive species *C. eberhardtii* strongly suggested that survival and resurrection are due to a set of adaptations where soluble sugars may play a pivotal role and contribute significantly along and together with the accumulation of total phenols and total glutathione. Further studies are needed to clarify the initial trigger(s) of the stress response network in *H. rhodopensis* and the interactions between the different players in this network.

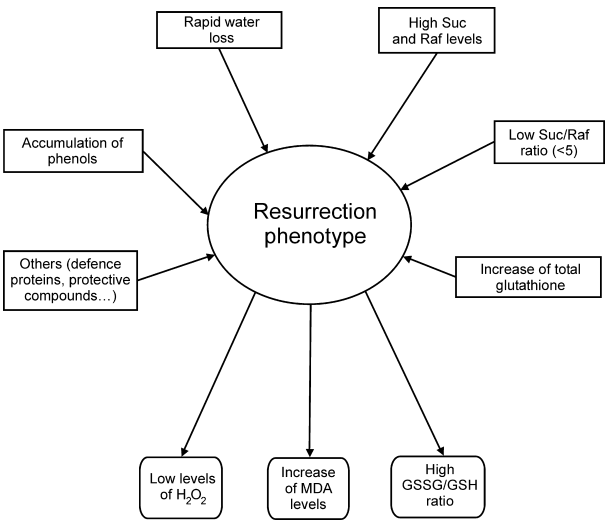

Fig. 8. The resurrection phenotype of *Haberlea rhodopensis*: a hypothetical scheme. Arrows pointing towards the central box might contribute to the resurrection phenotype; arrows pointing away might be considered a consequence of the resurrection phenotype.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Soluble carbohydrate content ($mg \cdot g^{-1}$ dry weight) in *Chirita eberhardtii* during dehydration.

Table S2. Soluble carbohydrate contents ($mg \cdot g^{-1}$ dry weight) in *Haberlea rhodopensis* during dehydration and recovery.

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REFERENCES

Abreu I., Mazzafera P. (2005) Effect of water and temperature stress on the content of active constituents of *Hypericum brasilienne* Choisy. *Plant Physiology & Biochemistry*, **43**, 241–248.

Alexieva V., Sergiev I., Mapelli S., Karanov E. (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*, **24**, 1337–1344.

Amiard V., Morvan-Bertrand A., Billard J.-P., Huault C., Keller F., Prud'homme M.P. (2003) Fructans, but not the sucrosyl-galactosides, raffinose and loliose, are affected by drought stress in perennial ryegrass. *Plant Physiology*, **132**, 2218–2229.

André C., Schafleitner R., Legay S., Lefèvre I., Aliaga A., Nomberto G., Hoffmann Hausman J.-F., Larondelle Y., Evers D. (2009) Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. *Phytochemistry*, **70**, 1107–1116.

Barr H.D., Weatherley P.E. (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Australian Journal of Biological Science*, **15**, 413–428.

Bartels D. (2005) Desiccation tolerance studied in the resurrection plant *Craterostigma plantagineum*. *Integrative and Comparative Biology*, **45**, 696–701.

Bolouri-Moghaddam M., Le Roy K., Xiang L., Rolland F., Van den Ende W. (2010) Sugar signalling and antioxidant network connections in plant cells. *FEBS Journal*, **277**, 2022–2037.

Burtt B.L., Wiehler H. (1995) Classification of the family Gesneriaceae. *Gesneriana*, **1**, 1–4.

Cheeseman J. (2009) Seasonal patterns of leaf H_2O_2 content: reflections of leaf phenology, or environmental stress? *Functional Plant Biology*, **36**, 721–731.

Couée I., Sulmon C., Gouesbet G., El Amrani A. (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany*, **57**, 449–459.

Dat J., Vandenabeele S., Vranova E., Van Montagu M., Inzé D., Van Breusegem F. (2000) Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences*, **57**, 779–795.

De Gara L. (2004) Class III peroxidases and ascorbate metabolism in plants. *Phytochemistry Reviews*, **3**, 195–205.

Deng X., Hu Z., Wang H., Wen X., Kuang T. (2003) A comparison of photosynthetic apparatus of the detached leaves of the resurrection plant *Boea hygrometrica* with its non-tolerant relative *Chirita heterotrichia* in response to dehydration and rehydration. *Plant Science*, **165**, 851–861.

Djilianov D., Genova G., Parvanova D., Zapryanova N., Konstantinova T., Atanassov A. (2005) *In vitro* culture of the resurrection plant *Haberlea rhodopensis*. *Plant Cell, Tissue and Organ Culture*, **80**, 115–118.

Dunand C., Crévecoeur M., Penel C. (2007) Distribution of superoxide and hydrogen peroxide in Arabidopsis root and their influence on root development: possible interaction with peroxidases. *New Phytologist*, **174**, 332–341.

Farrant J.M. (2000) Comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plants. *Plant Ecology*, **151**, 1–11.

Farrant J.M., Cooper K., Kruger L.A., Sherwin H.W. (1999) The effect of drying rate on the survival of three desiccation-tolerant angiosperm species. *Annals of Botany*, **84**, 371–379.

Farrant J.M., Vander W.C., Loffell D.A., Bartsch S., Whittaker A. (2003) An investigation into the role of light during desiccation of three angiosperm resurrection plants. *Plant Cell and Environment*, **26**, 1275–1286.

Farrant J.M., Lehner A., Cooper K., Wiswedel S. (2009) Desiccation tolerance in the vegetative tissues of the fern *Mohria caffrorum* is seasonally regulated. *Plant Journal*, **57**, 65–79.

Foyer C., Noctor G. (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment*, **28**, 1056–1071.

Gaff D.F. (1971) Desiccation-tolerant flowering plants in Southern Africa. *Science*, **174**, 1033–1034.

Ganchev I. (1950) Anabiotic desiccation resistance and other biological traits of Haberlea rhodopensis Friv. *Reports of the Institute of Botany at the Bulgarian Academy of Sciences*, **1**, 191–214.

Georgieva K., Maslenkova L., Peeva V., Markovska Y., Stefanov D., Tuba Z. (2005) Comparative study on the changes in photosynthetic activity of the homoiochlorophyllous desiccation-tolerant *Haberlea rhodopensis* and desiccation-sensitive spinach leaves during desiccation and rehydration. *Photosynthetic Research*, **85**, 191–203.

Georgieva K., Szigeti Z., Sarvari E., Gaspar L., Maslenkova L., Peeva V., Peli E., Tuba Z. (2007) Photosynthetic activity of homoiochlorophyllous desiccation tolerant plant *Haberlea rhodopensis* during dehydration and rehydration. *Planta*, **225**, 955–964.

Georgieva K., Lenk S., Buschmann C. (2008) Responses of the resurrection plant *Haberlea rhodopensis* to high irradiance. *Photosynthesis*, **46**, 208–215.

Ghasempour H.R., Gaff D.F., Williams R.P.W., Gianello R.D. (1998) Content of sugars in leaves of drying desiccation tolerant flowering plants, particularly grasses. *Plant Growth Regulation*, **24**, 185–191.

Grace S., Logan B. (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **355**, 1499–1510.

Gronwald J., Fuerst E., Eberlein C., Egli M. (1987) Effect of herbicide antidote on glutathione content and glutathione-*S*-transferase activity of sorghum shoots. *Pesticide Biochemistry & Physiology*, **29**, 66–76.

Hincha D.K., Livingston D.P., Premakumar R., Zuther E., Obel N., Cacela C., Heyer A.G. (2007) Fructans from oat and rye: composition and effects on membrane stability during drying. *Biochimica et Biophysica Acta*, **1768**, 1611–1619.

Illing L., Denby K.J., Collett H., Shen A., Farrant J.M. (2005) The signature of seeds in resurrection plants: a molecular and physiological comparison of desiccation tolerance in seeds and vegetative tissues. *Integrative and Comparative Biology*, **45**, 771–787.

Ivanov S., Kerchev P. (2007) Separation and quantification of the cellular thiol pool of pea plants treated with heat, salinity and atrazine. *Phytochemical Analysis*, **18**, 283–290.

Jiang G., Wang Z., Shang H., Yang W., Hu Z., Phillips J., Deng X. (2007) Proteome analysis of leaves from the resurrection plant *Boea hygrometrica* in response to dehydration and rehydration. *Planta*, **225**, 1405–1420.

Kato-Noguchi H., Takaoka T., Izumori K. (2005) Psicose inhibits lettuce root growth via a hexokinase independent pathway. *Physiologia Plantarum*, **125**, 293–298.

Kramer G.F., Norman H.A., Krizek D.T., Mirecki R.M. (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry*, **30**, 2101–2108.

Kranner I. (2002) Glutathione status correlates with different degrees of desiccation tolerance in three lichens. *New Phytologist*, **154**, 451–460.

Kranner I., Birtic S. (2005) A modulating role for antioxidants in desiccation tolerance. *Integrative & Comparative Biology*, **45**, 734–740.

Kranner I., Beckett R.P., Wornik S., Zorn M., Pfeifhofer H.W. (2002) Revival of a resurrection plant correlates with its antioxidant status. *The Plant Journal*, **31**, 13–24.

Ksouri R., Megdiche W., Debez A., Falleh H., Grignon C., Abdelly C. (2007) Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology & Biochemistry*, **45**, 244–249.

Lloyd G.B., McCown B.H. (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Proceedings of the International Plant Propagation Society*, **30**, 421–427.

Markovska Y.K., Tsonev T.D., Kimenov G.P., Tutekova A.A. (1994) Physiological changes in higher poikilohydric plants *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panc. during drought and rewatering at different light regimes. *Journal of Plant Physiology*, **144**, 100–108.

Markovska Y.K., Tutekova A.A., Kimenov G.P. (1995) Ultrastructure of chloroplasts of poikilohydric plants *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panc. during recovery from desiccation. *Photosynthesis*, **31**, 613–620.

Mayer V., Möller M., Perret M., Wever A. (2003) Phylogenetic position and generic differentiation of *Epithemateae* (Gesneriaceae) inferred from cpDNA sequence data. *American Journal of Botany*, **90**, 319–327.

Mihailova G., Petkova S., Georgieva K. (2009) Changes in some antioxidant enzyme activities in *Haberlea rhodopensis* during desiccation at high temperature. *Biotechnology & Biotechnological Equipment*, **23**, 561–565.

Moore J., Westall K., Ravenscroft N., Farrant J., Lindsey G., Brandt W. (2005) The predominant polyphenol in the leaves of the resurrection plant *Myrothamnus flabellifolius*, 3,4,5 tri-O-galloylquinic acid, protects membranes against desiccation and free radical-induced oxidation. *Biochemistry Journal*, **385**, 301–308.

Moore J., Le N., Brandt W., Driouich A., Farrant J. (2009) Towards a systems-based understanding of plant desiccation tolerance. *Trends in Plant Science*, **14**, 110–117.

Morelli R., Russo-Volpe S., Bruno N., Scalzo R.L. (2003) Fenton-dependent damage to carbohydrates: free radical scavenging activity of some simple sugars. *Journal of Agriculture and Food Chemistry*, **5**, 7418–7425.

Mowla S.B., Thomson J.A., Farrant J.M., Mundree S.G. (2002) A novel stress-inducible antioxidant enzyme identified from the resurrection plant *Xerophyta viscosa* Baker. *Planta*, **215**, 716–726.

Mulako I., Farrant J.M., Collett H., Illing N. (2008) Expression of *Xhdsi-1*^{VOC}, a novel member of the vicinal oxygen chelate (VOC) metalloenzyme superfamily, is up-regulated in leaves and roots during desiccation in the resurrection plant *Xerophyta humilis* (Bak) Dur. *Journal of Experimental Botany*, **59**, 3885–3901.

Müller J., Sprenger N., Bortlik K., Boller T., Wiemken A. (1997) Desiccation increases sucrose levels in *Rhamonda* and *Haberlea*, two genera of resurrection plants in the Gesneriaceae. *Physiologia Plantarum*, **100**, 153–158.

Navari-Izzo F., Meneguzzo S., Loggini B., Vazzana C., Sgherri C.L.M. (1997) The role of the glutathione system during dehydration of *Boea hygroscopica*. *Physiologia Plantarum*, **99**, 23–30.

Nishizawa A., Yabuta Y., Shigeoka S. (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology*, **147**, 1251–1263.

Noctor G., Foyer C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 249–279.

Oliver M.J. (1996) Desiccation tolerance in vegetative plant cells. *Physiologia Plantarum*, **97**, 779–787.

Oliver M.J., Tuba Z., Mishler B.D. (2000) The evolution of vegetative desiccation tolerance in land plants. *Plant Ecology*, **151**, 85–100.

Passardi F., Penel C., Dunand C. (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends in Plant Science*, **9**, 534–540.

Peeva V., Cornic G. (2009) Leaf photosynthesis of *Haberlea rhodopensis* before and during drought. *Environmental and Experimental Botany*, **63**, 310–318.

Peeva V., Maslenkova L. (2004) Thermoluminescence study of photosystem II activity in *Haberlea rhodopensis* and spinach leaves during desiccation. *Plant Biology*, **61**, 319–324.

Peters S., Mundree G., Thomson J.A., Farrant J.M., Keller F. (2007) Protection mechanisms in the resurrection plant *Xerophyta viscosa* (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. *Journal of Experimental Botany*, **58**, 1947–1956.

Petrova G., Tosheva A., Mladenov P., Moyankova D., Djilianov D. (2010) *Ex situ* collection of model resurrection plant *Haberlea rhodopensis* as a prerequisite for biodiversity and conservation studies. *Biotechnology & Biotechnological Equipment*, **24**, 1955–1960.

Pirie A., Mullins M. (1976) Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. *Plant Physiology*, **58**, 468–472.

Proctor M.C.F., Tuba Z. (2002) Poikilohydry and homoihydry: antithesis or spectrum of possibilities? *New Phytologist*, **156**, 327–349.

Ramel F., Sulmon C., Bogard M., Couée I., Gouesbet G. (2009) Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in *Arabidopsis thaliana* plantlets. *BMC Plant Biology*, **9**, doi: 10.1186/1471-2229-9-28.

Rouhier N., Lemaire S., Jacquot J.P. (2008) The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation. *Annual Review of Plant Biology*, **59**, 143–166.

Schwab K.B., Schreiber U., Heber U. (1989) Response of photosynthesis and respiration of resurrection plants to desiccation and rehydration. *Planta*, **177**, 217–227.

Scott P. (2000) Resurrection plants and the secrets of eternal leaf. *Annals of Botany*, **85**, 159–166.

Sgherri C., Stevanovic B., Navari-Izzo F. (2004) Role of phenolics in the antioxidative status of the resurrection plant Ramonda serbica during dehydration and rehydration. *Physiologia Plantarum*, **22**, 478–485.

Shiomi N., Onodera S., Chatterton N.J., Harrison P.A. (1991) Separation of fructooligosaccharide isomers by anion-exchange chromatography. *Agricultural Biology and Chemistry*, **55**, 1427–1428.

Smeekens S., Ma J., Hanson J., Rolland F. (2010) Sugar signals and molecular networks controlling plant growth. *Current Opinion in Plant Biology*, **13**, 274–279.

Solfanelli C., Poggi A., Loreti E., Alpi A., Perata P. (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant Physiology*, **140**, 637–646.

Stefanov K., Markovska Y., Kimenov G., Popov S. (1992) Lipid and sterol changes in leaves of *Haberlea rhodopensis* and *Ramonda serbica* at transition from biosis into anabiosis and vice versa caused by water stress. *Phytochemistry*, **31**, 2309–2314.

Stoyanova S., Geuns J., Hideg E., Van den Ende W. (2010) The food additives inulin and stevioside counteract oxidative stress. *International Journal of Food Sciences and Nutrition*, doi: 10.3109/09637486.2010.523416.

Swain T., Goldstein J.L. (1964) The quantitative analysis of phenolic compounds. In: Pridham J.B. (Ed.) *Methods in polyphenol chemistry*. Pergamon Press, Oxford, UK, pp 131–146.

Tausz M., Sircelj H., Grill D. (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? *Journal of Experimental Botany*, **55**, 1955–1962.

Toldi O., Djilianov D., Scott P. (2006) Transgenic approach for functional analysis of genes associated with desiccation tolerance in resurrection plants. In: Teixeira da Silva J.A. (ed), *Floriculture, ornamental and plant biotechnology* Vol III. Global Science Books, UK, pp 209–216.

Toldi O., Tuba Z., Scott P. (2009) Vegetative desiccation tolerance: is it a goldmine for bioengineering crops? *Plant Science*, **176**, 187–199.

Van den Ende W., Valluru R. (2009) Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? *Journal of Experimental Botany*, **60**, 9–18.

Vander Willigen C., Pammenter N.W., Jaffer M.A., Mundree S.G., Farrant J.M. (2003) An ultrastructural study using anhydrous fixation of *Eragrostis nindensis*, a resurrection grass with both desiccation-tolerant and -sensitive tissues. *Functional Plant Biology*, **30**, 281–290.

Veljovic-Jovanovic S., Kukavica B., Navari-Izzo F. (2008) Characterization of polyphenol oxidase changes induced by desiccation of Ramonda serbica leaves. *Physiologia Plantarum*, **132**, 407–416.

Vergauwen R., Van den Ende W., Van Laere A. (2000) The role of fructan in flowering of *Campanula rapunculoides*. *Journal of Experimental Botany*, **51**, 1261–1266.

Weber H., Borisjuk L., Heim U., Buchner P., Wobus U. (1995) Seed coat-associated invertases of fava bean control both unloading and storage functions: cloning of cdnas and cell type-specific expression. *Plant Cell*, **7**, 1835–1846.

Whittaker A., Bochicchio A., Vaccanza C., Lindsey G., Farrant J.M. (2001) Changes in leaf hexokinase activity and metabolite levels in response to drying in the desiccation-tolerant species *Sporobolus stapfianus* and *Xerophyta viscosa*. *Journal of Experimental Botany*, **52**, 961–969.

Whittaker A., Martinelli T., Farrant J.M., Bochicchio A., Vaccanza C. (2007) Sucrose phosphate synthase activity and the co-ordination of carbon partitioning during sucrose and amino acid accumulation in desiccation-tolerant leaf material of the C-4 resurrection plant *Sporobolus stapfianus* during dehydration. *Journal of Experimental Botany*, **58**, 3775–3787.

Xiao L.M., Koster K.L. (2001) Desiccation tolerance of protoplasts isolated from pea embryos. *Journal of Experimental Botany*, **52**, 2105–2114.

Yahubyan G., Gozmanova M., Denev I., Toneva V., Minkov I. (2009) Prompt response of superoxide dismutase and peroxidase to dehydration and rehydration of the resurrection plant *Haberlea rhodopensis*. *Plant Growth Regulation*, **57**, 49–56.

Yamasaki H., Grace S.C. (1998) EPR detection of phytophenoxyl radicals stabilized by zinc ions: evidence for the redox-coupling of plant phenolics with ascorbate in the H_2O_2-peroxidase system. *FEBS Letters*, **422**, 377–380.

Zhao J., Dixon R.A. (2010) The 'ins' and 'outs' of flavonoid transport. *Trends in Plant Science*, **15**, 72–80.