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# Cellular Biochemical Changes in *Selaginella tamariscina* (Beauv.) Spring and *Sellaginella plana* (Desv. ex Poir.) Heiron. as Induced by Desiccation

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**Abstrak:** Perubahan biokimia dalam dua spesies *Selaginella* iaitu *S. tamariscina* (Beauv.) Spring dan *S. plana* (Desv. ex Poir.) Heiron., yang dicetuskan oleh pengeringan dan diikuti oleh rehidrasi telah dikaji. Tumbuhan telah dibenarkan untuk dehidrat secara semula jadi dengan menyekat pengairan sehingga kandungan air relatif (RWC) pucuk bernilai <10%. Kemudiannya, tumbuhan yang dehidrat disiram air sehingga mencapai keadaan rehidrasi sepenuhnya iaitu RWC 90% ataupun lebih. Sifat-sifat menahan pengeringan telah diperhatikan dalam *S. tamariscina* manakala sifat sensitif terhadap pengeringan dilihat pada *S. plana*. Integriti membran dikekalkan dalam *S. tamariscina* tetapi bukan dalam *S. plana* seperti yang dilihat dalam ukuran kebocoran elektrolit relatif semasa fasa pengeringan dan seterusnya semasa fasa rehidrasi. Analisa pigmen telah menunjukkan konservasi beberapa klorofil dan karotenoid semasa pengeringan dan mencapai tahap kawalan yang mengikuti proses rehidrasi dalam *S. tamariscina*. Kandungan pigmen yang sangat rendah telah dijumpai dalam *S. plana* semasa fasa pengeringan dan pigmen tersebut tidak dipulih semula semasa rehidrasi. Penentuan zat terlarut yang serasi menunjukkan kenaikan dalam kandungan gula dan proline dalam *S. tamariscina* kering sahaja, yang menunjukkan kewujudan jentera-jentera perlindungan biokimia dalam spesies ini dan ketidakhadirannya dalam *S. plana* semasa keadaan dehidrat. Data-data ini menunjukkan satu elemen penting untuk toleransi terhadap pengeringan dalam tumbuhan vaskular rendah ialah kebolehan melindungi tisu-tisu daripada kemusnahan serius akibat pengeringan yang teruk.

**Kata kunci:** Elektrolit, Pigmen, Proline, Rehidrasi, Gula

**Abstract:** The biochemical changes in two *Selaginella* species namely, *S. tamariscina* (Beauv.) Spring and *S. plana* (Desv. ex Poir.) Heiron., as induced by desiccation and subsequent rehydration were explored. Plants were allowed to dehydrate naturally by withholding irrigation until shoot's relative water content (RWC) reached <10%. After which, dehydrated plants were watered until fully rehydrated states were obtained which was about 90% RWC or more. Desiccation-tolerance characteristics were observed in *S. tamariscina* while desiccation-sensitivity features were seen in *S. plana*. Membrane integrity was maintained in *S. tamariscina* but not in *S. plana* as evidenced in the relative

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electrolyte leakage measurements during desiccation phase and the subsequent rehydration stage. Pigment analyses revealed conservation of some chlorophylls and carotenoids during desiccation and reaching control levels following rehydration in *S. tamariscina*. Very low pigment contents were found in *S. plana* during desiccation phase and the pigments were not recovered during rehydration attempt. Meanwhile, compatible solute determination showed rise in total sugar and proline contents of desiccated *S. tamariscina* only, indicating presence of biochemical protection machineries in this species and absence of such in *S. plana* during dehydrating conditions. These data indicate that one key element for desiccation-tolerance in lower vascular plants is the ability to protect tissues from severe damages caused by intense desiccation.

**Keywords:** Electrolyte, Pigment, Proline, Rehydration, Sugar

## INTRODUCTION

One of the most significant evolutionary forces is to be able to live in a very dry environment. Atmosphere with a relative humidity of 50% at 20°C equals to a water potential of –100 MPa (Alpert 2006). This also corresponds to drying to <0.1 g $H_2O$ $g^{-1}$ dry biomass which is roughly equivalent to about 10% water content or less (Alpert 2005). Majority of modern day plants may not survive in such environment as it might result to the loss of their intracellular water content down to 90% (Alpert 2006). However, there are certain species of plants, called "resurrection" plants, having strong selection for desiccation tolerance.

Resurrection plants survive the loss of most of their water content down to <5% relative water content (RWC) until a quiescent stage is achieved (Peters *et al.* 2007). Since most of the protoplasmic water is lost, it is considered the severest form of water stress (Bartels 2005). Upon watering, the plants rapidly revive and return to their normal physiological functioning (Alpert 2005). They are also known as poikilohydric plants because they lack capability to prevent desiccation. They directly rely on the environment for their water status. As a consequence, their cells' water content tend to reach equilibrium with that of the environment (Alpert 2000; Scott 2000; Pandey *et al.* 2010).

Numerous studies were conducted to investigate desiccation tolerance mechanisms in different resurrection plants. Most of the available information are derived from the rich data obtained from studies conducted in angiosperm species (Sherwin & Farrant 1996, 1998; Farrant *et al*. 1999, 2003; Farrant 2000; Cooper & Farrant 2002; Moore *et al.* 2007). Little attention has been given to desiccation tolerance in the lower group of vascular plants (Oliver *et al*. 2000). Although desiccation-tolerant pteridophytes have been documented, for example fern *Polypodium polypodioides* (Layton *et al*. 2010) and fern allies *Selaginella lepidophylla* (Brighigna *et al*. 2002), and *S. bryopteris* (Pandey *et al*. 2010), much still needs to be done (Oliver *et al*. 2005).

One of the most primitive taxa of vascular plants is *Selaginella* (Kenrick & Crane 1997). In the recent review by Setyawan (2011), this sole surviving genus of the spikemoss family, Selaginellaceae, has 700–750 species throughout the world. The genus has not been intensively studied in the Philippines; taxonomically, morphologically, and physiologically. Regarding the species of

*Selaginella* found in the country, no formal proclamation has been made on which are desiccation-tolerant or desiccation-intolerant. This implies that common and distinct physiological features of desiccation-tolerant and intolerant *Selaginella* in the Philippines are not yet fully worked out.

The aim of this work was threefold: (1) to investigate the biochemical responses of two species of *Selaginella*, namely *S. tamariscina* and *S. plana*, to desiccation and rehydration treatments; (2) to assess which species will perform better with respect to its capability to resume normal physiological functioning during rehydration stage; and (3) to elucidate different physiological strategies of the species to maintain cellular integrity and limit the damage caused by desiccation.

## MATERIALS AND METHODS

### Plant Materials and Growing Conditions

Commercially obtained *S. tamariscina* and *S. plana* were allowed to acclimatise and grow in a 50 kg substrate with an equal mixture of river sand, coir dust, and garden soil placed in a wooden tray. The substrate was kept damp supplemented weekly with half strength of Hoagland's solution. They were maintained in screen house conditions with a daytime temperature range of 23°C to 42°C.

### Experimental Set-up

The experimental treatments were as follows:

1. Desiccated – plants dried to a constant air-dried state (10% RWC) or below;
2. Rehydrated – plants which have been fully recovered at 90% RWC or more; and
3. Control – well watered plants which had not been desiccated.

All measurements were performed on desiccated and rehydrated plants. Same measurements were carried out in control plants twice. The first set of control measurements was done simultaneously with the desiccated plants while the second control set was done simultaneously with the rehydrated plants. This was purposely carried out to provide a point of comparison since plants experience different environmental conditions at different times. All measurements were done in triplicate wherein composite sampling was employed.

### Dehydration and Rehydration Treatments

Whole plants previously acclimatised were slowly dried by withholding water and allowing the plants to dry out naturally under ambient screen house condition until below 10% RWC was reached. The plants were left in the dry state for no longer than three days. Same plants were rehydrated up to approximately 90% RWC or more to be used for the next cycle of experimentation. Rehydration was carried out through overhead watering using a mist spray to simulate rainfall. The

plants were well watered on the first day and then the soil was kept damp by daily watering for the remainder of the experiment.

**Plant Relative Water Content Determination**

Hydration states were determined by measuring the relative water contents of the shoot. Fully expanded similarly-sized fronds were selected from at least three plants per replicate. Using a sharp razor blade, individual fronds were detached at the leaf base. Fronds were immediately weighed to get the initial mass (Mi). In order to obtain the mass at full turgor (Mt), fronds were floated in distilled water inside a closed Petri dish for 24 h. Surface water was eliminated by blotting the fronds dry with a paper towel. Mt was recorded and the leaf samples were subsequently dried at 80°C for 24 h, and the dry mass (Md) was obtained. All mass measurements were done using an analytical scale, with precision of 0.01 g. Values of Mi, Mt, and Md were used to calculate RWC using the equation (Barrs & Weatherley 1962):

$$RWC\ \% = Mi–Md/Mt–Md \times 100.$$

**Electrolyte Leakage Measurement**

Electrolyte leakage, which gives an indication of the degree of membrane integrity, was measured following the procedure of Wang *et al*. (2010) with modifications. Fronds (0.5 g) were rinsed three times in distilled water to remove the contents of the cut cells. The fronds were soaked in 25 mL distilled water and shaken at room temperature for 24 h after which aliquot for leachate measurement was taken. The electrical conductivity of the solution (C1) was determined using a conductivity instrument (D-2, Horiba Ltd., Kyoto). The samples in the tube was then placed in boiling water (100°C) for 10 min and allowed to cool to ambient temperature. The electrical conductivity of this solution (C2) was then measured to obtain the maximum conductivity. The electrical conductivity of the distilled water (C3) was also measured. The relative electrolyte leakage (REL) was calculated using the equation:

$$REL\ \% = C1–C3/C2–C3 \times 100.$$

**Chlorophyll and Carotenoid Measurements**

Approximately 0.5 g of leaf tissues was frozen with liquid nitrogen and homogenised using mortar and pestle. The homogenised samples were contained in a test tube with cover and the chlorophyll was extracted with 10 mL 80% acetone. The test tubes were wrapped with aluminum foil and left in room temperature overnight. The crude extract was centrifuged at 3000 g for 5 min. The supernatant was kept while the pellet was discarded. The absorbance of the supernatants was read at 663.6 nm, 646.6 nm, and 440.5 nm, which are the major absorption peaks of chlorophylls *a*, *b,* and carotenoids, respectively (Porra *et al.* 1989). The total chlorophyll (*Chl a*+*b*) and total carotenoid (*Car*) contents were calculated using extinction coefficients provided by Porra *et al.* (1989). The chlorophyll and carotenoid concentrations were then expressed on the basis of µg chl/g dry sample (µg $g^{-1}$).

**Total Soluble Sugar Content Determination**

The total soluble sugar content (TSS) of leaves from dry, rehydrated, and control plants was estimated following the phenol-sulfuric acid colorimetric assay by Dubois *et al*. (1956), modified and improved by the Institute of Plant Breeding Analytical Services Laboratory, University of the Philippines Los Baños. Fifty mg dried and ground sample was extracted by adding 5 mL 80% ethanol with occasional shaking for 10 min. The extract was centrifuged at 3000 g for another 10 min and the supernatant was decanted into a 100 mL volumetric flask. The extraction process was repeated twice and supernatant of these extractions were pooled. The supernatant was diluted with distilled water, made up to volume, and mixed well. One mL 5% phenol reagent was added and followed by 5 mL 96% sulfuric acid in an aliquot of 0.10 mL. The solution was mixed well and let to react for 10 min at room temperature. The absorbance of the solution was immediately read at 490 nm using a spectrophotometer (UV-2700, Shimadzu Corporation, Kyoto). A standard curve for sucrose was constructed to determine the total soluble sugar concentration in each sample and expressed in percentage of the dry sample.

**Proline Content Determination**

Proline content was determined according to the method of Bates *et al*. (1973) and further modified by Cagampang and Rodriguez (1980). Dry powdered frond sample (50 mg) was extracted by the addition of 4 mL of chilled 3% sulphosalicylic acid solution and shaken for 30 min. The homogenate was filtered and the supernatant was collected in a glass tube. In a tube with 0.50 mL aliquot of the supernatant, 50 mL of 6M phosphoric acid, and 1 mL ninhydrin acid were added and shaken well. One mL glacial acetic acid was then added. The tube was incubated in boiling water bath for 20 min and then in an ice bath, then in room temperature. Absorbance of the solution was recorded at 520 nm against blank. A standard curve for proline was constructed to determine the proline concentration in each sample and expressed in mg/g dry sample (mg $g^{-1}$).

**Statistical Analysis**

Data obtained from the biochemical parameters were presented as means of replicates. Data were subjected to Analysis of Variance (ANOVA) using SPSS® version 16.0 software (SPSS Inc., Chicago, Illinois, USA) at 5% level of significance to determine significant differences among the treatments in each species. Multiple comparisons of treatments were carried out using Tukey HSD at 5% level of significance.

## RESULTS AND DISCUSSION

**Membrane Stability**

Conductometric measurement of solute leakage was carried out to assess membrane damage. No significant difference ($p$>0.05) in the REL was evident among the shoot of dried (12.0%) and fully hydrated (13.6%) *S. tamariscina* plants (Fig. 1). This indicates that membrane integrity was maintained during

drying in this species. Meanwhile, there was reduction in electrolyte leakage in rehydrated *S. tamariscina* as evidenced in its lower REL (9.1%) than in desiccated plants. This means that membrane integrity was even more intact during the rehydration phase.

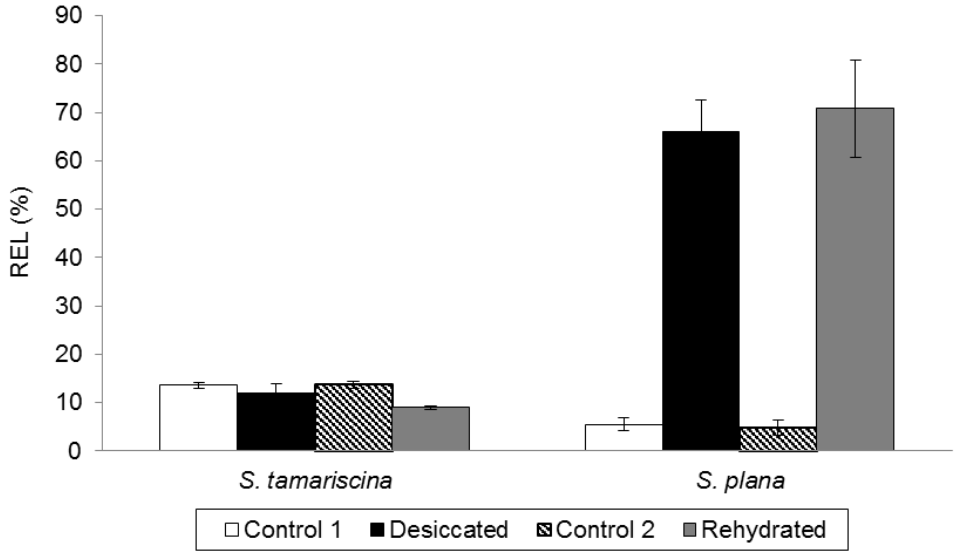


**Figure 1:** Effect of desiccation and rehydration on stability of cell membrane in *S. tamariscina* and *S. plana* reflected as REL. Error bars represent standard deviation within the test group or treatment ($p$<0.05), obtained from three replicates.

Although placed in a different plant group, the same trend was observed by Sherwin and Farrant (1996) when they monitored the rate of electrolyte leakage in the resurrection plant *Craterostigma wilmsii*. Leakages in control, dry, and rehydrated leaves were not variable. Similar to this species, protection mechanism was probably reinforced in *S. tamariscina* during rehydration as reflected in the lower REL noted in the rehydrated plants when compared with the dehydrated ones. This supports claim that as a desiccation tolerant plant rehydrates following dehydration, damage in the plasma membrane is immediately repaired. Crowe *et al*. (1992) asserted that drying causes the membrane to change from a liquid crystalline phase to gel phase during dehydration and then return to liquid crystalline phase during rehydration. These membrane phase transitions are the putative cause when leakage occurs in desiccation tolerant species (Oliver *et al*. 2005). In addition, rupture in the plasma membrane may occur as it contracts from the cell wall and by its attachment to it via the plasmodesmata during dehydration (Levitt 1980).

Highly significant rise in REL, however, was noted in both desiccated and rehydrated *S. plana* against the controls. It was also observed that there was a further substantial increase in REL upon rehydration attempt. This indicates that damage in the membrane incurred during the dehydration process was not

reversed or repaired during rehydration. In fact it would seem that leakage was even aggravated during rehydration in this species.

**Pigment Content**

Illustrated in Figure 2 is the total chlorophyll content (*Chl a+b*) in desiccated, rehydrated, and control plants. Statistical analysis showed that there was significant difference in the mean *Chl a+b* among test groups or treatments in *S. tamariscina* ($p<0.05$). The *Chl a+b* in desiccated plants dropped to 1133.8 µg $g^{-1}$ of dry shoot tissues from 1770.9 µg $g^{-1}$ ($p<0.05$). Hence, approximately 64.0% total chlorophyll of the fully hydrated (control) plants was retained during the desiccated state. When hydration resumed, however, total chlorophyll content (1617.5 µg $g^{-1}$) roughly reached control levels. This indicates that chloroplasts of this species likely recovered and became functional during this state.

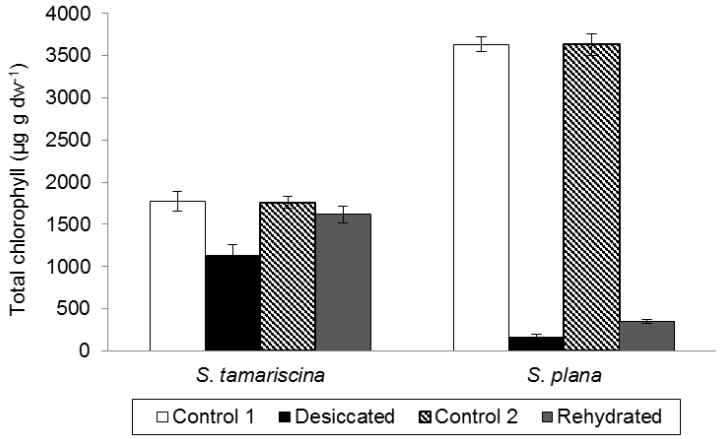


**Figure 2:** Total chlorophyll (*Chl a+b*) contents of *S. tamariscina* and *S. plana* following desiccation and rehydration. Error bars represent standard deviation within the test group or treatment ($p<0.05$), obtained from three replicates.

On the other hand, it is apparent that there was significant loss in *Chl a+b* in *S. plana* during the dry state (Fig. 2). Total chlorophyll content dropped from 3633.3 µg $g^{-1}$ to 167.9 µg $g^{-1}$ after desiccation ($p<0.05$), which was maintained even during the rehydration attempt. In this species, the total chlorophyll in rehydrated plants was not significantly different from the desiccated ones indicating that there was no element of regeneration ($p>0.05$).

Decrease in chlorophyll content of leaves is thought to be linked to the protection of plants against UV light and from damage as a result of oxygen free radical generation during desiccation (Sherwin & Farrant 1998). Chlorophyll content of the leaves did not drop too much in desiccated *S. tamariscina* indicating that no complete dismantling of photosynthetic apparatus was observed. It is assumed that *S. tamariscina* did not lose all its chlorophyll because the plant itself is protected from irradiation damage through different means. Since it was observed that it formed a ball upon desiccation, the

functional leaves were protected inside. The adaxial side of the leaves remained green while the abaxial side did not. It must be noted, however, that the total chlorophyll in desiccated *S. tamariscina* was significantly lower than the control suggesting degradation probably happened but was not severe.

The changes in carotenoid content during dry state are shown in Figure 3. Compared to *S. tamariscina*, carotenoids in *S. plana* were almost degraded. From 122.9 µg $g^{-1}$, carotenoids in *S. plana* declined to 9.9 µg $g^{-1}$ or approximately 8.1% of the total ($p$<0.05). Whereas, approximately 41.9% of the total carotenoid content of control *S. tamariscina* (124.2 µg $g^{-1}$) was retained in the dried state. Upon rehydration, control levels were even exceeded unlike in the case of *S. plana*.

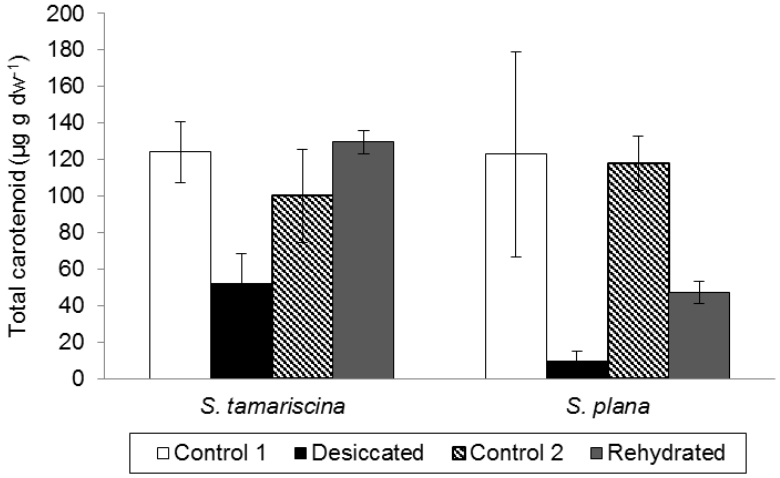


**Figure 3:** Total carotenoid contents in shoot of *S. tamariscina* and *S. plana* following desiccation and rehydration. Error bars represent standard deviation within the test group or treatment ($p$<0.05), obtained from three replicates.

Thus, chlorophyll and carotenoid results show that some of the photosynthetic apparatus during desiccation in *S. tamariscina* was retained, if not completely upheld. Desiccation-tolerant plants can be classified through pigment analysis, whether they retain their photosynthetic pigments or not. Oliver *et al*. (2000) and Tuba *et al.* (1998) stated that desiccation-tolerant plants that retain their chlorophyll content during dry state are homoichlorophyllous while those that dismantle their chlorophyll are termed poikilochlorophyllous. On this basis, Farrant (2000) classified the resurrection angiosperms *C. wilmsii* and *Myrothamnus flabellifolius* as homoichlorophyllous species because they retained some of their chlorophyll content, 82% and 60% chlorophyll, respectively. Since *S. tamariscina* retained 64% of its chlorophyll in the dehydrated state, it can be classified then as homoichlorophyllous species. Upon rehydration, this partial loss of chlorophyll was regained. Being a desiccation-tolerant plant, *S. tamariscina* has innate characteristics protecting its photosynthetic apparatus. This can be supported by the observation that when non-irrigation of some plants

was prolonged up to 30 days, the plants remained as they were visually during the third day in <10% RWC and did not bleach at all. Moreover, *S. tamariscina* curved and folded its rosette shoot upward during drying which aided to protect its adaxial surface and safeguard the inner rosette shoot. A similar morphological strategy was observed in *C. wilmsii, C. plantagineum*, and *M. flabellifolius* (Farrant 2000; Scott 2000). This strategy would stop photochemistry even though photosynthetic pigments are present since the functional leaves are covered by the outer or aged leaves (Farrant 2000).

**Osmotic Adjustments**

Plants under osmotic stress synthesise compatible solutes as these compounds are known to stabilise proteins and help in osmotic balance. Examples of these compatible solutes are soluble sugar and proline contents which were analysed in the two plants. Results show that soluble sugar was significantly highest ($p$<0.05) in desiccated *S. tamariscina* representing 22.1% of the dried tissue (Fig. 4). This is approximately two-fold higher than the sugar level of the controls. These data indicate that there is build-up of soluble sugars in *S. tamariscina* during desiccation. The same observation was reported by Wang *et al*. (2010) in one variety of *S. tamariscina* collected from China. Marked increase was noticed on the fifth day of drying up to the seventh day reaching approximately 100% increase against the fully hydrated tissue. According to Dinakar *et al*. (2012), if carbohydrates have a protective role during desiccation then accumulation must be very fast and the concentration must be sufficiently high. On the other hand, no significant increase in soluble sugar content was observed in *S. plana* during both desiccated and rehydrated states.

Increase in soluble sugars in plants undergoing osmotic stress is a common occurrence in the living world. Bacteria, yeasts, and seeds of higher plants concentrate high amount of soluble sugars in the dry tissues which correlates to their ability of surviving desiccation (Crowe *et al*. 1992; Nedeva & Nikolova 1997). Sucrose, trehalose, and raffinose are some sugars commonly observed in desiccated resurrection plants (Smirnoff 1992; Ingram & Bartels 1996; Crowe *et al*. 1998; Scott 2000; Peters *et al*. 2007; Liu *et al.* 2008). In terms of minimum concentration required, trehalose is the most effective osmoprotectant sugar (Crowe *et al*. 1992). Resurrection *Selaginella, S. lepidophylla,* accumulates trehalose at high levels, as much as 20% of the dry weight (Crowe *et al*. 1998).

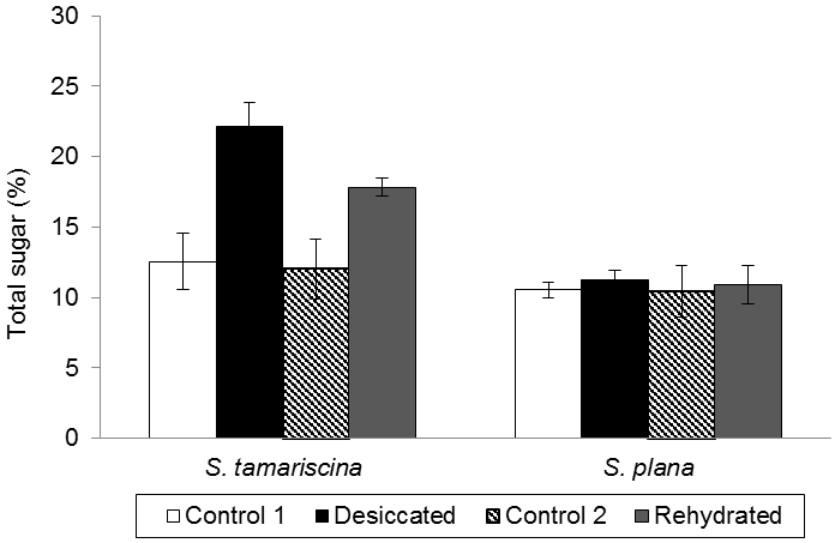


**Figure 4:** Total soluble sugar contents of *S. tamariscina* and *S. plana* following desiccation and rehydration. Error bars represent standard deviation within the test group or treatment ($p$<0.05), obtained from three replicates.

Although trehalose is considered a major factor that determines the anhydrobiotic ability of resurrection plants (Zentella *et al*. 1999), sucrose and other sugars, however, may also act as osmoprotectants (Crowe *et al*. 1992). In water-deprived *Xerophyta viscosa*, sucrose concentration in the leaves increased to nearly 5-fold at 5% RWC from the original sucrose content while raffinose increased to approximately 3-fold at same RWC (Peters *et al*. 2007). Others suggest that there is redirection of carbon flow from reserve substances such as starch or octulose to soluble saccharides (Ingram *et al*. 1997). In *Craterostigma* species, leaves of well-watered *C. wilmsii* and *C. plantagineum* have high amount of eight carbon sugar 2-octulose (Bianchi *et al*. 1991; Norwood *et al*. 2000). This eight-carbon sugar is converted to sucrose upon drying (Willige *et al*. 2009). This massive conversion of stored carbohydrates upon dehydration will concentrate sucrose in the dried tissue which consequently will comprise about 40% of the plant's dry weight. In some cases, sucrose may make up as much as 50% of the dry weight (Crowe *et al*. 1998). In another desiccation-tolerant plant *Ramonda* sp., instead of 2-octulose, starch is converted to sucrose that serves the same function (Müller *et al*. 1997).

Crowe *et al*. (1998) stated two hypotheses concerning the roles of sugars in desiccated plants; they form supersaturated liquid known as biological glasses to stabilise internal structures and they prevent fusion of membranes and denaturation of proteins in the cell rendering maintenance of cell integrity during desiccation. The process of forming biological glasses is known as vitrification (Hoekstra 2005). This process is obligatory to survival during desiccation as this protects organelles from damage. This biological glass forms cavity inside the cell to prevent cellular collapse. It also restricts production of free radicals by slowing

molecular mobility in the cytoplasm to prevent chemical reactions to occur (Koster 1991; Ingram & Bartels 1996; Hoekstra 2005). Moreover, sugars protect membranes by altering the properties of the dry membranes to resemble those of fully hydrated ones. It is suggested that the hydroxyl groups of sugars substitute for water and provide the required hydrophilic interactions for membrane, the "water replacement hypothesis" (Crowe *et al*. 1992). Similarly, sugars stabilise proteins through the formation of hydrogen bonds between sugar hydroxyl groups and polar residues in proteins (Crowe *et al*. 1992). This mechanism of direct bonding of sugars with biomolecules is indeed imperative in the stabilisation of proteins, membranes, and whole cells under conditions of dehydration (Peters *et al*. 2007).

The present study showed that desiccated *S. tamariscina* had the highest proline content ($p$<0.05); approximately 15.0% increase in proline content was noted in desiccated plant compared to control plant (Fig. 5). In contrast, rehydrated *S. tamariscina* was shown to have lower proline content relative to the desiccated samples. These results validate the important role of proline during desiccation as shown in its increase during stress and its decrease when the plant was relieved from stress.

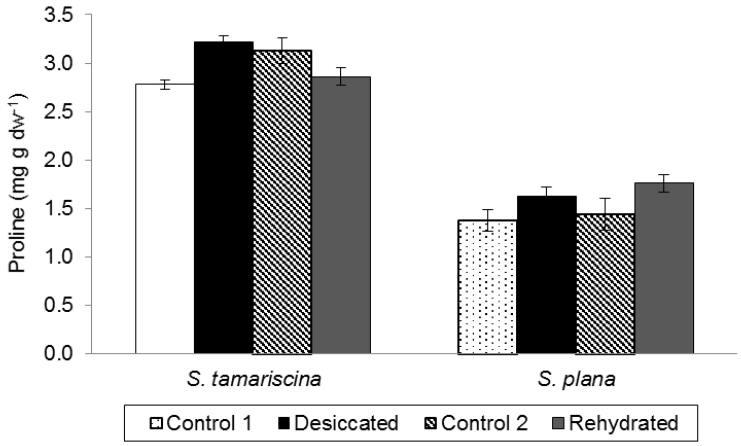


**Figure 5:** Proline contents of *S. tamariscina* and *S. plana* following desiccation and rehydration. Error bars represent standard deviation within the test group or treatment ($p$<0.05), obtained from three replicates.

Proline is also known to accumulate in response to a wide range of abiotic stresses in many plants (Hare & Cress 1997). Its presumed roles are in stabilising protein structures against denaturation, interacting with phospholipids to protect cell membranes, and in functioning as a hydroxyl radical scavenger in stressed plants (Santoro *et al*. 1992; Ingram & Bartels 1996; Claussen 2005).

Pandey *et al*. (2010) and Wang *et al*. (2010) used proline as one of their parameters to assess desiccation tolerance in *S. bryopteris* and *S. tamariscina*, respectively. Proline in dried *S. bryopteris* frond was more or less 5-fold higher than in fully hydrated fronds (Pandey *et al*. 2010). On the other hand, Wang *et al.*

(2010) observed that relatively stable proline levels were noted from zero to third day of drying in *S. tamariscina*. It was only during the fifth day that sharp increase in proline concentration was observed which continued and was highest during the seventh day of drying. Moreover, proline concentrations during the 12th hour of rehydration and initial phase of drying were comparable indicating that high concentration of proline during fully hydrated and rehydrated phases is not a requirement.

Figure 5 also shows that the second control of *S. tamariscina* plants had higher proline concentration than the first control plants and was not significantly different compared to the desiccated plants. This is in contradiction with the initial statement that proline accumulates in response to desiccation. If the second control is taken into account and compared it with the desiccated data, statistics suggest that proline did not rise during desiccation. However, it should also be noted that the desiccated and the second control data were taken at different time intervals and perhaps at different prevailing environmental conditions. That is, the second control data were taken at a time corresponding to the time when rehydrated samples were analysed. Since proline is also implicated in other stressful environmental conditions like salinity stress (Amirjani 2010), drought stress (Mafakheri *et al*. 2010), high light intensity, and temperature (Claussen 2005), it might be that the increase in proline concentration in the second control of *S. tamariscina* plants were due to environmental stress which is not osmotic in nature. While desiccated and rehydrated *S. plana* was observed to have higher proline content than the fully hydrated ones (controls), this increase, however, was not significantly different from the control plants.

Despite evolutionary relatedness, it is apparent that *S. tamariscina* and *S. plana* behave differently toward desiccation stress. Most of the *Selaginella* species, including *S. plana*, are tropical growing in damp shady habitats (Kramer & Green 1990) but others such as *S. lepidophylla* and *S. sartorii* are adapted for seasonal drought or xerophytic conditions (Setyawan 2011) just like *S. tamariscina*. They grow on dry rocky cliffs or on soil that dries periodically. Besides physiological adaptations, desiccation-tolerant plants have also evolved the ability to overcome the drought-induced stress morphologically. To name a few, leaf curling, excessive cell volume reduction, and cell wall folding or shrinkage as the plant dries but they re-expand as they are moistened (Farrant *et al.* 2007). The aerial parts of *S. tamariscina* undergo gradual morphological rolling and wilting forming a ball in the dry state but as it is re-watered, the aerial parts fully open and recover in just 12 h (Wang *et al.* 2010). All these things together are manifestations of active strategy of *S. tamariscina* for protecting itself and avoiding damages brought by desiccation.

## CONCLUSION

Desiccation-tolerance mechanisms have been found in *S. tamariscina* while desiccation-sensitive characteristics were found in *S. plana*. In *S. tamariscina*, evident protection mechanisms are engaged such as low electrolyte leakage and retention of some of the chlorophylls and carotenoid contents during the

desiccation and rehydration phases. The former indicates stability of the membranes while the latter means protection of the photosynthetic apparatus. Compatible solutes like sugars and proline also increased during desiccation inferring decreased rates of chemical reactions, reduced diffusion of molecules, and prevention of oxidative damage. Meanwhile, there appears to be no mechanism of subcellular protection operating in *S. plana*. As the drying process progressed, being intolerant of desiccation, drying up of tissues became irreversible.

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