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# A study on mechanisms of response and adaptation to desiccation tolerance in *Selaginella wightii*, a pteridophyte species of semiarid region in south India

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## Abstract

Water deficiency is the most significant abiotic stress factor for land plants. Most plants are unable to survive desiccation to the air dry state. However there are a few species across the plant kingdom that tolerate desiccation known as resurrection plants. Desiccation tolerance (DT) is the ability of the organisms to dry completely and resume normal function when rehydrated. Several Pteridophytes species with DT have been discovered in ecological niches with seasonally limited water availability and unreliable rainfalls including forests with microclimatic zones. *Selaginella* is one of the most primitive vascular resurrection plants, which can survive a desiccated state and then recover when water becomes available. This study deals with Pteridophyte species collected from Devarayanadurga *State* Forest, Tumakuru district, Karnataka, India. (Coordinates 13.3707° N, 77.1773° E) and identified as *Selaginella wightii (SW).* The Relative water content was established from the fresh tissues in independent experiments. Detached fronds from fully hydrated species were subjected to subsequent desiccation and rehydration. During desiccation, the RWC of detached fronds decreased rapidly from 90% to a stable dried state of 10% after 12h. Desiccated fronds showed intense inward curving during rehydration. The RWC of the detached fronds regained to 100% after 4 h, along with its original morphology. The Chlorophyll content being regained and proline and sucrose content increased. Activities of antioxidant enzymes (SOD, POD, CAT, and GR) also increased during desiccation. Further scanning electron microscopy reveals the leaf morphology and fine structure to analyse the cellular changes occurring during the desiccation which helps in elucidation of the effects of desiccation on cellular integrity.

**Keywords:** desiccation tolerance, *Selaginella wightii*, relative water content, antioxidant enzymes, SEM

## 1. Introduction

Climate-change crop production and productivity are now affecting global food security with a population estimated to exceed 9.8 billion by 2050. Competition for water supplies to support agriculture is becoming a major concern with the ongoing and expected global climate change. Availability of Water to plants is the most important variable under changing environmental conditions determining plant growth and survival. Different Plant species have special acclimation and adjusting characteristics at various levels which increase tolerance. Semiarid localities are prone to water scarce periods of varying periods and time. Desiccation tolerance (DT) is referred as resurrection plants which have an ability to equilibrate their cellular water content with dry atmospheric air conditions and also to the loss of complete protoplasmic water and regain its originality when water is available (Proctor & Pence 2002) [27] Resurrection plants are able to resume their normal biological, physiological functions and morphology within several hours or a few days by their complex continuous processes of alterations, depending on the size, the dried vegetative tissues of the species until equilibrium with low relative air humidity (Aidar *et al.*, 2017) [1]. Many DT species have been extensively studied and correlated for understanding the role of molecules and physiology behind the desiccation tolerance process: the bryophyte-*Tortula ruralis*, club mosses-*Selaginella lepidophylla* and *Selaginella tamariscina*, dicots-*Craterostigma plantagineum*, *C. wilmsii*, *Boea hygrometrica*, *Myrothamnus flabellifolia,* the monocots-*Xerophyta viscosa*, *X. humilis*, and *Sporobolus stapfianus* (Ingram and Bartells 1996; Alpert and Oliver 2002; Moore *et al* 2009; Cushman and Oliver 2011; Oliver *et al* 2011a, b) [15, 2, 19, 8, 21, 22].

Ferns and ferns allies are a group of non-flowering vascular plants dating back to 360 million years and proved to be one of the earliest land plants, collectively called 'Pteridophytes.' (Benniamin *et al.*, 2008) [3]. in ecological centers with varying seasons and unreliable water supply and scarce rainfall, DT species have been described. (Porembski *et al.*, 2001) [25]. In determining the structural and functional variables of any ecosystem, the Pteridophyte community plays an important role by indicating its soil conservation strategies, moisture balance, microclimatic level, and other related aspects. There are at least 1,300 plants tolerant to vascular desiccation, of which 1,000 are fern and fern allies and 300 are angiosperm species. These plants have defence mechanisms that involve changes in dehydration and rehydration process-related metabolism, physiological and biochemical networking that affects the ability to tolerate desiccation. (Suguiyama *et al.*, 2014) [31], although it is considered that the mechanisms of desiccation tolerance of fern-allies such as Selaginella species are well established in comprehension (Deeba *et al.*, 2016) [9]. Most of the data on desiccation-tolerant ferns has been derived from the physiological and morphological responses of a few species, such as *Polypodium virginianum* (Bewley *et al.*, 1993b) [4], *Mohria caffrorum* (Farrant et al., 2009) [12], Hymenophyllaceae or filmy ferns (Bravo *et al.*, 2016) [5], *Pleopeltis polypodioides* (John and Hasenstein 2017) [16].

Pteridophyte species like *Selaginella* have continued to evolve in changing micro-environmental conditions that are prevalent in its micro ecological niche and adopting from desiccated to hydrated states and vice versa. *Selaginella* are more moderate in terms of rehydration and employ both constitutive and induced mechanisms which are more beneficial for stable and durable expression of desiccation tolerance (Yathisha Neeragunda Shivaraj 2018) [34]. However, *Selaginella* does not follow either of the two modules for survival and follow a more moderate approach of rehydration that induces both mechanisms. Therefore a clear understanding of the mechanism of establishment of DT in *Selaginella* is essential to establish the DT phenotype in Pteridophytes. The present study is an overview of a potential resurrection plant *Selaginella wightii*, a Pteridophyte with semiarid habitat, its distribution, characteristics, physiological, morphological and biochemical mechanisms during hydrated, dehydrated and rehydrated conditions.

## 2. Materials and Methods

### 2.1 Collection of *Selaginella wightii.*

*Selaginella wightii* growing naturally on rocks in Devarayanadurga State forest of Tumakuru District, Karnataka, India at Coordinates 13.3707° N, 77.1773° E were collected for the study. The collected samples were brought to lab in sterile polythene bags and were maintained under greenhouse conditions for experimental purpose.

All the chemicals were procured from Sigma Aldrich except where mentioned.

### 2.2 Relative Water Content Analysis

The *Selaginella wightii* plant of equivalent age; same sized aerial parts and collected from the same habitual conditions were used for this study. The young and healthy fronds of *Selaginella wightii* plants were detached and immersed in double distilled water in a petri dish (150mm X 20mm Size) to hydrate fully until it did not gain further weight and was found to be stabilized such fronds are considered as hydrated state (HS).The HS fronds were allowed to desiccate (air dry) to lose water under room temperature until no further weight loss occurred, such fronds are considered as desiccated state (DS).Further the DS fronds were rehydrated in a petri dish (similar dimensions as mentioned above) with double distilled water for saturation till no further weight gain. The light conditions (a diurnal rhythm of 11 h day and 13 h dark cycle was maintained) were similar for all the three sets of fronds. All the three samples were treated the same way for all experimental purposes. The HS, DS and RS fronds were ground using liquid nitrogen and stored at -80°C for the further analysis.

All plant material required for morphological and biochemical analysis were treated in the same way described above. RWC is a technique to measure the cellular water content of the plants. The RWC is expressed as percentage in the samples, calculated as the difference between fresh weight and dry weight divided by the fresh weight (Plancot *et al.*, 2018) [24].

### 2.3 Ultra-structural Analysis

Unprocessed freshly harvested leaves were used for Scanning Electron Microscope (SEM). To minimize dehydration of the samples in the SEM chamber, a cold stage was used to bring the sample temperature down to 2 C while a double-sided sticky tape was used to mount the sample on the cold stage. The epidermis of the leaves and vertical sections of the stems in hydrated, desiccated and rehydrated states was imaged using an TESCAN VEGA3 (TESCAN Brno, Brno - Czech Republic).

### 2.4. Chlorophyll Measurements

0.5g of HS, DS and RS tissues of *Selaginella wightii* was weighed and frozen using liquid nitrogen and homogenised. From the homogenised samples chlorophyll was extracted using 10 mL of 80% acetone. The test tubes (Borosil) were incubated in room temperature overnight covered with aluminium foil, then the crude extract was centrifuged at 3000 g for 5 min using Centrifuge 5400R (Eppendorf, CA, USA) and the supernatant was collected while the pellet was discarded. The absorbance of the supernatant was read at 663.6 nm, 646.6 nm and 440.5 nm by using BioSpectrometer Kinetic (Eppendrof, CA, USA), as they are the major absorption peaks of chlorophylls *a*, and *b* respectively. The total chlorophyll (*Chl a+b*) contents were calculated using extinction coefficients provided by (Porra *et al.*, 1989*)* [26]. The chlorophyll concentration was then expressed on the basis of µg chl/g dry sample (µg g–1).

### 2.5 Determination of Soluble Sugar and Starch.

The frozen *Selaginella wightii* samples of HS, DS and RS was ground in a mortar with ice-cold 1M perchloric Acid ($HClO_4$), the extract was centrifuged at 12000 g (Eppendrof, CA, USA) for 2 min at 4–8° C. The supernatant was neutralized with 5M Potassium carbonate ($K_2CO_3$) and precipitated potassium perchlorate ($KClO_4$) was removed by centrifugation. The supernatant was kept on ice and used for the estimation of sucrose, while the pellet was used for the determination of starch. Sucrose and starch were estimated enzymatically according to the method given by (Pandey *et al*,. 2010*)* [23]. Sucrose and starch content were expressed as µmol/G FW.

### 2.6 Proline Estimation

Free proline accumulation in *Selaginella wightii* HS, DS and RS were determined using the method of Lee *et al.*, 2000) [17]. The 0.04g frozen *SW* sample was homogenized with 3% sulfosalicylic acid and after 72 hours proline released was measured. The homogenate was centrifuged at 3000 g (Eppendorf, CA, USA) for 20 minutes. The supernatant was treated with acetic acid ninhydrin, boiled for 1 hour and then absorbance at 520 nm was determined BioSpectrometer Kinetic (Eppendrof, CA, USA),. Contents of proline were expressed as mg $g^{-1}$ $dw^{-1}$.

### 2.7 Estimation of Lipid Peroxidation

Lipid peroxidation was estimated depending on the intermediate metabolite malondialdehyde (MDA), using 2-thiobarbituric acid (TBA) as described by Lee (2000) [17]. 0.2g of the tissues (HS, DS and RS) was extracted using 2 ml of 0.25% TBA made in 10% TCA. Extract was heated in a boiling water bath at 95°c for 30 minutes then cooled suddenly. The samples were centrifuged at 10,000g (Eppendorf, CA, USA) for 10 minutes. The supernatant was collected and the absorbance was read at 532nm and 600nm using BioSpectrometer Kinetic (Eppendrof, CA, USA), further by subtracting the absorbance value taken at 600 nm, correction of non-specific turbidity was carried out. The lipid peroxidation level was expressed in n mol $g^{-1}$ FW of

MDA calculated using an extinction coefficient of 155 mM $cm^{-1}$.

### 2.8 Analysis of Antioxidant Enzymes.

The antioxidant enzymes activity superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) were determined in HS, DS and RS of *Selaginella wightii*. The frozen Tissues were grounded separately in 6 mL of extraction buffer-1 (50 mM PBS, pH 7.8 for assay of SOD and CAT) and 6 mL extraction buffer-2 (100 mM PBS, pH 7.0 for assay of POD and GR) at 4 °C. The homogenates were collected and centrifuged at 15000 g (Eppendorf, CA, USA) at 4 °C for 20 min. The ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) was used as control to determine SOD. A 6 mL reaction solution of SOD consisted of 50 mM PBS (pH 7.8), 130 mM methionine, 750 µM nitro blue tetrazolium chloride (NBT), 100 µM EDTA-$Na^{2+}$, 20 µM riboflavin, and 0.1 mL of enzyme extract. The reaction solution was incubated for 10 min under fluorescent light with an intensity of 50 µmol $m^{-2}$ $s^{-1}$ for 20 min. The absorbance was determined at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit photochemical reduction of NBT by 50% and expressed in Units $mg^{-1}$ protein. CAT activity was assayed by measuring the initial rate of disappearance of $H_2O_2$ by the technique described by change and Maethly (1995) [6]. The decline in absorbance at A240 was recorded by BioSpectrometer Kinetic (Eppendrof, CA, USA), and the activity was expressed as the number of µ mol of $H_2O_2$ catalyzed by a unit of CAT per min and were expressed in units/mg protein. POD activity was analyzed using a modified method of Rao *et al* (1995) [30]. The reaction solution contained 100 mM PBS (pH 7.0), 50 mM o-methoxyphenol, 40 mM $H_2O_2$, and 0.1 mL of enzyme extract and were expressed in µmol $min^{-1}$ $g^{-1}$ protein. GR activity was determined according to the method described by (14 Halliwell and Foyer 1978) [14]. The reaction solution consisted of 50 mM Tris-HCl, 0.5 mM GSSG, 5 mM $MgCl_2$, and 0.2 mM NADPH. GR activity was determined at 340 nm, within 3 min and expressed as the number of µmol of NADPH oxidization and was expressed in Units $mg^{-1}$ protein.

### 2.9 Statistical analysis

Data obtained were subjected to a one-way analysis of variance (ANOVA). Significant differences among the test groups ($P \leq 0.05$) was obtained by Tukey's honestly significant difference (HSD) *post hoc* test using SPSS software (SPSS20.0, SPSSInc., USA). Values shown in the Figures are the means ± standard errors (SEs) of three independent replicates.

## 3 Results and Discussion

### 3.1 Selection and Defining Morphology

Selaginella wightii was collected during both monsoon and non-monsoon period and was identified based on morphological characters. The Selaginella wightii was identified based on the following phenotypic characters: Lithophyte type habitat, Small plants, roughly 12 x 6 cm, stem without leaves of up to 1 mm in diameter, often rooted All over the main stem bearing several main branches often uniformly and irregularly branched. Dense, spirally arranged leaves, greenish-black, linear, up to 2.5x 0.2 mm, long acuminate apex, membranaceous margin, ciliate border. Cones borne on ultimate branches, very substantially different branching. Growing in dry locations along roadsides between 350-1500 m on rock crevices. Maintenance of life in the absence of water requires a complex and finely tuned set of mechanisms working in close coordination and the results obtained in this study clearly indicates that SW is a potent desiccation tolerant plant.

### 3.2 Relative Water Content (RWC)

During water stress condition, changes in the leaf water potential and its components occurs (Wang *et al.*, 2010) [32]. It is noted that the transpiration rate and water relation parameters are interlinked, as there is no equipment to quantify plant water potential, determination of the RWC is a valid parameter to quantify the plant water status (Georgieva K *et al.*, 2007) [13]

RWC is a measure of the relative cellular water volume that shows any changes that could be affecting interactions between macromolecules and organelles. RWC of *SW* was estimated in detached fronds in HS, DS and RS stages in three independent experiments under laboratory conditions and photos were captured at all the 3 stages using a digital camera (Nikon D3300 Digital single lens reflex Camera). The original total water content being at 100%, hydrated (A) the weight before the water loss was 1.67g. After 4 hours of water loss of the detached frond by air dry the weight was found to be 0.30g and after rehydration the weight was 1.62g. Therefore the percentage of relative water content after 4 hours of desiccation (B) and rehydration (C) was found to be 17% and 97.08% respectively. The difference in percentage of relative water content in HF, DF and RF reveals that SW exhibits resurrection traits. (Plancot *et al.*, 2018) [24].

**Fig 1:** Morphological changes in detached fronds of *Selaginella wightii* in control-hydrated (A) RWC 100%, desiccated (B) 17% and rehydrated (C) 97%.

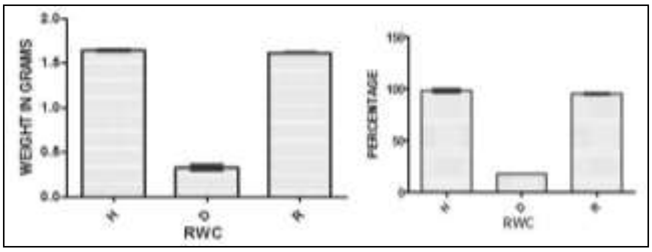

**Fig 2:** Relative water content in weight grams and percentage in all three different stages hydrated (control), desiccated, rehydrated in *Selaginella wightii.*

### 3.3 Membrane flexibility and morphology

During water deficit stress conditions in desiccation-tolerant plants, morphology is greatly affected. The most obvious response during desiccation is leaf curling and folding. (Farrant *et al.*, 2007) [11] UV radiation causes oxidative stress damage that can be minimised by folding the leaves, so desiccation is an essential adaptation. During water deficit tension Desiccation-tolerant plant vegetative tissues are inward curled. The folding in the leaf blades is half along the midrib under desiccated conditions, and only the abaxial surface is exposed to direct sunlight (Farant *et al.*, 2000) [10].

In the understanding the status of plant cells during desiccation and also upon rehydration, the preservation of cellular organisation and structures is of critical concern. The promising factor in assessing the effects of desiccation and rehydration on cellular integrity is the understanding of the fundamental mechanisms involved in the survival of extreme water deficit plant cells. In order to describe leaf morphology and fine structure, the resurrection species has been examined by the Scanning Electron microscope (SEM) to examine the cellular changes that occur during the desiccation and rehydration processes. In order to know their possible function in plant desiccation-tolerance, characteristics related to the completely hydrated leaf and ultra-structural changes occurring and physiological events occurring during morphological adaptations are studied.

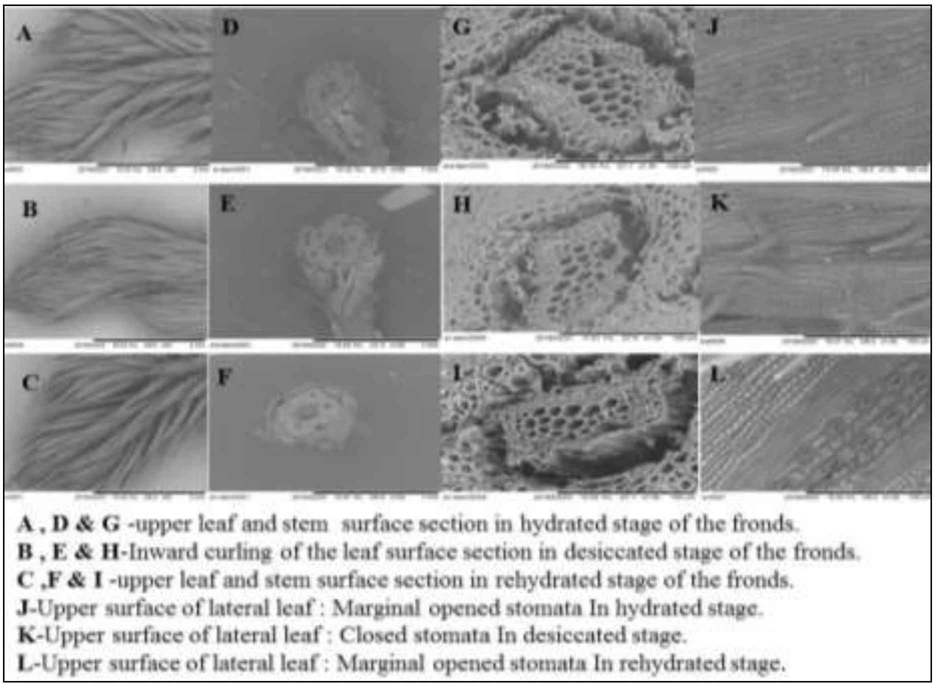

**Fig 3:** Scanning Electron Microscope study shows the changes occur in the morphology of *Selaginella wightii* in response to hydration, desiccation and rehydration.

Here we clearly observe the changes in leaf morphology by inward curling of the leaf fronds in desiccation stage in 40-60X magnification & 10kv resolution (A-F). Further, leaf folding pattern studies at 700 X-5kX magnifications in hydrated, desiccated and rehydrated fronds (G, H, I). Stomata are specialized epidermal structures which are crucial to the survival and productivity of plants; two guard cells around a pore contain these structures. Each stoma is a gas exchange molecular valve, mainly CO2 and O2, which is essential for optimal photosynthesis and which limits water loss by modulating the degree of transpiration. SEM analysis clearly showed the opening of stomata during hydration (J) and rehydration (K) stages, in which transpiration occurs at regular intervals and stomata remains closed / shrunk in desiccation (L) stages to prevent water loss.

### 3.4 Photosynthetic capacity of resurrection plants: Chlorophyll pigment Content

During dehydration, chloroplasts are modified by being orbicular with alteration and stacking in internal membrane layers. Water deficit stress is well known to impede the photosynthesis process due to the closure of stomata and the discrepancy between light capture and energy consumption (Chaves *et al.*, 2009) [7].

Photosynthetic activities of the ferns and fern-allies are reported to decline following dehydration (Farrant *et al.*, 2009) [12]. From our study it can be noted that there is significant difference in Chl a and chl *b* among hydrated, desiccated and rehydrated states. The concentrations of Chl a and chl b decreased during desiccation from 12.08μg $g^{-1}$ to 08.20μg $g^{-1}$ of chlrophyll a and 2.98μg $g^{-1}$ to 1.12μg $g^{-1}$ of chlrophyll b respectively. Upon rehydration, chlrophyll a and chlrophyll b concentration gradually increased and returned to almost similar levels of HF i.e., 10.81μg $g^{-1}$ and 2.19μg $g^{-1}$. Our results imply that significant difference in chlorophyll. The same pattern was observed in chlorophyll b, where the difference between the hydrated to desiccated was 60.15% in and upon recovery difference was 2% in rehydrated fern. Our results show that during desiccation the chlorophyll loss was 40%. However *SW* regains most of its chlorophyll. Since there is no complete loss of chlorophyll and *SW* can be categorized as homoiochlorophyllous in nature.

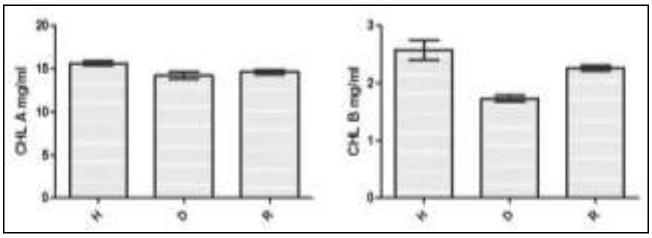

**Fig 4:** Chlorophyll a/b in *SW in* HS, DS and RS stages. Error bars represent standard error within the test group ($p \leq 0.05$), obtained from three replicates.

### 3.5 Proline Accumulation

Accumulation of amino acids has been observed in many studies in plants exposed to desiccation stress (Widodo *et al.*, 2009) [33]. Proline is an osmolyte, ROS scavenger, and acts as molecular chaperone stabilizing the protein structure, thereby protects the cells from damage occurring due to stress. Proline levels in HS, DS and RS fronds of *SW* were expressed in terms of µ mol/g FW. It was clearly evident that the proline levels increased significantly during Desiccation. In hydrated stage it was found to be 0.347µ mol/g FW and in DF it increased to 0.409µ mol/g FW and during rehydration it almost regained its original level i.e., 0.222µ mol/g FW, the percentage increase in DF was 37.5% and in RS was 16.37% compared to HS. The results obtained showed a significant raise in proline at desiccation stage indicates that proline serves as an osmolyte, to playing an important role in plant cells for adaptation to water deficit stress.

### 3.6 Lipid Peroxidation

Increased levels of reactive oxygen species (ROS) causes lipid peroxidation leading to the disruption of integrity of plant cell membranes. As a consequence, membrane function will be disturbed and imbalance occurs due to leakages of ionic constituents from the plant cell. (Mundree *et al.*, 2002) [20]. Lipids are the main components of cell prone to damage by free radicals (peroxidation of unsaturated fatty acids in membranes).

Lipid peroxidation is the clear indication of oxidative stress in response to desiccation stress which will result in membrane damage and inactivation of enzymes which will result in cell viability loss. (Mittler *et al.*, 2001) [18]. Lipid peroxidation in *SW* was estimated as reactive metabolites of 2-TBA mainly MDA. In HS it was found to be 0.68n mol g-1 FW and 2.31n mol g-1 FW in DS, indicating a rigorous increase which indicates a major role played during desiccation stress. Further it reduced to 0.85n mol g-1 FW in RS (21% more compared to HS) as a consequence of rehydration process. The result obtained clearly indicates that significant damage was caused to the membrane integrity.

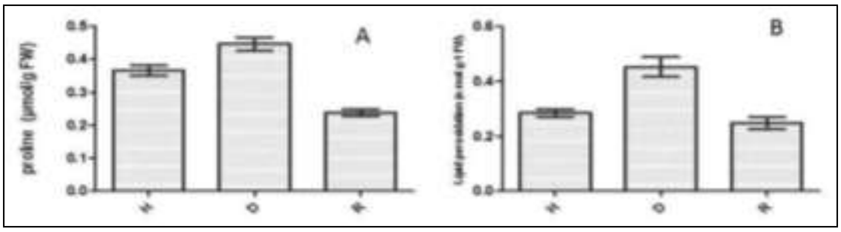

**Fig 5:** (A) Concentration of proline and (B) MDA in HS,DS and RS stages of *SW*. Error bars represent standard error within the test group ($p \leq 0.05$), obtained from three replicates.

### 3.7 Soluble Starch and Sugars

Studies suggest that desiccation tolerant ferns protect their membrane during dehydration by accumulating several sugars such as sucrose and members of the raffinose family of oligosaccharides (Rabert *et al.*, 2015) [28]. It is commonly pragmatic that the accumulation of soluble sugars during the desiccation process has is significance to provide tolerance during stress. During desiccation starch is broken down to its simplest form which functions as osmotic adjustments during dehydration and stabilization of proteins in the dehydrated cells. Protein denaturation and membrane fusions are prevented by sucrose and trehalose (Ramanjulu S *et al.*, 2002) [29]. Sucrose content was expressed as µmol/g FW. Sucrose content in HS, DS and RS stages were found to be 32.9 49.38, and 39.46µmol/g FW respectively i.e., around 40.5% and 8.13% increase respectively in DF and RF. The starch concentration was found to be 79.31, 23.26 and 43.3µmol/g FW which corresponds to 86% (DF) and 58% (RF) respectively when compared to control (hydrated stage). In this study, Starch content decreases and sucrose content increased in DS of *SW*, it is therefore evident that the principle carbohydrate in the fern is broken down during desiccation.

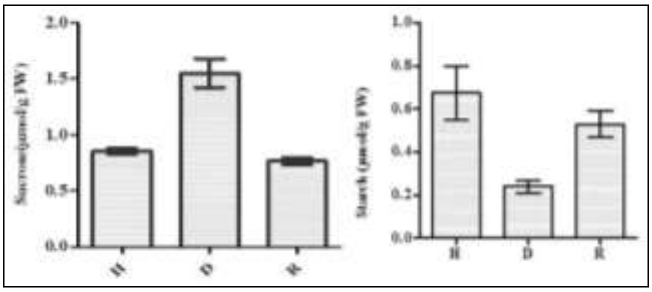

**Fig 6:** Changes in the level of (A) Sucrose (B) Starch, in HF, DF and RF tissues of *Adiantum raddianum.* Error bars represent standard error within the test group ($p \leq 0.05$) obtained from three replicates.

### 3.8 Antioxidant enzymes protection

In order to reduce the deleterious effects of ROS including enzymes, plants have different enzymatic and non-enzymatic protection systems: toxic ROS concentrations cause significant damage to protein structures, inhibit the activity of multiple enzymes of essential metabolic pathways, and contribute to macromolecular oxidation. It evolves as a consequence of reactive oxygen species (ROS) overproduction. ROS contains singlet oxygen (1O2), superoxide ($O^{2-}$), peroxide of hydrogen ($H_2O_2$) and radical hydroxyl (HO). In plants, the generation of ROS due to desiccation could be lethal. During stressful environments, antioxidant pathways are created in order to remove excessive oxidative stress. In *S. wightii* various defence enzymes like CAT, SOD and APX, along with invertase and proline, were found to be significantly increased during dehydration.

The activities of four antioxidant enzymes were analysed in HS, DS and RS stages of *SW* superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR), are the part of the plant antioxidation system that counteracts free radical stress. Enzyme activities of CAT, SOD and GR and peroxidase activity were high under dehydrated and hydrated conditions.

Concentration of SOD in *SW* fronds increased from 3.905 Units mg$^{-1}$ protein in HS to 5.25 Units mg$^{-1}$ protein in DS and upon rehydration; in RS it was found to be 4.20 Units mg$^{-1}$ protein. The percentage increase in DS was 29.3% and 7.2% in RS when compared to HS.

The peroxidase (POD) concentration in HS of *SW* was 0.0424 µmol min$^{-1}$ g$^{-1}$ protein which increases in DS to 0.101 µmol min$^{-1}$ g$^{-1}$ protein in DT an increase of 81.7%. Thereafter, in RS it was 0.087 µmol min$^{-1}$ g$^{-1}$ protein an increase of 54.4% when compared to control (HS).

The Catalase (CAT) activity in *SW* also increased in DS the trend was similar to other antioxidant enzymes tested. In HS it was found to be 0.57 units/mg protein which increased to 0.76 units/mg protein which accounts to an increase of 28.5% in DS and in RS it was at 0.626 units/mg protein which was 9.36% increase as compared to HS.

The Glutathione reductase (GR) activity of *SW* was found to be 410.98 Units mg$^{-1}$ protein in HS which increased to 625.96 Units mg$^{-1}$ protein in DS an increase of 41.4% and marginally reaching HS to 549.05 Units mg$^{-1}$ protein in RS around 28.7% increase as compared to HS.

The results obtained clearly shows an increase in all the four antioxidant enzymes that are activated in response to the damage caused due to extreme water deficit/desiccation which leads to oxidative stress which has deleterious effect on the physiology and also survival of the fern.

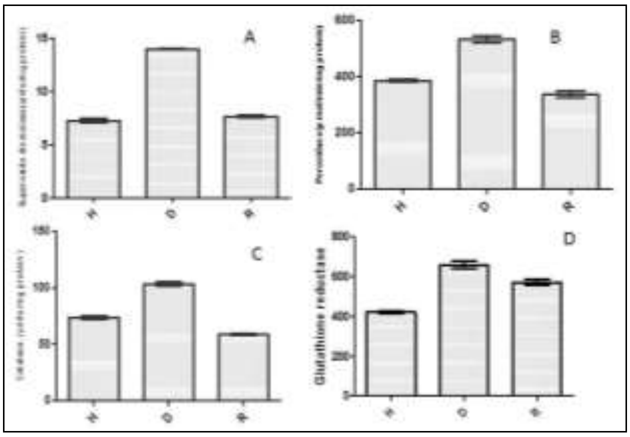

**Fig 7:** Specific activity of (A) Superoxide dismutase (B) Peroxidase (C) Catalase (D) Glutathione reductase in HS, DS and RS stages of *SW*. All the four antioxidant enzymes increased in DS compare to HS and minimized in RS. Error bars represent standard error within the test group ($p \leq 0.05$), obtained from three replicates.

## 4. Conclusion

The existence of vegetative desiccation tolerance in *S wightii* has been confirmed by our study on morphological, biochemical and physiological responses during hydration, desiccation and rehydration. *S wightii* recovered its full RWC and its original morphology. SEM images clearly show leaf folding and opening and closure of stomata. *S wightii* after desiccation synthesizes chlorophyll de novo. The increase in superoxide radicals is an indication of desiccation-related oxidative stress. In addition, SOD, POD, CAT and GR antioxidant enzymes showed increased concentrations during desiccation. Similarly, in the acquisition of desiccation tolerance in *S wightii* the signaling and osmoprotective molecule proline was increased and MDA levels reached concentrations equivalent to those of rehydration regulation, demonstrating the recovery of membrane functionality. During desiccation, breakdown of starch and increasing sugar levels suggest that the metabolism of carbohydrates. Hence it is proposed that *S wightii* due to its conjunctional properties depicting DT traits could be a model DT plant.

## Conflicts of Interest Statement

The authors declare that they have no conflicts of interest.

## Acknowledgement

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