

Physiological and biochemical responses involved in vegetative desiccation tolerance of *Cheliantes Argentina* fern

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Abstract

Cheilanthes argentea is a fern belonging to *Pteridaceae* family which is found mainly in the rock crevices of sub-tropical hilly areas. It has been recognized to possess desiccation tolerant properties because it survives complete desiccation and resurrects when water is available. An extensive field survey was conducted to collect *C. argentea* from the Nandhi hills State Forest, Chikkaballapura district of Karnataka (Coordinates 13.3702° N, 77.6835° E), the detached fronds obtained from green house maintained plants were hydrated and subsequently desiccated under *in-vitro* condition. The Relative Water Content decreased in desiccated stage to 4% of its original weight. The fronds regained 96% of its original weight and Relative Water Content during rehydration. The rehydrated fronds largely regained its original morphology. Also, 80% of the total chlorophyll content was retained during desiccation. Further, activities of antioxidant enzymes namely Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) and Glutathione reductase (GR) increased significantly during desiccation. Lipid peroxidation was found to be higher during desiccation when compared to other stages. Proline and sucrose content was higher during desiccation while the concentration of starch decreased due to desiccation. The results obtained in this study demonstrates that *C. argentea* is potent to tolerate desiccation with its physiological and biochemical defense mechanisms.

Keywords: *c argentea*, desiccation, rwc, antioxidants, proline, lipid peroxidation sucrose and starch

1. Introduction

Food safety is currently widely talked over global concerns. Many as one quarter of the globe's area is found in arid and semiarid regions and the major limitation for crop production is non-availability of water. Water deficiency is the main and the most significant abiotic stress factor affecting the crop plants. Water stress is a restrictive ecological factor that limits its yield and growth of such a plant. Most of the plants cannot survive air dry condition or desiccation and majority of the plants are susceptible (41-70% water loss), losing physiological capacity (Oliver *et al.*, 2005) [23]. Desiccation sensitive plants undergo stress leading to multiple damage and loss of protein confirmation. Chromatin stability and oxidative damage occurs extensively (Tapia and Koshland, 2014) [30].

However, a few species across the phylogenetic spectrum can tolerate desiccation, and are known as resurrection plants (Moore *et al.*, 2013) [21]. These plants are able to withstand long periods of total water deficit, and use complex systems which cause them to lose more than 80% of water content and are still capable to revive upon water availability (Olivar *et al.*, 2005). Resurrection plants endure remarkable contraction of their vegetative organs (eg. leaves) upon desiccation and the cell wall plays a vital role in permitting this reversible wall dehydration to occur (Moore *et al.*, 2013) [21]. DT plants grow in specialized niches by adapting to faster growth and high dry mass productivity mainly due to adaptations related to relevant water loss. These include modifications in the stomata, cuticle, roots, and the vascular system and up regulation of antioxidants (Dinakar and Bartels, 2012) [10].

Desiccation Tolerance species have been discovered time and again in various ecological niches from all continents

with perineal water paucity and untrustworthy rainfalls. They inhabit inselberg soils with very lesser water retention capacity and also grow as outcrops in tropical/subtropical forests. They have been identified from southern Africa (incl. Madagascar), Brazil, East-West Africa, North America, Australia and the Eastern / Western Ghats of India (Poremski and Candotto Barthlott, 2001) [26]. Desiccation tolerance has been reported in lower plants like bryophytes terrestrial microalgae and lichens (Candotto *et al.*, 2015) [6] as well higher angiosperms- but rarely in pteridaceae (Alpert 2005) [1].

The genus *C. argentea* is tropical in nature and is traditionally used to treat peptic ulcer and stomach disorders. Desiccated state has been observed in these plants which resurrect when water is available. Reported some *Cheliantes* species survive 95% of total cellular water content loss for prolonged time periods. They fully recover their metabolic activity within 12-72h of rehydration. Although documentation of several species of *Pteridaceae* with vegetative DT has been conducted; DT properties of *C. argentea* has not been studied. In this study we present a comprehensive evaluation of morphological and biochemical properties of *C. argentea* that helps it to counter desiccation stress.

2. Materials and Methods

2.1 Sampling and Identification

C. argentea an evergreen perennial fragile looking fern. Was collected from Nandi hills forest of Chikkaballapur District (Coordinates 13.3702° N, 77.6835° E) in sterilized polythene bags during February 2021 (winter season), and brought to the laboratory for further study. Phenotypic characters like stipe color, length, and size of the foliage,

texture, and field data (growing condition) like soil requirement, sun exposure and water needs were recorded for morphological identification.

2.2 Relative Water Content (RWC) Analysis

Three independent biological replicates of *C. argentea* were used for measurement of relative water content. The areal parts of the fronds were harvested before dehydration (100% RWC), *C. argentea* of homogeneous age, with similar size of aerial parts and from the same habitat were selected. Plants were cared in pots for healthy plant growth, 30 days prior to *in vitro* desiccation stress treatment. All samples were collected at noon to avoid apparent differences in protein abundance caused by circadian or light-dark regulation. From the healthy and young *C. argentea* plants, detached fronds were immediately allowed to hydrate in a petridish (150mm X 20mm Size) this was followed with addition of double distilled water, for a period of 4 hours until there was no further increase in weight; such plants were considered to be in hydrated fronds (HF) and it served as a control. The hydrated plant tissues were dewatered for 48 hours at room temperature until no further weight loss occurred, such plants were considered to be in desiccated fronds (DF). The desiccated plant was immersed in double distilled water to rehydrate for 12 hours until no additional weight gain took place under similar light or dark cycle; these plants were considered to be in rehydrated fronds (RF) Pandey *et al.*, 2010) [24]. The dry weight of the rehydrated plant was obtained after baking at 105°C for 30 min in a hot air oven (RT-150 RELITECH, India), followed by treatment at 80°C until no further weight gain and stabilized. All plant material required for morphological and biochemical analysis were treated in the same way described above. The hydrated, desiccated and rehydrated plants (leaves for biochemical analysis, leaf + stem for ultrastructural analysis) were ground using liquid nitrogen with pestle and mortar and stored at -80°C for further analysis. The water content in the samples was calculated as the difference between fresh weight and dry weight divided by the fresh weight (Yobi *et al.*, 2017) [35]. RWC was expressed in percentage.

2.3 Measurement of Chlorophyll Content

0.5g of *C. argentea* leaf HF, DF and RF tissues was homogenized using mortar and pestle. Chlorophyll was extracted with 15 mL 80% acetone from the homogenised samples. Aluminium foil are wrapped to the test tubes and was left in room temperature overnight, then crude extract was centrifuged for 10 minutes at 5300 rpm 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 (Eppendorf CA USA), which are the major absorption peaks of chlorophylls *a*, *b* respectively (Porra *et al.*, 1989) [27]. Total chlorophyll (a+b) were calculated according to Arnon, (1949) [3]; Lichtenthaler, (1987) [18]; Bulgari, (2019) [5], and expressed on a fresh weight basis.

2.4 Estimation of Lipid Peroxidation

Lipid peroxidation in *C. argentea* tissues were quantified spectrometric ally by following the protocol of Heath and Packer (1968) [17]. 0.2 g of HF, DF and RF leaves were ground with pestle and mortar in 2 mL of 0.25% thiobarbituric acid prepared in 10 % trichloro acetic acid.

The ground tissues were heated for 30 minutes at 95°C and centrifuged for 10 minutes at 10,000 g in centrifuge 5400 R (Eppendorf, CA, USA). Later, the supernatant was carefully transferred and the absorption at $\lambda=532$ nm and $\lambda=600$ nm was measured. The obtained absorbance at $\lambda=600$ nm was subtracted from $\lambda=532$ nm to adjust non-specific turbidity. Extinction coefficient of $1.55 \text{ m}^{-1} \text{ Mv}^{-1} \text{ cm}^{-1}$ MDA was used in the study. Lipid peroxidation was reported as MDA formed and expressed in $\text{nmol g}^{-1} \text{ FW}$ (Pandey *et al.*, 2010 [24]; Dixit *et al.*, 2001) [11].

2.5 Proline Estimation

Free proline accumulation of *C. argentea* in HF, DF and RF was determined by extraction and spectrophotometric estimation. Approximately 600 mg of hydrated, desiccated and rehydrated leaf samples were homogenised in mortar and pestle with liquid nitrogen and 10 ml of 3% aqueous sulfosalicylic acid. Equal volumes of filtered homogenate, glacial acetic acid and acid-ninhydrin, were boiled for an hour in a water bath. 4ml of toluene was added and tubes were placed on ice to terminate the reaction. The chromophore-containing layer was used to measure the absorbance $\lambda=520$ nm after room temperature achieved. Proline concentration was expressed on a fresh weight basis ($\mu\text{mol g}^{-1} \text{ FW}$) (Gururani *et al.*, 2015) [14].

2.6 Analysis of Antioxidant Enzymes

Activities of four antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and glutathione reductase (GR) were conducted in HF, DF and RF. *C. argentea* tissues were ground separately in 6 mL of extraction buffer-1 extraction buffer-2 (100 mM PBS, pH 7.0 for POD and GR assays) at 4°C. The collected homogenates were centrifuged at 15000 g at 4°C for 20 min. upon which the SOD was assayed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). A 6mL reaction solution of SOD consisted of 50mM PBS (pH 7.8), 130mM methionine, 750 μM nitro blue tetrazolium chloride (NBT), 100 μM EDTA- Na^{2+} , 20 μM riboflavin, and 0.1mL of enzyme extract. The reaction solution was incubated for 10min under fluorescent light with an intensity of 50 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for 20min. At 560nm the absorbance was determined. 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} proteins. CAT activity was assayed by measuring the initial rate of disappearance of H_2O_2 by the technique described by (Change and Maehly, 1995) [7]. The decline in A240 was recorded by BioSpectrometer Kinetic (Eppendorf, CA, USA), and the activity was expressed as the number of μmol 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) [28]. The reaction solution contained 100 mM PBS (pH 7.0), 50mM o-methoxyphenol, 40mM H_2O_2 , and 0.1mL of enzyme extract and were expressed in $\mu\text{mol min}^{-1} \text{ g}^{-1}$ protein. GR activity was determined according to the method described by (Halliwell and Foyer, 1978) [15]. The reaction solution consisted of 50mM Tris-HCl, 0.5mM GSSG, 5mM MgCl_2 , and 0.2mM NADPH. GR activity was determined at 340nm, within 3min and expressed as the number of μmol of NADPH oxidation and were expressed in units mg^{-1} protein (Wang *et al.*, 2010) [33].

2.7 Determination Sucrose and Starch

Fronde material of HF, DF, RF tissues of *C. argentea* was grounded in a mortar with ice-cold 1M HClO₄. Then, the extract was centrifuged at 12000g for 2 min at 4°C. Next, the supernatant was neutralized with 5MK₂CO₃ and precipitated KClO₄ was removed by centrifugation. The supernatant was then kept on ice and used for the estimation of sucrose, while the pellet was used for the determination of starch. The supernatants were re-suspended in 0.2 ml buffer. The suspension was treated with 14 units amyloglucosidase and 0.4 units amylase at 55°C for 2 hours (or overnight at room temperature). Reaction buffer consisted of 100 mM HEPES pH 7.0, 5 mM MgCl₂, 0.5 mM Dithiothreitol, 0.02% (w/v) bovine serum albumin. In a photometric cuvette, 1.0 mM ATP and 0.4 mM NADP and 50 µl extract were added. The absorbance was recorded at A340 nm. Further, 0.5 units glucose-6-phosphate dehydrogenase, 0.5 units phosphoglucose isomerase, 3 units hexokinase and 100 units invertase were added and the absorbance measured at A340 nm. Difference in the readings gives the concentration of specific glucose which appeared after its liberation from sucrose hydrolysis catalysed by invertase. The sucrose concentration was then calculated using 6.22 10E³M⁻¹cm⁻¹ as extinction coefficient of NAD (P) H. In this assay, glucose hydrolysed from starch was measured as an indicator of the concentration of starch. The pellet obtained from the extraction was washed and homogenized three times in 0.2 M MES buffer (pH 4.5) and was then added 0.2 ml buffer. The suspension was treated with 14 units amyloglucosidase and 0.4 units amylase at 55°C for 2 hours (or overnight at room temperature). The reaction mixture was centrifuged and glucose in the supernatant as below: Reaction buffer consisted of 100 mM HEPES pH 7.0, 5 mM MgCl₂, 0.5 mM dithiothreitol 0.02% (w/v) bovine serum albumin. To the cuvette, 1.0 mM ATP and 0.4 mM NADP and 50 µl extract was added. The absorbance was recorded at λ=340 nm. Further, 0.5 units glucose - 6-phosphate dehydrogenase was added and the absorbance measured at λ=340 nm. The difference in the readings gave the concentration of glucose hydrolysed from starch which was calculated using 6.22 as extinction coefficient of NAD (P) H (at 1mM and 1cm) Sucrose and starch were enzymatically estimated using the Jones method used given by (Coombs *et al.*, 1985) [8].

2.8. Statistical Analysis

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

3. Results

3.1. Collection and Morphological Identification of *C. argentea*

C. argentea was collected in the month of February, 2020 and was identified based on phenotypic characters. The identified *C. argentea* had the following phenotypic characters: it was evergreen to greenish like other dry land ferns and was comparatively shorter in size. It formed a low clump of star shaped structure with rich matte green on top, and a striking waxy silver-white shade underneath with

fuzzy texture when felt upon. The fern grew to about 4''-12'' in height and 6''-9'' in width, It grew averagely well in drained and aerated rock soil, rock out crops, crevices, stone walls and other rocky sites.

3.2 Relative Water Content

Relative Water Content (RWC) is one of the important techniques to measure the appropriate plant water balance deficit and associated physiological consequences. RWC of *C. argentea* was measured in three experimental studies using detached fronds in lab conditions. These plants were observed separately and at each stage photos of fronds were captured using a digital camera (Nikon D3300 Digital SLR Camera). In hydrated stage the RWC increased up to 100% (no further increase in fresh weight took place) which decreased to 4% in the desiccated stage. The fresh weight again increased during rehydration in 12 hours was found to be 96% of its original weight. The very high percentage of recovery shows that it is one of the key properties that of *C. argentea* uses to counter desiccation.

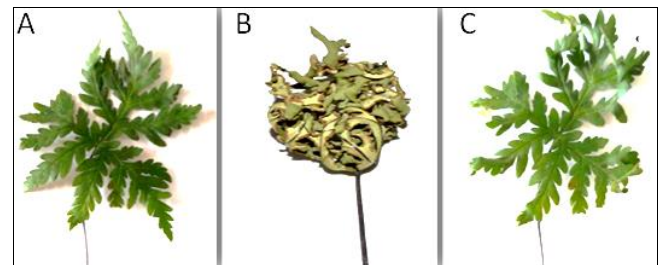


Fig 1: Morphological changes of *C. argentea* detached fronds in 1. Hydrated frond (control) (H, RWC 100%) 2. Desiccated frond (D, RWC 4%). 3. Rehydrated fronds (RWC 96%).

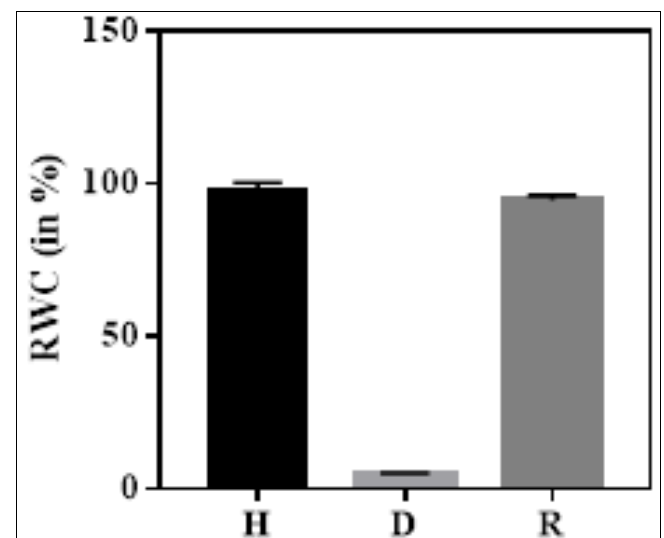


Fig 2: Relative water content (RWC in Percentage). In Hydrated (H), Desiccated (D) and Rehydrated (R) fronds. Error bars indicate a standard error in the test group ($p \leq 0.05$) between three replicates.

3.4 Chlorophyll content in *C. argentea* in response to desiccation

Figure 02 depicts the illustration of total chlorophyll content (Chlorophyll *a+b*) in hydrated, desiccated and rehydrated fronds of *C. argentea*. There were significant differences observed in the Chlorophyll *a+b* among hydrated, desiccated and rehydrated fronds. The Chlorophyll *a+b* in desiccated fronds dropped to 0.6 µg g⁻¹ from 0.9 µg g⁻¹ in the hydrated fronds which shows that

approximately 80% of the total chlorophyll of the hydrated fronds was retained during the desiccation. Further, during rehydration chlorophyll content was found to be $0.83\mu\text{g g}^{-1}$ which was 92% of that of the hydrated fronds. This indicates that chlorophyll became fully functional during rehydration as it was not completely destroyed due to desiccation. Further, it can be assumed that *C. argentea* demonstrates a mechanism of chlorophyll protection in order to prevent total destruction during desiccation.

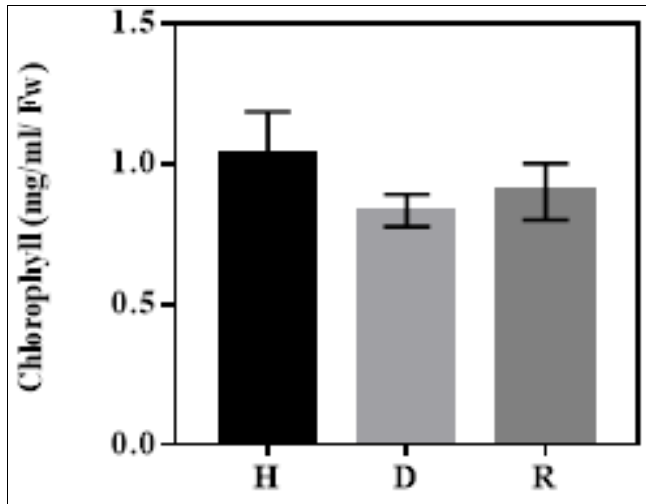


Fig 3: Total Chlorophyll content in hydrated, desiccated and rehydrated fronds of *C. argentea*. Error bars indicate a standard error in the test group ($p \leq 0.05$) based on three replicates.

3.5 Proline accumulation in *C. argentea*

The proline concentrations in hydrated, desiccated and rehydrated fronds of *C. argentea* will be measured in terms of $\mu\text{mol / g FW}$. It was clear that the proline concentrations increased significantly in desiccated fronds. In hydrated stage a fresh weight of $0.0184 \mu\text{mol/g FW}$ was recorded which increased in the desiccated stage to $0.024 \mu\text{mol/g FW}$. During rehydration a fresh weight of $0.014 \mu\text{mol/g FW}$ of proline was observed. Our results showed that a significant increase in proline, approximately 1.5 fold of proline level in desiccation stage which evident the role of proline in DT.

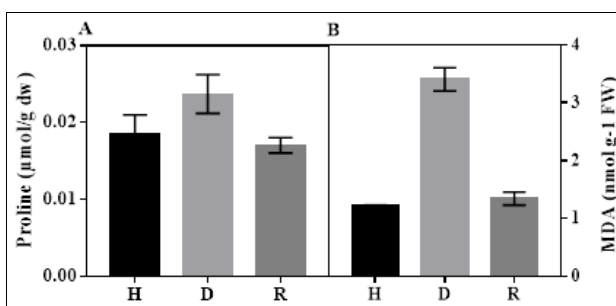


Fig 4: *C. argentea* (A) Proline levels (B) Lipid peroxidation. Error bars indicate a standard error in the test group ($p \leq 0.05$) based on three replicates.

3.6 Lipid Peroxidation in *C. argentea*

Lipid peroxidation in *C. argentea* was determined to be 2-TBA reactive metabolites, specifically MDA. In hydrated fronds (H) MDA concentration of $0.191 \text{ n mol g}^{-1}$ was recorded. In desiccated fronds (D) MDA concentration was found to be 3.2 n mol g^{-1} , which further reduced to $1.45 \text{ n$

mol g^{-1} in rehydrated fronds (R). Hence, our results reveal that there is a considerable increase in MDA levels in desiccated stage in related to hydrated and rehydrated stages.

4.6 Activity of Antioxidant Enzymes in Response to Desiccation of *C. argentea*

The antioxidant activities of four anti-oxidant enzymes namely SOD, POD, CAT and GR was analysed in three different stages of *C. argentea* fronds. The antioxidant enzymes demonstrated differential responses during desiccation and rehydration. The activities showed a similar trend by increasing gradually to peak during desiccation and return to approximately original levels during rehydration. SOD activities. In hydrated stage 2.49% in desiccated stage it increases to 3.08% in Rehydrated stage it was decreases up to 1.3%. The peroxidase (POD) concentration HF of *C. argentea* in was $0.303 \mu\text{mol min}^{-1} \text{g}^{-1} \text{protein}$ which increased in Desiccated stage to $0.35 \mu\text{mol min}^{-1} \text{g}^{-1} \text{protein}$. Thereafter, it decreased in rehydrated stage to about $0.259 \mu\text{mol min}^{-1} \text{g}^{-1} \text{protein}$ GR activities of *C. argentea* found to be $0.563 \text{ Units mg}^{-1} \text{protein}$, in hydrated fronds which increased to $1.527 \text{ Units mg}^{-1} \text{protein}$ in desiccated stage and decreased marginally to $0.926 \text{ Units mg}^{-1} \text{protein}$ in rehydrated fronds which indicates a 3 fold increase in desiccation stage when compared to hydrated stage. CAT activities in *C. argentea* increased in desiccated fronds and followed by the similar variations compared to other antioxidant enzymes. In hydrated fronds it was found to be $64 \text{ units/mg protein}$ which significantly increased to $94.7 \text{ units/mg protein}$ in desiccated fronds and in rehydrated fronds it was at $45 \text{ units/mg protein}$. This result indicates that there is 30% increase in antioxidant enzyme activity in desiccated fronds. All antioxidant enzymes level significantly improved in desiccated fronds compared to hydrated and rehydrated fronds. It clearly shows that antioxidants are overexpressed during desiccation to overcome oxidative stress.

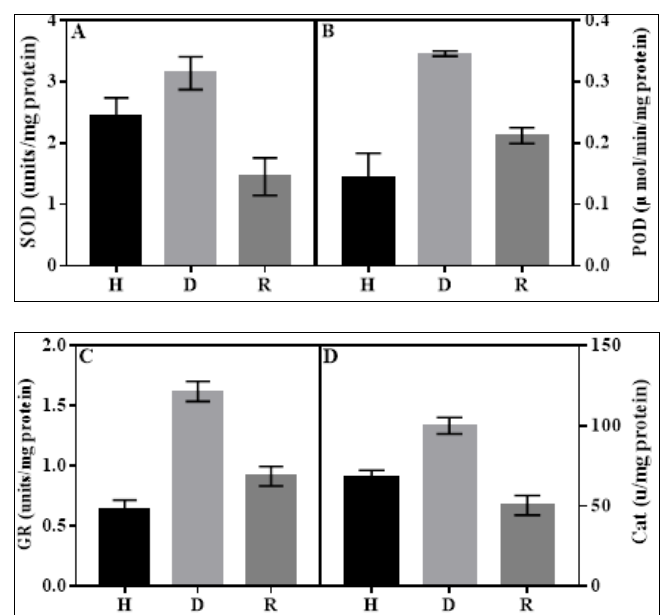


Fig 5: Specific activity of (A) Superoxide dismutase (B) Peroxidase (C) Glutathione reductase (D) Catalase in hydrated, desiccated and rehydrated fronds of *C. argentea*. Error bars indicate a standard error in the test group ($p \leq 0.05$) based on three replicates.

4.7 Starch and Sucrose

Sucrose level was measured as a fresh weight of $\mu\text{mol} / \text{g}$ and there is gradual increase in desiccated stage compared to hydrated and rehydrated fronds. Sucrose content in hydrated, desiccated and rehydrated fronds was found to be 1.15 $\mu\text{mol/g}$, 1.5 $\mu\text{mol/g}$ and 0.85 $\mu\text{mol/g}$ fresh weight respectively. The starch concentration in hydrated, desiccated and rehydrated fronds was found to be 2.5835 $\mu\text{mol/g}$, 1.807 $\mu\text{mol/g}$, and 2.416 $\mu\text{mol/g}$ fresh weight respectively. Degradation of starch and increase in sucrose content was observed in desiccated fronds.

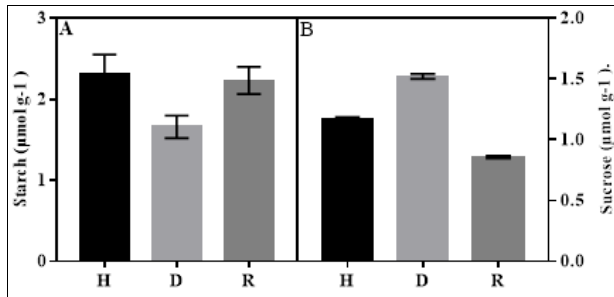


Fig 6: percentage of (A) sucrose and (B) starch content in hydrated, desiccated and rehydrated fronds. Error bars indicate a standard error in the test group ($p \leq 0.05$) based on three replicates.

5. Discussion

Water deficiency causes numerous morphological modifications in desiccation tolerant resurrection plants including a noticeable change in inward leaf curling. Relative Water Content (RWC) is one of the important techniques to measure the appropriate plant water balance status in cellular water associated with physiological consequence (Nar *et al.*, 2009) [22]. In the present study, *C. argentea* was subjected to relative water content study to confirm whether the species was able to resurrect or not. Our study reveals that there is a significant curling of fronds during water loss. Similar results were also observed in *Selaginella bryopteris*, *Selaginella delicatulla*. The fronds were entirely out-stretched while watered which slowly folds inward while drying but firmly wrapped inwards such that only the abaxial areas of the fronds are accessible to the sun when no water was available. Leaf curling prevents photo oxidative injuries due to light stress, reduces the exposure region and hence it is a significant morphological adjustment towards sustaining desiccation (Brighigna *et al.*, 2002) [4].

Reduction in chlorophyll concentration of plants is related to both the defense by plants from Ultraviolet radiation and damage done from oxygen free radical formation during desiccation (Sherwin and Farrant, 1998) [30]. Similarly, In desiccated stage, the chlorophyll concentration of the leaves did not reduce excessively in *C. argentea*. Signifies that there was no complete change of the photosynthetic system during desiccation which helps in the The leaves are covered. The adaxial part of a leaf surface continues to remain green whereas the abaxial part wasn't. It should be noted, however, that maximum chlorophyll in DF was just 20% lesser in desiccation fronds, indicating that destruction which took place did not affect the photosynthesis. Further, based on how they retain their photosynthetic pigments during desiccation or not Desiccation-tolerant plants can be classified (Oliver *et al.*, 2005) [23]. Now it is understood that desiccation-tolerant plants which maintains their chlorophyll

content during desiccation are homochlorophyllous, whereas those which destroy their chlorophyll are poikilochlorophyllous (Farrant 2000) [13], due to the retention of some of their chlorophyll content *C. wilmsii* and *Myrothamnus flabellifolius* which retained 82% and 60% chlorophyll are considered as homoichlorophyllous species. We observed that *C. argentea* retained 80% of its chlorophyll content in desiccated stage therefore can be classified as homoichlorophyllous species. Moreover, *C. argentea* curved and folded inwardly during desiccation, rescuing its adaxial layer and to protect the inside of the rosette shoot. After rehydration, a significant reduction of chlorophyll is recovered to almost the original level. A similar physiological changes also been studied in *C. wilmsii*, *C. plantagineum*, and *M. flabellifolius* (Farrant 2000 [13], Scott 2000) [29]. This mechanism avoid the photochemical degradation of chlorophyll content (Farrant 2000) [13].

The studies on plants exposed to desiccation stress have observed accumulation of amino acids including proline. Proline is a well known ROS scavenger and molecular chaperone that protects the damage to cells from stress by stabilizing the structure of proteins (Widodo *et al.*, 2009) [34]. The proline content in resurrection plants increases under desiccation stress and has been demonstrated *Selaginella bryopteris* (Pandey *et al.*, 2010) [24], *Selaginella tamariscina* (Wang *et al.*, 2010) [33], *Craterostigma wilmsii*, (Vicré *et al.*, 2004) [32]. It is crucial in mechanically stabilizing the cells and membranes in the desiccated fronds (Pandey *et al.*, 2010 [24], Vander *et al.*, 2004). Proline also helps in scavenging free radicals, cellular structure stabilization (Gururani *et al.*, 2015 [14]. and acts by alleviating cytoplasmic acidosis and as a protein compatible hydrotrope (Pandey *et al.*, 2010) [24]. Our results show that proline could have played a major role as an osmolyte during desiccation as its concentration significantly increased during desiccation.

Lipids are the major cellular components susceptible to free radical attack leads to peroxidation of unsaturated fatty acids in membranes. Lipid peroxidation signals oxidative stress in response to desiccation, leading to membrane disruption and then further inhibition of enzymes responsible for cell viability damage. (Mittler 2001) [20]. In this study our results reveal that there is a significant increase of MDA levels in the desiccation stage of *C. argentea*. Increase in TBA is known to increase the cell viability by safeguarding against the damage caused by free radicals by strengthening the lipid-Cell membrane cross linking (lipid peroxidation) (Mattos and Moretti. 2016) [19]. Oxidative stress in plants is a complex physiological phenomenon. In plants, ROS generation is triggered by desiccation (Halliwell 2006) [16]. It is developed due to overproduction of reactive oxygen species (ROS). The singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (HO) are also among the most toxic ROS. To eliminate excessive oxidative stress, antioxidant mechanism is activated by desiccation tolerant plants during water limiting conditions. Several antioxidant mechanisms have already been recorded in vascular desiccation tolerant plants that defend the desiccated plants from significant damage resulting from Reactive oxygen species and free radical injury (Farrant *et al.*, 2007) [25]. Interaction of four antioxidant enzymes (SOD, CAT, POD, and GR) of the *C. argentea* species were found to increase

during desiccated stage. Increased activities of the Anti-oxidation enzymes in our study supports the hypothesis that oxidative stress due to ROS accumulation triggered by desiccation is responsible for cell death and increased accumulation of antioxidant enzymes is needed to keep the plants physiologically and metabolically active.

In our study on *C. argentea* soluble sugars concentration increased and concentration of soluble decreased during desiccation. Our results indicate increase in sugar concentration and decrease in starch during desiccation is responsible for metabolic stability of the cells. Sugars secure the cells during desiccation by retaining hydrophilic activities in proteins and membranes by replacing water from the sugar of Hydroxyl groups. Hence, hydrogen bonding sugars bind to proteins and membranes to resist protein denaturation. Sugars also contribute to vitrification in the cytoplasm of desiccated cells by formation of biological gas which prevents collapse of the cells during desiccation (Vicré *et al.*, 2004) [32].

The severe water loss during desiccation leads to the conversion of starch to sugars. Some of the most important sugars are sucrose and trehalose, which have been suggested to contribute to the retention of turgor under stress and for the avoidance of denaturation of proteins and cell fusion in the cell (Crowe *et al.*, 2004) [9]. Decrease in the amount of glucose and fructose, is likely to guard nucleophilic interactions which may exist among aldehyde and amino groups found in proteins and amino acids resulting in protein stability

Roots as well as leaf tissues, serve as big store house of sucrose which is generated in response to drying. Glucose and fructose monosaccharides are adequately available throughout the hydrated environment and their quantities reduce due to desiccation. (Peters *et al.*, 2007) [25]. In this study, the concentration of starch decreased and the sugar content increased during desiccation in *C. argentea*. Previous studies have shown that starch is degraded during desiccation and acts as a carbon supply for sucrose. During desiccation it is also known that conversion of starch to sugar is high and the same has been reported in the desiccation stage of *C. argentea*.

Conclusion

Our investigation of morphological, biochemical and physiological responses during hydration, desiccation and rehydration confirmed the occurrence of vegetative desiccation tolerance in *C. argentea*. *C. argentea* recovered its complete RWC and leaf regained its original morphology upon rehydration. *C. argentea* synthesizes chlorophyll de novo after desiccation. Increase in superoxide radicals under water deficit is an indicator of the oxidative stress due to desiccation. Furthermore, antioxidant enzymes SOD, POD, CAT and GR showed increase in concentrations during desiccation. Similarly, signalling and osmoprotective molecule proline was also involved in the acquisition of desiccation tolerance in *C. argentea*. Relative electrolyte leakage and MDA levels reached concentrations equal to that of control on rehydration, giving evidence that membrane functionality recovered. Break down of starch and increasing sugars levels during desiccation indicates that carbohydrate metabolism is controlled during desiccation. The results point out that *C. argentea* is a PDT species which recovers its complete physiological and biochemical activity after rehydration. Hence, we propose

that *C. argentea* could be used as a DT model to study mechanisms underlying tolerance to desiccation.

Conflicts of Interest Statement

The authors declare that they have no conflicts of interest.

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