

Unravelling desiccation and rehydration tolerance mechanism in the fern, *Adiantum latifolium*

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ABSTRACT

Desiccation-tolerance (DT), the ability to lose virtually all free intracellular water and then recover normal function upon rehydration, is one of the most remarkable features of ferns. The phenomenon of desiccation tolerance in pteridophytes is well known and many species can withstand drying with water content to 10–20% of their dry weight and return to normal metabolism and growth following rehydration. The time required to recover from desiccation increases with duration of desiccation. Hence the present investigation was carried to reveal the biochemical adaptations against desiccation and also subsequent rehydration in *Adiantum latifolium* Lam. a fern. Experimental design for desiccation periods was 1 D (24 h), 2 D (48 h) and 3 D (72 h) in a desiccator contain PEG. Desiccation caused an enhanced production of reactive oxygen species (ROS) and increased lipid peroxidation. The analytical data were substantiated by histochemical localization. Total phenolic content increased with desiccation. Fractionation of phenols revealed a pool of phenolic acids. Further, high induced level of polyphenol oxidase and phenylalanine ammonia lyase enzyme activates provide tolerance to desiccation. Further studies are planned to analyze the molecular mechanism involved in providing desiccation tolerance in the fern.

KEY WORDS: DESICCATION-TOLERANCE; REHYDRATION; REACTIVE OXYGEN SPECIES; LIPID PEROXIDATION

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INTRODUCTION

Pteridophytes, the seedless vascular plants characterized with independent heteromorphic alternation of generation and primitive vasculature. These form the conspicuous flora of tropical plant world. The pteridophytes consist of ferns and fern allies; of these, the ferns are predominantly distributed in a wide range of habitats. Out of 1250 species of pteridophytes occurring in India, 173 species have been found to be used as food, flavor, dye, medicine, bio-fertilizers, oil, fiber and bio-gas production (Manickam and Irudayaraj, 1992). Besides sugar, starch, proteins and amino acids, ferns contain alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols, sesquiterpenes as potential raw materials used in various industries. In addition to medicinal properties they play an important role in the ecological niches of forest ecosystem i.e., integral part of biogeochemical cycling of minerals; also they help as ecological indicators of pollution, (Srivastava and Paul, 2016; Bergenon and Pellerin, 2014).

Among the vascular land plants there are some 60 to 70 species of ferns and fern allies exhibit some degree of vegetative desiccation tolerance. Oxidative stress is a major hazard of desiccation which in turn leads to the formation of ROS (Reactive Oxygen Species). Most of the studies in ferns indicate that enzyme conservation may be important in the physiological reactivation process; *de novo* synthesis of some enzymes during rehydration also contributes to full physiological recovery in these plants. Through the years of evolution and climatic changes, this plant group showed a resistance and persistence in maintaining its diversity in the earth. The stress tolerance capacity of these plants is the reason for its established biodiversity in the earth (Pereira et al., 2014; Scoffoni et al., 2014; Maia et al., 2014)

The primary response of an organism to an oxidative stress condition includes the activation of antioxidant enzymes, and the use of water-soluble antioxidant compounds and lipid-soluble antioxidant molecules. Antioxidant enzymes include catalase (CAT) and ascorbate peroxidase (AP), which are able to reduce H_2O_2 to water, and peroxiredoxins (PRXs), expressed mainly in the organelles and involved in detoxification of H_2O_2 and alkyl hydroperoxides. Antioxidant mechanisms are present in all living organisms, and have been studied with particular emphasis in host-pathogen interactions and to clarify how organisms cope with extreme environmental conditions. Rabara et al., (2015) reported that when water uptake and loss cannot keep balance by primary adaptive responses, different drought mechanisms through abscisic acid and other signaling pathways, may be exploited to avoid and/or tolerate dehydration plants.

Similarly, various genes that function as stress sensors in signaling transduction pathways, which comprise a network of protein-protein reactions, transcription factors (TFs) and promoters, are activated in *Arabidopsis* and other plants (Liu et al., 2016).

In this scenario, the present study was undertaken to evaluate the stress tolerance mechanism in terms of desiccation tolerant capacity of a fern *Adiantum latifolium* Lam. Commonly known as the maidenhair fern.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material *Adiantum latifolium* collected from the Kattakada Taluk of Trivandrum district, Kerala, was used for the present study. It was identified using the standard flora of Manickam and Irudayaraj (1992). The identification of the fern was further confirmed by herbaria of University of Calicut and a voucher specimen was kept in the Department herbarium (CCB 047).

DESICCATION TREATMENT

The fresh and mature sporophyte of *Adiantum latifolium* cleaned carefully and the samples were desiccated in a desiccator over polyethylene glycol (PEG) in a controlled environment chamber (Fig. 2 a & b). The selected plants were subjected to three different desiccation regimes like 1D (24 h), 2D (48h) and 3D (72h) (Fig. 3 a, b & c). After desiccation a set of desiccated samples were subjected to rehydration for 30 min. Thus the samples were divided into two groups namely desiccated and desiccated subsequently rehydrated. Control plants were maintained in an optimal water conditions in each case during the whole experimental period.

ESTIMATION OF RELATIVE WATER CONTENT (RWC)

The water status at each desiccated stage was calculated as relative water content (RWC).

$$RWC = \left(\frac{FW - DW}{FW} \right) \times 100$$

(FW - Fresh Weight, DW - Dry Weight)

QUANTIFICATION OF PHENOL

Total phenol content was estimated by the method of Mayr et al. (1995).

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC) OF PHENOLIC ACIDS

Quantitative fractionation of various phenolic acids in the samples was studied by RP-HPLC analysis. Phenolic acids extracted from fresh and desiccated tissues in aqueous methanol were used for the study.

PREPARATION OF THE SAMPLE

1g leaf tissue was refluxed in boiling 80% methanol for 10min. The tissue was homogenized, filtered through cheesecloth and centrifuged at 15000 rpm for 10 min. The resultant supernatant was made up to 5 ml with 80% methanol and used for RP-HPLC analysis.

PROCEDURE OF RP-HPLC

A modified method of Beta *et al.* (1999) was followed for HPLC analysis. An HPLC system (Waters Associates) equipped with a 7725 Rheodyne injector and Waters 510 HPLC pump, 486 tunable absorbance detector and Millennium 2010 software data module were used for the study. An HPLC column of 4.6x250 mm id reverse phase (RP) C8 was used for the fractionation of phenolic acids. Potassium hydrogen phosphate and acetonitrile in a ratio of 75:25 was used as the mobile phase for the isocratic elution. An elution period of 20 min with a flow rate of 0.8 ml min⁻¹ was given. 10µl of the sample was injected and the absorbance at 254 nm was recorded. Standard phenolic acids such as gallic, vanillic, p-hydroxybenzoic, ferulic, chlorogenic, sinapic, paracoumarate and cinnamic acids were injected in to the column separately. Comparing with the retention time of the standard identified phenolic acids in the sample. Height of the peaks was taken for quantification. Concentration of the standard and height of the standard peak were taken as the standard parameters.

QUANTIFICATION OF HYDROGEN PEROXIDE (H₂O₂)

H₂O₂ concentration of the experimental tissues was estimated as per the procedure of Bellincampi *et al.* (2000).

LOCALIZATION OF HYDROGEN PEROXIDE (H₂O₂)

Histochemical localization of H₂O₂ was done by staining the tissues with Tetramethylbenzidine (TMB) reagent, as per the method of RosBarcelo (1998).

QUANTIFICATION OF LIPID PEROXIDATION (LPX)

The level of lipid peroxidation in the cells was measured in terms of malondialdehyde (MDA) content determined by the thiobarbituric acid (TBA) reaction as described by Zhang and Kirkham (1996).

ISOLATION AND ASSAY OF PHENYLALANINE AMMONIA LYASE (PAL) AND POLYPHENOL OXIDASE (PPO)

Isolation of PAL was made by the method of Morrison *et al.* (1994) and the activity of PAL was estimated by the method of Whetten and Sederoff (1992). Isolation and the assay of PPO determined as per the method of Mayr *et al.* (1965).

STATISTICAL ANALYSES

The values were mean of six independent analysis ± SD and significance of the differences in all parameters tested was determined by two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Pteridophytes gain water in their cells both through external (ectohydric) capillary movement and internal (endohydric) transport. When fully hydrated, their water content is very high, attained several times more than their dry mass. The relative water content of control and 76 h desiccated fern were 80% and 31% respectively (Table 1). Unlike other plants, water content of ferns is highly related to environmental conditions and weakly regulated by their internal and morphological structures. Alpert and Oechel (1987) found that those species that occurred in microsites with lower water availability were able to attain maximum net photosynthetic gain at lower

Table 1: Influence of desiccation (24, 48 and 72h) stress on the levels of relative water content (RWC) % in *Adiantum latifolium*. Values are mean ±SD of six independent replications

Stages of desiccation	RWC (%)
Control	80±0.23
24hour desiccated	62±0.81
48 hour desiccated	48±0.19
72hour desiccated	31±0.34

Table 2: Hydrogen peroxide (H₂O₂), lipid peroxidation (LPX) and total phenolic content in *Adiantum latifolium* subjected to desiccation (24, 48 and 72hours) and rehydration. Values are mean \pm SD of three independent replications

Name of the assay	Control	24D	24R	48D	48R	72D	72R
H ₂ O ₂ ($\mu\text{molg}^{-1}\text{DW}$)	3.9 \pm 0.53	5.64 \pm 0.89	4.85 \pm 0.24	11.03 \pm 0.71	7.54 \pm 0.59	16.04 \pm 0.53	9.35 \pm 0.82
LPX ($\mu\text{molg}^{-1}\text{DW}$)	3.41 \pm 0.23	6.87 \pm 0.15	7.65 \pm 0.54	8.82 \pm 0.48	6.73 \pm 0.35	12.91 \pm 0.49	7.19 \pm 0.98
Total Phenol (mg g ⁻¹ tissue)	3.2 \pm 0.21	5.4 \pm 0.35	4.1 \pm 0.42	11.6 \pm 0.98	6.2 \pm 0.87	15.9 \pm 0.76	10.2 \pm 0.57
D-Desiccated	R-Rehydrated						

water content and to recover better from prolonged desiccation than those taxa in less xeric microsites.

The total phenolic content in the tissues varied among the experimental ferns. After desiccation (72h), the total phenols increased by 5 fold; following rehydration it was decreased substantially than the value found in desiccated phase (Table 2). The level of phenols may be supported by the activity profile of phenylalanine ammonia lyase (PAL) during desiccation. The waxing and waning pattern of phenols was further investigated by fractionating the phenols by reverse phase high performance liquid chromatography (RP-HPLC). The figures 1a and b represent the HPLC chromatogram of phenolic extracts from leaf tissues of *A. latifolium* with control and desiccated conditions.

Phenolic acids such as cinnamic acid, caffeic acid, ferulic acid, sinapic acid, coumaric acid, hydroxy benzoic acid, chlorogenic acid, gallic acid and vanillic acid were used as standards for detecting the compounds. It is evident from the figures 1a and b that phenolic extracts of the samples, contain the peaks of most of the standards, indicating the functional compartmentation of phenolic acids during desiccation when com-

pared with the control. The major phenolic acid noticed in the plant under control and desiccated condition was represented in the table 3. Interestingly, the desiccated fern shows significant profile of phenolic acids such as sinapic acid(4105.56 $\mu\text{g/g}$), ferulate (5136.73 $\mu\text{g/g}$), phlorogucinol (3006.56 $\mu\text{g/g}$), cinnamate (617.413 $\mu\text{g/g}$), gallate(529.21 $\mu\text{g/g}$), vanillate (617.413 $\mu\text{g/g}$), catechol (705.615 $\mu\text{g/g}$) and hydroxyl benzoic acid(3301.59 $\mu\text{g/g}$). The antioxidant activity of phenolic acids is related to the acid moiety and the number and relative positions of hydroxyl groups on the aromatic ring structure. Hydroxycinnamic acids are more effective antioxidants than hydroxybenzoic acids due to increased possibilities for delocalization of the phenoxyl radical. Hydroxylation in the 2- and 4-positions or in the 3-, 4- and 5-positions confers the greatest antioxidant activity. Adjacent hydroxyl groups, as found in protocatechuic acid, are less favourable for antioxidant activity than those meta-orientated with respect to each other.

Higher plants respond to various stresses by activating secondary metabolic pathways such as phenyl propanoid pathway (Zadworna and Zenkteler, 2006) which plays an important role in plant desiccation. Browning

Table 3: Phenolic acid profile of the control and desiccated *A. latifolium*.

	Control		Desiccated	
	Retention time(minutes)	Amount ($\mu\text{g/g}$ tissue)	Retention time(minutes)	Amount ($\mu\text{g/g}$ tissue)
Catechol	3.66	640.03	3.656	705.615
Cinnamic Acid	3.663	560.03	3.656	617.413
Gallate	3.663	480.02	3.656	529.29
Vanillate	3.663	560.027	3.656	617.413
Sinapic Acid	4.284	4043.30	4.296	4105.56
Ferulate	4.310	4877.08	4.602	5136.73
Hydroxybenzoic Acid	5.284	2339.99	5.350	3301.589
Phlorogucinol	4.284	2557.85	4.296	3006.56

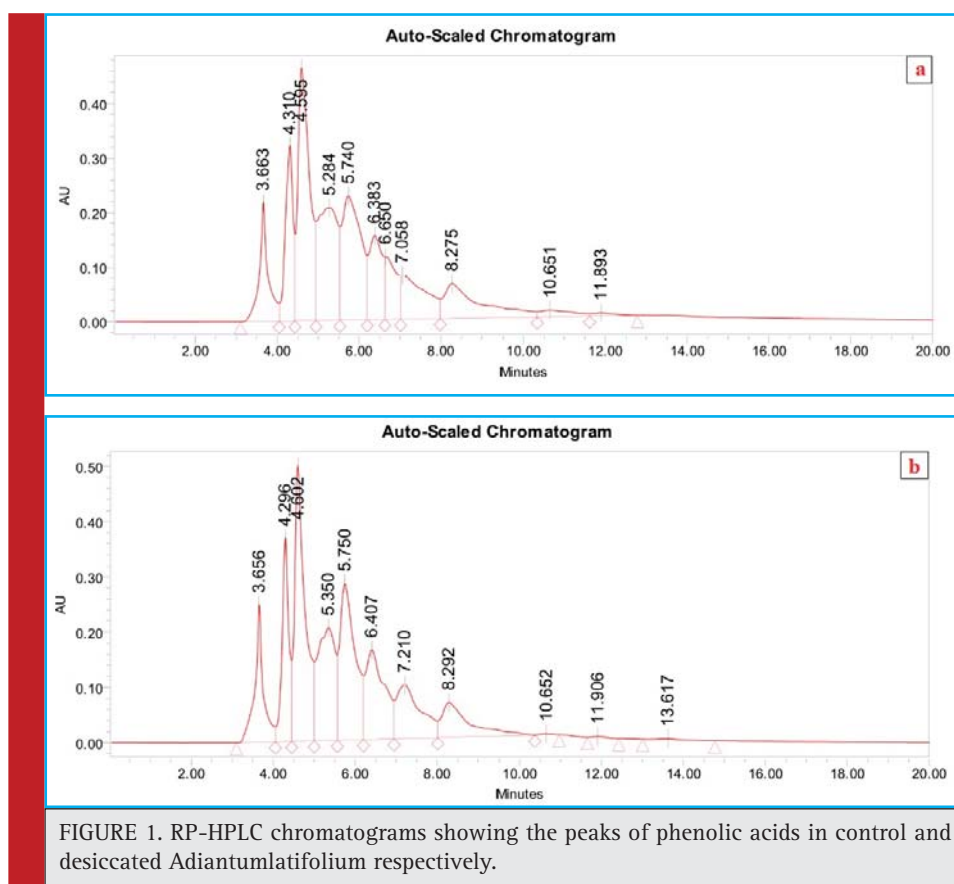


FIGURE 1. RP-HPLC chromatograms showing the peaks of phenolic acids in control and desiccated *Adiantum latifolium* respectively.

of plant tissue probably connected with phenolic accumulation, is a morphological symptom of drought stress (Hossain et al., 2013a and Moharramnejad et al., 2015).

The total phenolic content in the desiccated fronds showed significant increase. It is possible to suggest that negative impact of desiccation stress was reduced by total phenols. Increasing evidences indicate that phenols enhanced water stress tolerance via the induction of antioxidant defence systems in the plants (Jiang and Zhang, 2004). Moreover, Oliver and Bewley (1997) stated that vascular plants, even belonging to resurrection, could survive desiccation, only when they are dehydrated slowly. Longer rapid desiccation could cause even irreparable damages as suggested by Proctor et al., (2007a) would allow insufficient time for cytological osmotic adjustment, which is critical for plant survival during water stress. In all the fronds, after rehydration following desiccation, phenolic levels decreased, but not below control values. It seems that rehydration was too quick to put in motion the cascade of protection mechanisms in them (Nadernejad et al., 2013; Solti et al., 2014)

H_2O_2 , the reactive oxygen species (ROS) is derived from the $O_2^{\bullet-}$ by dismutation reaction. In the present study H_2O_2 was quantified in the fronds of control plants and experimental. Table 2 demonstrates the H_2O_2 con-

tent accumulated in the desiccated and rehydrated tissues of *A. latifolium*. From the data it is clear that, the desiccated tissues showed higher deposition of H_2O_2 than the control. The assay data of H_2O_2 in the desiccated tissues corroborates with the deep blue colour deposits in the histochemical analysis (Fig.2 a, b, c and d). Apart from the synthesis of H_2O_2 in cell system as a normal metabolic process, the over production of H_2O_2 in the tissues of desiccated plants is possibly a manifestation of oxidative stress. It can be postulated that H_2O_2 play a central role in plant as a signaling cascade or tolerance to stress (Bhattacharjee 2013; Sathiyaraj et al., 2014).

Irrespective of the comparison regarding the deposition of ROS- H_2O_2 between desiccated and control the higher accumulation of ROS remains as a physiological impact of the pteridophytes against desiccation stress. The level of H_2O_2 formation in the pteridophytes can be considered as an indicator of magnitude of abiotic stress in the species. Thus the results clearly indicates physiological impairedness of plants, due to this oxidative burst caused by the higher level of H_2O_2 production and it also highlights the reaction response of the pteridophytes against abiotic stress. The role of abiotic stress response in the formation of H_2O_2 in plant tissue has

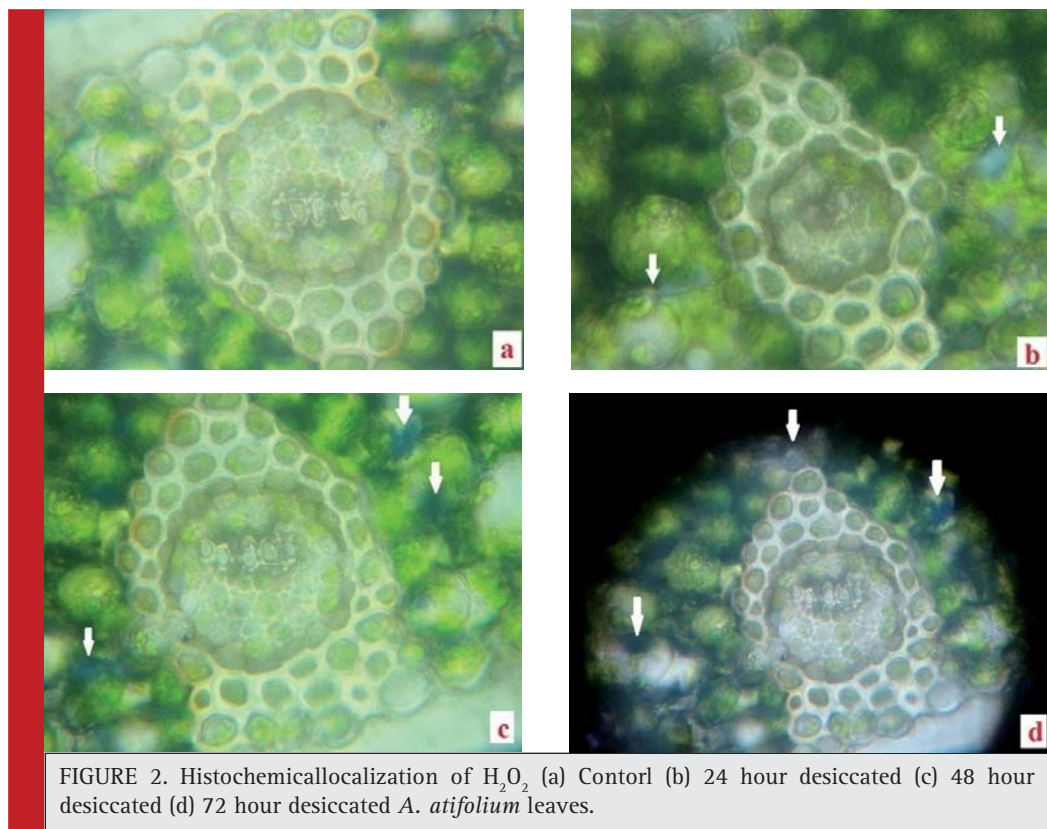


FIGURE 2. Histochemical localization of H_2O_2 (a) Control (b) 24 hour desiccated (c) 48 hour desiccated (d) 72 hour desiccated *A. latifolium* leaves.

been conclusively proved by several studies (Pukacka and Ratajczak, 2006).

The present data conclusively proved the excess generation of H_2O_2 in the species as a desiccation effect. As a constitutive metabolite of cell wall for lignin formation, the presence of H_2O_2 on cell system of plants is a common physiological feature. No lignin content and the tendency of plant cell to accumulate H_2O_2 in the excessive level, requires further analysis at biochemical level, for detecting the role of the marker enzyme involved in the process (Wang et al., 2014a).

Accumulation of H_2O_2 concomitant with lipid peroxidation resulted in significant decrease in cell membrane stability. It has been suggested that decrease in cell membrane stability reflects the extent of lipid peroxidation caused by ROS. Dat *et al.*, (1998) reported that by

heat acclimation pre-treatment, accumulation of H_2O_2 in plants can be reduced, compared with those without heat acclimation pre-treatment, which may be indicative of an enhanced antioxidant potential in leaf tissues and would contribute to enhance thermo tolerance. Desiccation stress injury may involve different mechanisms, depending on environmental conditions, before or during the dehydration stress. Genetic differences may also be important for elevated levels of H_2O_2 contributing to stress injury in plants.

Effect of desiccation stress on plant tissues were determined in terms of membrane lipid peroxidation, i.e., estimated as the content of thiobarbituric acid-reactive substances (TBARS). Increase in membrane lipid peroxidation was seen in all the desiccated fronds but at a slow pace right from 24 hour onwards, when compared with

Table 4: Phenylalanine ammonia lyase (PAL) and poly phenol oxidase (PPO) content in *Adiantum latifolium* subjected to desiccation (24, 48 and 72h) and rehydration. Values are mean \pm SD of three independent replications

Name of the assay	Control	24D	24R	48D	48R	72D	72R
PAL (Umg-1 protein)	37 \pm 0.62	59 \pm 1.23	48 \pm 1.01	78 \pm 1.48	54 \pm 0.98	96 \pm 0.89	63 \pm 1.16
PPO (Umg-1 protein)	3.25 \pm 0.14	10.79 \pm 0.98	6.89 \pm 0.36	6.81 \pm 0.28	3.77 \pm 0.13	2.48 \pm 0.11	1.23 \pm 0.05

control plants (Table 2). Within the experimental period of 72 hour, it increased to a maximum of $12.91 \mu\text{mol g}^{-1}$ DW. Thus, desiccation appeared to cause some damage in membrane configuration, but this was reversed and or repaired on rehydration to control values suggesting the active phase of antioxidant machinery in scavenging the ROS in the system.

Sairam et al., (2002) reported that, osmotic stress causes severe damage to the membrane integrity of various tissue types in different plant species. Moreover, membrane integrity is frequently correlated with plant tolerance to stress such as heat, salt, drought and other osmotic stress. Lipid peroxidation is an effective indicator of cellular oxidative damage, and was estimated by the levels of TBARS. The observed increase in TBARS concentration in stressed plants, might indicate lipid peroxidation of cell membrane components, caused by ROS generated by the oxidative stress (Sairam et al., 2002), i.e., LPX data strongly corroborates with the level of H_2O_2 content in the species (Zhou et al., 2014)

The key enzyme of phenyl propanoid metabolism known as phenylalanine ammonia lyase (PAL) was isolated from the control, desiccated and rehydrated fronds of *A. latifolium* and assayed spectrophotometrically in order to ascertain their role in secondary metabolite initiation. Being the initial enzyme of PPM, the presence of PAL, in plant tissue becomes an indispensable part for the synthesis of phytoalexins, lignin and phenolics thus involved in the defence response of plant cells (Morrison et al., 1994). Thus, PAL has been generally recognized as a marker of environmental stress in different plant species (MacDonald and D' Cunha, 2007).

Effects of desiccation on PAL activity in *A. latifolium* was shown in Table 4. A significant increase in PAL activity was observed in the fern at all tested desiccation periods, and the highest activity of $96 \mu\text{mg}^{-1}$ proteins observed at the 72 h desiccation period compared to the control. In few studies, PAL activity was induced by various biotic and abiotic stresses, such as wounding, chilling, heavy metal and infection by viruses, bacteria or fungi (MacDonald and D' Cunha, 2007). The responses of PAL activity subjected to desiccation could be a protective response to the cellular damage provoked by desiccation stress (John et al., 1971). The results suggest that changes in activity of enzyme strongly depend on desiccation periods (Huang et al., 2015)

D-DESICCATED R-REHYDRATED

PPOs are copper containing metalloenzymes that catalyze the oxidation of hydroxy phenols to their quinone derivatives. Polyphenol oxidase (PPO) showed varied expression in the desiccated fern compared to the control. PPO activity decreased from the 1st to 3rd day after

desiccation i.e., 10.79 ± 0.98 and 2.48 ± 0.11 respectively (Table 4). Initially the activity of endogenous polyphenol oxidase was higher in desiccated and rehydrated samples followed by a decrease in subsequent days of desiccation and rehydration (Table 4). Sofo et al., (2005) reported that a decrease in PPO activity following abiotic stress was associated with improved antioxidant capacity. The suppression of PPO increased the drought tolerance in tomato plants (Thipyapong et al., 2004a).

CONCLUSION

The results obtained in this study demonstrate that desiccation treatment from 24 to 72 h duration, in *Adiantum latifolium*, generated an oxidative stress condition, and morphological and biochemical alterations. The activation of different biochemical mechanisms helps to explain the high tolerance to desiccation of this species. In this context, *in vitro* rehydration experiments demonstrated the rapid capacity of the species to recover from oxidative stress, and provide the functional basis that helps to explain, at least in part, the position of this species at the highest tropical zones.

REFERENCES

- Alpert P., and Oechel W. C. (1987). Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting micro distributions. *American Journal of Botany* 74 : 1787-1796.
- Bellincampi D., Dipierro N., Salvi G., Cervone F., and De Lorenzo G. (2000). Extracellular H_2O_2 induced by oligogalacturonides is not involved in the inhibition of the auxin regulated ro 1B gene expression in tobacco leaf plants. *Plant Physiology* 122 : 1379-1385.
- Bergenon A., and Pellerin S. (2014). Pteridophytes as indicators of urban forest integrity. *Ecological Indicators* 38 : 40-49.
- Beta T., Rooney L. W., Marovatsanga L. T., and Taylor J. R. N. (1999). Phenolic compounds and kernel characteristics of Zimbabwian sorghums. *Journal of Science Food and Agriculture* 79: 1003-1010.
- Bhattacharjee S. (2013). Heat and chilling induced disruption of redox homeostasis and its regulation by hydrogen peroxide in rice (*Oryza sativa* L., Cultivar Ratna). *Physiology and Molecular Biology of Plants* 19: 199-207.
- Dat J.F., Foyer C.H., and Scott I. M. (1998). Changes in salicylic acid and antioxidants during induction of thermo tolerance in mustard seedlings. *Plant Physiology* 118: 1455-1461.
- Hossain M. A., Mostofa M. G., Fujita M. (2013a). Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. *Molecular Plant Breeding* 4: 50-70.
- Huang Y. W., Zhou Z. Q., Yang H. X., Wei C. X., Wan Y. Y., and Wang X. J. (2015). Glucose application protects chloro-

- plast ultrastructure in heat-stressed cucumber leaves through modifying antioxidant enzyme activity. *Biologia Plantarum* 59: 131-138.
- Jiang M. Y., and Zhang J. H. (2004). Abscisic acid and antioxidant defense in plant cells. *Acta Botanica Sinica* 46: 1-9.
- John M., Bardzik H. V., Marsh J. R., and Havis J. R. (1971). Effects of water stress on the activities of three enzymes in Maize seedlings. *Plant Physiology* 47 : 828-831.
- Liu C, Zhang X, Zhang K, An H, Hu K, Wen J, Shen J, Ma C, Yi B , Tu J and Fu T (2015) Comparative Analysis of the *Brassica napus* root and leaf transcript profiling in response to drought stress. *Int. J. Mol. Sci.* 2015, 16: 18752-18777.
- Mac Donald M. J., and D'Cunha G. B. (2007). A modern view of phenylalanine ammonia-lyase. *Biochemistry and Cell Biology* 85: 273-282.
- Maia J., Dekkers B.J.W., Dolle M., Ligterink W., and Hilhorst H.W.M. (2014). Abscisic acid (ABA) sensitivity regulates desiccation tolerance in germinated Arabidopsis seeds. *New Phytologist* 203: 81-93.
- Manickam V.S., and Irudayaraj V. (1992). Pteridophytic flora of the Western Ghats-South India. B.I.
- Mayr V., Treutter D., Santos-Buelga C., Bauer H., and Feucht W. (1995). Developmental changes in the phenol concentration of golden delicious apple fruits and leaves. *Phytochemistry* 38: 1151-1155.
- Moharramjad S., Sofalian O., Valizadeh M., and Shiri A .M. (2015). Proline, glycine betaine, total phenolics and pigment contents in response to osmotic stress in maize seedlings. *Journal of Bio Science and Biotechnology* 4 : 313-319.
- Morrison T. A., Kessler J. R., Hatfield R. D., and Buxton D. R. (1994). Activity of two lignin biosynthesis enzymes during development of a maize internode. *Journal of the Science of Food and Agriculture* 65: 133-139.
- Nadernejad N., Ahmadimoghadam A., Hossyinfard J., and Poorseyedi S. (2013). Evaluation of PAL activity, phenolic and flavonoid contents in three pistachio (*Pistacia vera* L.) cultivars grafted on to three different root stocks. *Journal of Stress Physiology and Biochemistry* 9 : 84-97
- Oliver M. J., and Bewley J. D. (1997). Desiccation tolerance of plant tissues: a mechanistic overview. *Horticultural Reviews* 18: 171-214.
- Pereira W.V.S., Faria J.M.R., Tonetti O.A.O., and Silva E.A.A. (2014). Loss of desiccation tolerance in *Copaiferalangs dorffii* Desf. seeds during germination. *Brazilian Journal of Biology* 74: 501-508.
- Proctor M. C. F., Ligrone R., and Duckett J. G. (2007a). Desiccation tolerance in the moss *Polytrichum formosum*: physiological and fine-structural changes during desiccation and recovery. *Annals of Botany* 99: 75-93.
- Rabara R.C, Tripathi P, Reese R.N, Rushton D.L, Alexander D, Timko M. P, Shen Q.J and Rushton P.J (2016). Tobacco drought stress responses reveal new targets for Solanaceae crop improvement *BMC Genomics* 2015, 16:484, 1-22.
- Ros Barcelo A. (1998). Hydrogen peroxide production is a general property of the lignifying xylem from vascular plants. *Annals of Botany* 82: 97- 103.
- Sairam R. K., Rao K. V., and Srivastava G. C. (2002). Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* 163: 1037-1046.
- Sathiyaraj G., Srinivasan S., Kim Y. J., Lee O. R., Balusamy S. D. R., and Khorolaragcha A. (2014). Acclimation of hydrogen peroxide enhances salt tolerance by activating defense-related proteins in *Panax ginseng* CA. Meyer. *Molecular Biology Reports* 41: 3761-3771.
- Scoffoni C., Vuong C., Diep S., Cochard H., and Sack L. (2014). Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. *Plant Physiology* 164: 1772-1788.
- Sofo A., Dichio B., Xiloyannis C., and Masia A. (2005). Antioxidant defenses in olive tree during drought stress: changes in activity of some antioxidant enzymes. *Functional Plant Biology* 32: 45-53.
- Solti A., Mihailova G., Sarvari E., and Georgieva K. (2014). Antioxidative defence mechanisms contributes to desiccation tolerance in *Haberlea rhodopensis* population naturally exposed to high irradiation. *Acta Biologica Szegediensis* 58 :11-14.
- Srivastava S., and Paul A. K. (2016). Associated microflora of medicinal ferns: biotechnological potentials and possible applications. *International Journal of Bioassays* 5: 4927-4943.
- Thipyapong P., Hunt M.D., and Steffens J.C. (2004a) Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. *Planta* 220:105-117.
- Wang Y., Zhang J., Li J. L., and Ma X. R. (2014a). Exogenous hydrogen peroxide enhanced the thermo tolerance of *Festuca arundinacea* and *Lolium perenne* by increasing the antioxidative capacity. *Acta Physiologiae Plantarum* 36: 2915-2924.
- Whetten R. W., and Sederoff R. R. (1992). Phenylalanine ammonia lyase from loblolly pine. Purification of the enzyme and isolation of complementary DNA clones. *Plant Physiology* 98: 380-386.
- Zadworna B A., and Zenkteler E. (2006). Ultrastructure of endodermis and stele cells of dehydrated *Polypodium vulgare* L. rhizomes. *Acta Biologica Cracoviensia Series Botanica* 49(2): 73-81.
- Zhang J. X., and Kirkham M. B. (1996). Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. *Plant Science* 113: 139-147.
- Zhou J., Xia X. J., Zhou Y. H., Shi K., Chen Z., and Yu J. Q. (2014). RBOH1-dependent H₂O₂ production and subsequent activation of MPK1/2 play an important role in acclimation-induced cross-tolerance in tomato. *Journal of Experimental Botany* 65: 595-607.