Advances in Environmental Biology, 11(6) June 2017, Pages: 19-26

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Differential Antioxidant Isozyme Response from Desiccation Induced Oxidative Stress of the Forked Fern (*Dicranopteris linearis*)

[1]Kavitha C.H and [2]Murugan K

[1]Department of Botany, St John's College, Anchal.
[2]Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum, 695 034, Kerala, India.

Address For Correspondence:
Kavitha C.H, Department of Botany, St John's College, Anchal
E-mail: kavithach1979@gmail.com

Received 28 January 2017; Accepted 12 May 2017; Available online 18 June 2017

ABSTRACT

Desiccation is an important abiotic stress that reduces productivity of plants globally. The effects of desiccation on plants include reduced growth, toxicity, osmotic stress, mineral deficiencies, photosynthetic imbalance, and consortium of these effects. Antioxidant enzymes involves in scavenging reactive oxygen species in the cellular system and safe guard the cells from oxidative burst. The present study aims to unravel the relationship between the antioxidant indices and desiccation tolerance of the forked fern. The response parameters evaluated includes expression of isozymes of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POX). The *in vivo* ferns were subjected to desiccation artificially using poly ethylene glycol from 2 to 10 days. The results showed that the expression of SOD isozymes in fronds was upregulated. CAT isozymes were induced well and overexpressed readily in leaves during desiccation stress. The expression pattern of POX isozymes was dissimilar to CAT. Similarly, APX and GR also showed remarkable expression. Thus it is possible to suggest that SOD, APX, CAT, POX and GR enzymes play significant role in determining response by up regulating their expression mitigate to desiccation stress in the ferns.

KEYWORDS: *Dicranopteris linearis*, Desiccation, Antioxidant enzymes, Isozymes expression

INTRODUCTION

Desiccation-tolerance is a phenomenon exhibited by plants called resurrection plants which have certain outstanding ability to withstand extreme dehydration and rapid rehydration of vegetative tissues without much cell damage. The basic mechanisms underlying such rapid and ecologically beneficial changes in cellular activity achieved in these plants are not well documented. The main effect of abiotic stress in plants is the induction of imbalance in the free radical production and scavenging occurring inside cells leading to a condition oxidative stress. The production of reactive oxygen species (ROSs) is a common phenomenon in plants under drought stress. These reactive oxygen species generations lead to lipid peroxidation [1, 2], protein degradation [3] and nucleic acid damages[4]. To alleviate adverse effects of reactive oxygen species, plants have evolved an antioxidant defence system that includes enzymes like superoxide dismutase, peroxidase and catalase [5].

The enzyme-catalyzed reactions to remove excess ROSs is the most predominant mechanism for the removal of superoxide and H_2O_2 along with the utilisation of non-enzymic low molecular weight antioxidants such as ascorbate and reduced glutathione (GSH).Within the antioxidant network, catalases (CATs) and ascorbate peroxidases (APXs), are the main enzymes involved in H_2O_2 removal. In parallel, peroxidases, glutathione S-transferases and glutathione reductases reduce H_2O_2 and organic hydroperoxides by ascorbate-

dependent thiol-mediated pathways. Many studies have reported effects of drought on the expression and activities of the major antioxidant enzymes such as superoxide dismutase SOD, CAT, APX, and so forth. Expression and/or activities are either measured after drought in comparison with well watered controls, or they are compared between plants that have differing drought sensitivity. A summary of data on total extractable enzyme activities provides little evidence for a striking, consistent response between different plant species, but the reported effects generally involve unchanged or increased activities [6]. In terms of extractable activities, the information provided by such analyses is potentially useful to understand the physiology underlying the response and/or as a marker in breeding programs. However, the interpretations that can be drawn from such analyses are complicated by several factors, notably the existence of multiple isoforms for these enzymes as well as the now-evident complexity of plant anti oxidative systems, meaning that only a partial picture of ROS metabolism is obtained.

In this context, the aim of the present investigation is to analyse the changes in the isoenzyme patterns of the major enzymes involved the scavenging of ROSs in the desiccation stressed fronds of the terrestrial forking fern *Dicranopteris linearis*. This will help in acquiring an unambiguous picture on the role of anti-oxidative enzymes in mitigating the desiccation stress felt by the fern.

MATERIALS AND METHODS

Plant material:

Dicranopteris linearis (Burm.f.) Underw. commonly known as forking fern belongs to Gleicheniaceae and is widespread in tropical and subtropical regions of the world .It grows horizontally at ground level with stalked compound fronds. It is found extensively growing along the road cuttings in shaded or open areas where water availability is scarce. The sporophyte of the fern is up to 3 m tall, with characteristic dichotomously divided leaves and rhizome is several meter long creeping, brown and covered with septate, branched hairs.

Desiccation treatment protocol:

Fresh *D. linearis* was fully hydrated and equilibrated in a controlled environment chamber for 48 h at 20°C and a radiant flux intensity 75 µM /m^2/ s. The samples were desiccated in a desiccator over polyethylene glycol (PEG) in a controlled environment chamber using the same light and temperature regimes as described above. The selected species were subjected to five different desiccation regimes (a) 2 day (b) 4 day (c) 6 day (d) 8 day and (e) 10 day. Control plants were maintained in an optimal water conditions in each case during the whole experimental period.

Native page and activity staining of various antioxidant enzymes:

Native polyacrylamide gel electrophoresis (PAGE) was performed at 4°C for SOD, CAT, POX, APX, and GR. Samples were mixed with 20% glycerol (v/v) and 0.25% bromophenol blue before loading onto the gels. An equal amount of protein (20 µg) was loaded in each lane. The gels were run at a constant 200 V at 4°C in a Bio-Rad Mini protein electrophoresis system. Isoforms of SOD were resolved in a 10% native polyacrylamide gel and visualized by NBT staining [7].The different isoforms of SOD were identified by selective inhibition with H_2O_2 and potassium cyanide (KCN) following the method described earlier by Miszalski *et al* [8]. The isoforms of Cu/Zn SOD and Fe-SOD were inhibited by staining the gel in solution containing 5 mM H_2O_2 and selective inhibition of Cu/Zn SOD was carried out by incubating the gel in solution containing 3 mM KCN. CAT isoforms were separated in a 7.5% native polyacrylamide gel and were visualized by staining with 0.03% (v/v) H_2O_2, 1% ferric chloride, and 1% potassium ferricyanide following the method of Woodbury *et al* [9] APX isoforms were separated in a 10% native polyacrylamide gel and were stained following the method of Mittler and Zilinskas [10]. Isoforms of POX were resolved in a 7.5% polyacrylamide gel and visualized by staining with a solution containing 50 mM sodium acetate buffer (pH 5.4), 10 mM *O*-dianisidine and 10 mM H_2O_2. GR isoforms were separated in a 7.5% native polyacrylamide gel and detected by incubating the gels in 50mM KPO_4 (pH 7.5) containing 0.24 mM monotetrazolium, 0.34 mM 2,6-dichloro phenol indo phenol, 3.4 mM oxidized glutathione (GSSG) and 0.5 mM NADPH. The gels were scanned and analyzed using a scanner.

Lipid peroxidation:

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 [11]. The absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of malondialdehyde was calculated using the molar extinction coefficient of 155 mM cm^{-1}.

Results:

ROS generation and its detoxification mechanisms vary in response to abiotic stress in plant species [12].

Advances in Environmental Biology, 11(6) June 2017, Pages: 19-26

To avoid excessive ROS accumulation, plants possess a complex antioxidant defense system including non-enzymatic systems, for example, carotenoids, ascorbic acid, and glutathione in addition to ROS-scavenging enzymes such as SOD, CAT, POX, APX, and GR to protect the cellular membranes and organelles from detrimental effects of ROS.

Lipid peroxidation:

Efficiency of antioxidant defense may be correlated with the degree of membrane damage due to peroxidation of unsaturated fatty acids in the lipids structure of cell membranes. Oxidative stress factors induce enormous amounts of ROSs and alkyl peroxides, if these molecules are not effectively detoxified by specific antioxidant enzymatic and non-enzymatic compounds, oxidative membrane damage results. This leads to the formation of lipid peroxide derivatives, the most common and toxic of these being the malondialdehyde (MDA). The present results reflect that desiccation stress exerted on forked fern was significantly mitigated by antioxidants so that the membrane damage by lipid peroxidation was marginal. Interestingly the enhanced membrane lipid peroxidation, manifested in increased generation of malondialdehyde and other related, thiobarbituric acid-reactive substances, occurs upon exposure to 2nd day of desiccation stress condition compared to 4, 6, 8 and 10 days (Fig.1) ($P < 0.05$).This may be due to the sudden drastic impact of stress in the fern or delay in the induction of antioxidant machinery in the cells system.

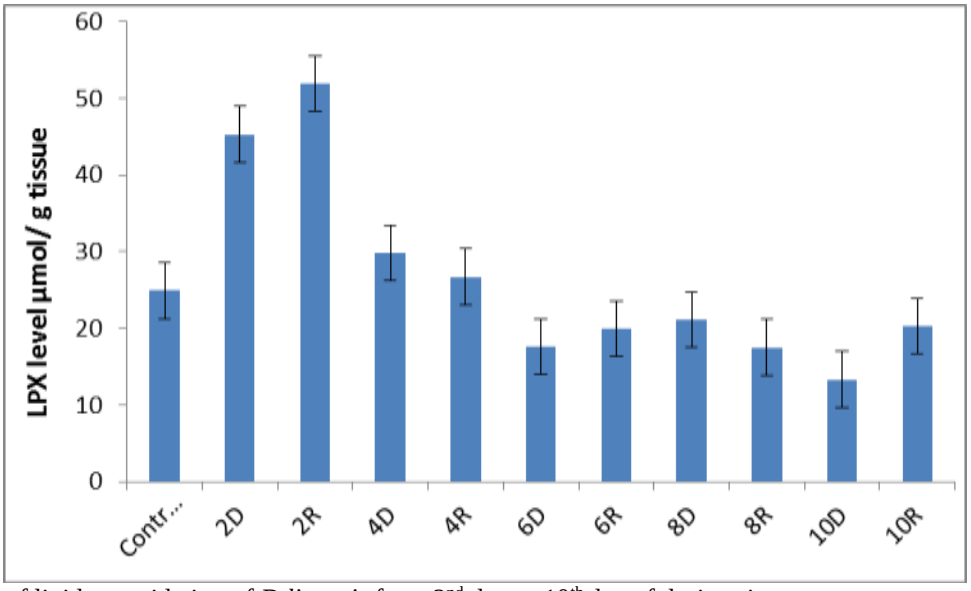

Fig. 1: Level of lipid peroxidation of *D.linearis* from 2nd day to 10th day of desiccation.

Isozyme of SOD:

Under desiccation stress conditions, five major SOD isozyme bands 1, 2, 3, 4 and 5 were clearly observed in the fronds of *Dicranopteris linearis* when compared to control (one band only) (Fig. 2). The SOD isozyme 1 was increasingly expressed from 2nd day to 8th day of desiccation stress, and then became slightly weaker in the 10th day. SOD isozyme 2 and 3 were prominent throughout the desiccation periods. The isozyme 4 was feebly expressed. Isozyme 5 was up regulated from 2nd day to 8th day and was visible marginally on 10th day desiccated ferns.

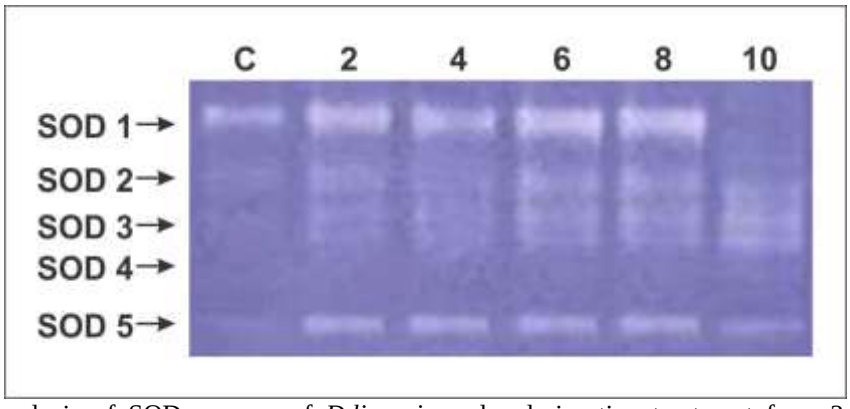

Fig. 2: Isozyme analysis of SOD enzyme of *D.linearis* under desiccation treatment from 2 to 10 day of desiccation.

Isozyme of CAT:

During desiccation stress, single CAT isozyme band was clearly observed from 2nd to 8th day and less prominent at 10th day. The expression of CAT isozyme was induced by desiccation stress and was strong and stable in the fronds (Fig. 3). However, the CAT enzyme expression was weak in the control.

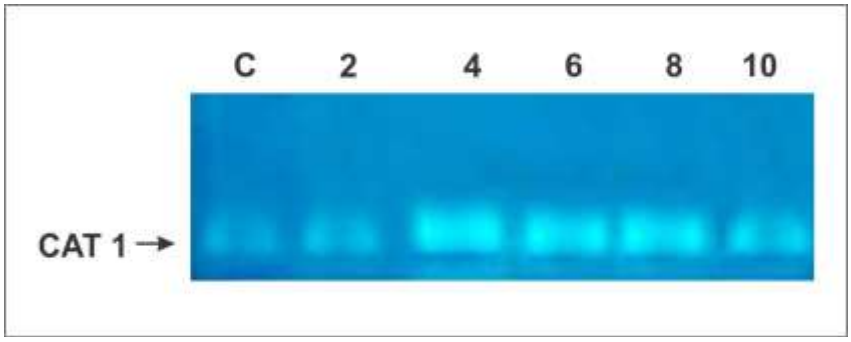

Fig. 3: Isozyme analysis of CAT enzyme of *D.linearis* under desiccation treatment from 2 to 10 day of desiccation.

Isozyme of POX:

As shown in Fig. 4, POX isozymes were clearly seen as compared to control (single band only). On the 2nd day of desiccation stress, the expression of POX isozyme 1 was increased in the fronds up to 6th day. On the 10th day the expression became weak. The isozyme 2 expression was upregulated from the 2nd day to 10th day (Fig. 4). The expression of the POX 3 was noticed throughout the periods of desiccation stress.

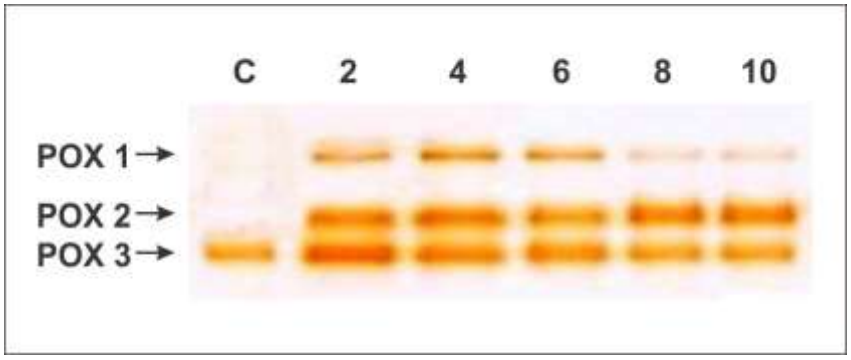

Fig. 4: Isozyme analysis of POX enzyme of *D.linearis* under desiccation treatment from 2 to 10 day of desiccation.

Isozyme of APX:

The activity of APX 1 was increased significantly from 2nd to 8th day of desiccated ferns compared to the control (Fig. 5). However, APX 2 expressed from 2nd to 8th day and was marginally reduced in 10th day desiccated plants.

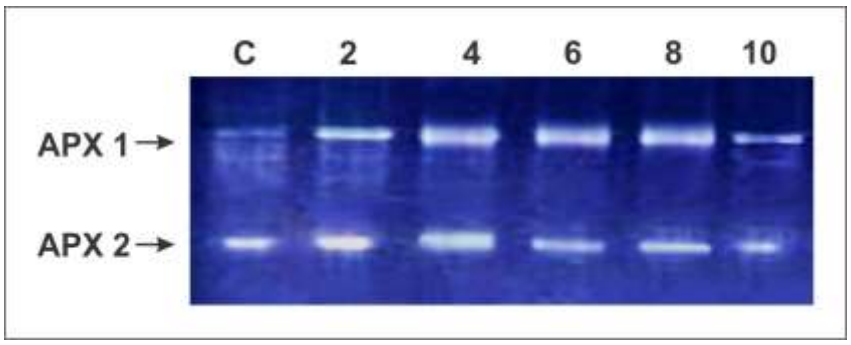

Fig. 5: Isozyme analysis of APX enzyme of *D.linearis* under desiccation treatment from 2 to 10 day of desiccation.

Isozyme of GR:

As evidenced from figure 6, there were marginally increased changes in GR activity in plants desiccated up to 10th days. Activity staining specific for GR showed three isoforms in a native gel (Fig. 6). The band intensity of GR 3 isoform was significant with 2nd and 4th days.

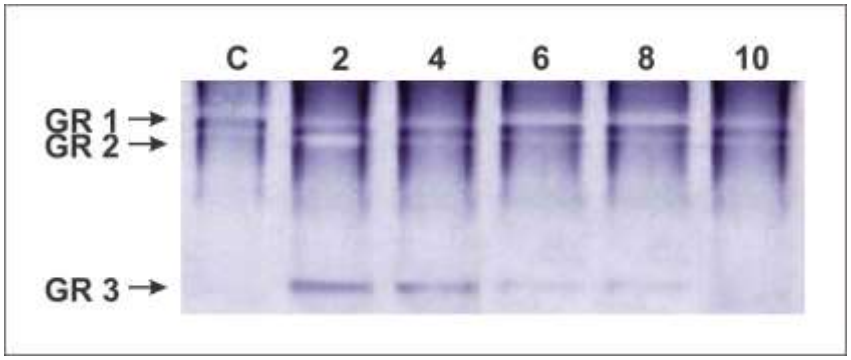

Fig. 6: Isozyme analysis of GR enzyme of *D.linearis* under desiccation treatment from 2 to 10 day of desiccation.

Discussion:

Drought stress is one of the major environmental factors that adversely affect plant growth and productivity [13, 14]. The osmotic imbalance caused by desiccation stress can trigger many responses such as growth inhibition, synthesis of nontoxic molecules and increase in osmotic potential of the cell in natural and artificial habitats. Desiccation stress in plants leads to damage *via* overproduction of ROS or free radicals. Counterbalance against desiccation of plants was connected with antioxidant enzymes and regulation of these ROSs level. Increased POX activities and SOD in response to desiccation stress were reported by Kukreja *et al* [15]. The SOD activity is responsible for dismuting $O_2^{\cdot-}$radical in to H_2O_2 in plants [16]. It is well known that H_2O_2 is a complex signal transmission network in cell system. There are diverse types of stress reactions mediated by H_2O_2; the homeostasis of H_2O_2 is mostly due to its scavenging by CAT. Thus, H_2O_2 accumulation was possibly the trigger of SOD activation. So, the balance between ROS synthesis and its scavenging by anti-oxidative mechanism is crucial for survival and tolerance of plants against stress.

Stress-induced antioxidant activities are dependent on the intensity and duration of the treatment and also the plant species. For example, SOD activity increased or remained unchanged in the early phase of desiccation but decreased with prolonged water stress [17]. Many studies documented that desiccation stress alters the amount and the activities of the antioxidant enzymes involved in scavenging ROS [18]. In the present study, SOD 1 and 5 isozyme bands, POX 1 and 2 isozyme bands and APX 1 and 2 isozyme bands were clearly detected after native polyacrylamide gel electrophoresis (PAGE) analysis in the fronds of the fern under 2-10 days of desiccation stress. These isozymes were involved in removing ROS in the fronds of the fern. The differential expression of these antioxidant enzymes was diverse under continuous desiccation stress. The concentration of H_2O_2 should correlate with SOD activity and the ability of species to adapt to the environment.

Naderi *et al* [19] reported antioxidant enzyme changes in response to osmotic stress in wheat seedling. POX, CAT, GR and APX activities were increased significantly in the severe stress compared with control condition about 31, 61, 129 and 149 percent, respectively. Whereas, SOD activity increased significantly by 41% in the mild stress compared with control treatment. The highest enzymatic activity was belonged to tolerant group under severe stress conditions for almost all of isozymes reported. Eslami *et al* [20]reported some antioxidant enzymes banding patterns and their correlation in common bean genotypes under water deficit stress. Gonzalez-Parraga *et al* [21] pointed out the role of antioxidant enzymatic defenses against oxidative stress (H_2O_2) and the acquisition of oxidative tolerance in *Candida albicans*. Weng *et al* [22] evaluated effects of drought stress on antioxidant enzymes in seedlings of different wheat genotypes using PEG-6000 solution for 1~6 days. Expression of SOD isozymes showed variation among the cultivars. CAT isozymes were induced well and increased readily in Changwu134 and Xinong928 cultivars than in Xinong2208 and Shaan253. POX isozymes expression was similar to CAT.

ROS synthesis its oxidative burst and detoxification mechanisms vary in response to biotic and abiotic stress [12]. For balancing of excessive ROS accumulation, plants possess a complex antioxidant defense system including non-enzymatic molecules such as carotenoids, ascorbate, tocopherol and reduced glutathione and ROS-scavenging enzymes like SOD, CAT, POX, APX, and GR in order to protect the cellular membranes and organelles from detrimental damages of ROS. SODs dismutate $O_2^{\bullet-}$ into H_2O_2 and have been considered as the first line of defense against oxidative stress in plants [23]. SOD group includes Mn, Fe, and Cu/Zn isoforms occur in different cell compartments such as the cell wall, cytoplasm, mitochondria, and chloroplasts [24]. For

example, Cu/Zn SODs are within chloroplasts [8], in the cytosol [25], and in mitochondria [8], Fe-containing SOD in chloroplasts [8], Mn-SODs located in mitochondria, peroxisomes [8], and their localization in chloroplasts has been also reported by Slooten *et al* [26]. In plants, subcellular fraction as well as *in vitro* activity staining studies has established that CAT isoforms are dominantly localized in peroxisomes [27]. APX isozymes were reported from chloroplast, cytosol, mitochondria, and peroxisomes [28, 29]. Seckin *et al* [30]; Ellouzi *et al* [31] Srivastava *et al*[32] reported that the salt loving species have effective anti-oxidative machineries than glycophytes. Over production of ROS in cells can leads to oxidative damage to lipid membrane, proteins, and DNA, thereby affecting the structural integrity of membranes, enzyme activities, and the function of chloroplast [33]. However, in the present study in ferns, the tolerance to desiccation derives largely from the constitutively maintained higher anti-oxidative enzymatic activities. It is logic to assume that if there is over ROS accumulation in the fern that may leads to lipid peroxidation. But in contrasts the present data on the lipid peroxidation level shows that it was marginal after an initial up regulation during 2nd day of desiccation indicating the activity of an efficient anti-oxidative mechanism. Nazar *et al*[34]; Shobbar *et al*[35]; Shaheen *et al*[36]; Ben Rejeb *et al*[37] reported that high level of LPX which increase progressively with salinity in mung bean, rice, egg plants and Arabidopsis. In the desiccated fern, the SOD level is quite high than control plants. These results suggest that the enzyme SOD is constitutively at remarkable level to scavenge the desiccation induced production of $O_2^{\bullet-}$. Similarly, the over expression of CAT is seen at all levels, whereas the POX isozymes increased tremendously. On the other hand, the up regulation of APX increased from 2 d to 10th day. The increase activity of CAT, APX and POX might be involved in the detoxification of H_2O_2 in the species. It has been reported that the turnover value of CAT is high and can scavenge millions of H_2O_2 molecules [23]. The unregulated expression of APX suggests that the APX constitutively present in this plant might be involved in detoxify the H_2O_2 in chloroplast and cytosol. POXs are involved in many functions in plant cells such as ROS generation and regulation, H_2O_2 level regulation, oxidation of various substrates and also involved in loosening of the cross-linking of cell wall compounds [38] The present induced increase in both APX and POX was comparable with some halophytes [39, 40]. In contrast, *Puccinellia tenuiflora*, both the enzyme activity remained stable under salinity [33]. The GR is a redox regulatory enzyme like APX and it is essential for maintaining the redox state of ascorbate and glutathione [39]. GR plays an important role in the control of endogenous H_2O_2 content through an oxido-reduction cycle (Halliwell-Asada pathway) involving glutathione and ascorbate (Bose *et al*., 2014). GR isoform was found to up regulated by desiccation. GR is a versatile enzyme its level remains stable [33] or increases [23] under stress conditions among plants.

Pompeu *et al* [41] reported the antioxidant isoenzyme responses to different concentrations of nickel-induced stress in tobacco cell suspension culture. SOD 1, 2 isoenzymes, a Mn-SOD (band I) and a Fe-SOD (band II), as well as one CAT isoenzyme and four GR isoenzymes were observed in the study. Activity staining analysis revealed that CAT activity plays a major role in the early response to Ni induced oxidative stress, particularly when the Ni concentration used was low, whilst a specific GR isoenzyme appears to respond to the Ni-induced oxidative stress. Abedi and Pakniyat [42] antioxidant enzyme changes in response to drought stress in cultivars of oilseed rape revealed an enhancement of the activities of SOD and guaiacol peroxidase whereas CAT activity decreased. Native PAGE detected eight SOD isozymes and 5 POX isoforms. The intensities of POX-4 and -5 were enhanced under drought stress**.**

Conclusion:

The present study in the forked fern demonstrates that antioxidant enzymes isozyme forms responded differently to desiccation stress. The remarkable expression of these enzymes could be associated with antioxidant protection through increasing activities and maintaining the pool of ROSs. Isozymes of SOD for $O_2^{\bullet-}$ scavenging and increasing APX activities for H_2O_2 scavenging has been observed. Thus, a tolerant fern preferably should have greater expression of most of the antioxidant enzymes, such as SOD and Halliwell-Asada Pathway enzymes, such as APX. Similarly, the differences in CAT and POX activities or their isozymes could also account for the species in desiccation tolerance. Manipulation of those antioxidant enzymes that exhibited differential responses to drought stress for species differing in drought tolerance may lead to improvement in tolerance. The direction involvement, cellular location, characteristics of these enzymes, and the isoforms related to drought tolerance in lower plant species are largely unknown, and deserve further investigation.

Conflict of Interests:

The authors declare that there is no conflict of interest is regarding the publication of this paper.

ACKNOWLEDGMENT

The authors hereby acknowledge the University Grant Commission regional office, Bangalore for providing FDP status to the teacher fellow for completing the Ph.D. work (Order No.F.No.FIP/12th plan/KLKE021 TF 06).

REFERENCES

[1] Sreeni-Nivasuhu, N., S. Ramanjulu, K. Ramachandra, H.H. Prakash, H. Shekar-Shetty, H.S. Savithri and C. Sudhakar, 1999. Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. Plant Sci., 141: 1-9.
[2] Chen, W.P., P.H. Li and T.H. Chen, 2000. Glycinebetaine increase chilling tolerance and reduce chilling-induced lipid peroxidation in *Zea mays* L. Plant Cell Envi., 23: 609-618.
[3] Jiang, M and J. Zhang, 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. J. Plant Cell Physiol., 42: 1265-1273.
[4] Hagar, H., N. Ueda and S.V. Shal, 1996. Role of reactive oxygen metabolites in DNA damage and cell death in chemical hypoxic injury LLC-PK1 cell. Ame. J. Physiol., 271: 209-215.
[5] Agarwal, S and V. Pandey, 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia.* Biol. Planta., 48: 555-560.
[6] de Carvalho, M.H.C., 2013. Drought stress and reactive oxygen species. Production, scavenging and signalling. Plant Signal Behav., 3: 156-165.
[7] Beauchamp, C and I. Fridovich, 1971. Superoxide dismutase: improved assay applicable to acrylamide gels. Annu. Biochem., 44: 276-287.
[8] Miszalski, Z., ´ I. Slesak, E.N iewiadomska, R. Baczek-Kwinta, U. Lüttge and R. Ratajczak, 1998. Subcellularlocalization and stress responses of superoxide dismutase isoforms from leaves in the C3-CAM intermediate halophyte *Mesembryanthemum crystallinum* L. Plant Cell Environ, 21: 169-179.
[9] Woodbury, W., A.K. Spencer and M.A. Stahman, 1971. An improved procedure using ferricyanide for detecting catalase isozymes. Anal. Biochem, 44: 301-305.
[10] Mittler, R and B.A. Zilinskas, 1993. Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate-dependent reduction of nitroblue tetrazolium.Anal.Biochem, 212: 540-546.
[11] Zhang, J.X and M.B. Kirkham, 1996. Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. Plant Sci., 113: 139-147.
[12] Ozgur, R., B. Uzilday, A.H. Sekmen and I. Turkan, 2013. Reactive oxygen species regulation and antioxidant defence in halophytes; Review. Funct. Plant Biol., 40: 8-9.
[13] Muhammad, N., M. Birgit, G.R. Thomas, W. Krzysztof and S. Angela, 2014. Increased drought stress resilience of maize through endophytic colonization *by Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. Environ. and Experi. Bot., 97: 30-39
[14] Vinocur, B and A. Altman, 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotech., 16: 123-132.
[15] Kukreja, S., A.S. Nandval, N. Kumar, S.K. Sharma, V. Unvi and P.K. Sharma, 2005. Plant water status, H_2O_2 evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. Plant Biol., 49: 305-308.
[16] Bano, A., F. Ullah and A. Nosheen, 2012. Role of abscisic acid and drought stress on the activities of antioxidant enzymes in wheat. Plant Soil Environ., 58(4): 181-185.
[17] Tatari, M., R.F.G hazvini, N. Etemadi, A.M. Ahadi and A. Mousavi, 2012. Analysis Of Antioxidant Enzymes Activity, Lipid Peroxidation And Proline Content Of *Agropyron desertorum* Under Drought Stress. South West J Hortic. Biol. Environ., 3(1): 9-24.
[18] Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita, 2013. Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. Int. J. Mol. Sci., 14: 9643-9684.
[19] Naderi, R., M. Valizadeh, M. Toorchi and M.R. Shakiba, 2014 . Antioxidant enzyme changes in response to osmotic stress in wheat (*Triticum aestivum* L.) seedling. Acta Biolo.Szegediensis, 58(2): 95-101.
[20] Eslami, P., M. Valizadeh, M. Norouzi and M. Shakouri, 2015. Some Antioxidant enzymes banding patterns and their correlation in common bean genotypes under water deficit stress .Biological Forum – An International Journal, 7(1): 1474-1478.
[21] Gonzalez-Parraga, P., J.A. Hernandez and J.C. Arguelles, 2003. Role of antioxidant enzymatic defences against oxidative stress (H_2O_2) and the acquisition of oxidative tolerance in *Candida albicans.* Yeast, 20: 1161-1169.
[22] Weng, M., L. Cuiα, F. Liuα, M. Zhang, L. Shan, L. Yang and X. Deng, 2015. Effects of drought stress on antioxidant enzymes in seedlings of different wheat genotypes. Pak. J. Bot., 47(1): 49-56.
[23] Bose, J., A. Rodrigo-Moreno and S. Shabala, 2014. ROS homeostasis in halophytes in the contest of salinity stress tolerance. J. Exp. Bot., 65: 1241-1257.
[24] Mittova, V., M. Volokita, M. Guy and M. Tal, 2000. Activities of SOD and the ascorbate glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*.Physiol.Plant., 110: 42-51.

[25] Sakamoto, A., T. Okumura, H. Kaminaka and K. Tanaka, 1995. Molecular cloning of the gene (SodCc1) that encodes a cytosolic copper zinc-superoxide dismutase from rice (*Oryza sativa* L.). Plant Physiol., 107: 651-652.
[26] Slooten, L., K. Capiau, W. Van Camp, M. Van Montagu, C. Sybesma and D. Inzé, 1995. Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco over expressing manganese superoxide dismutase in the chloroplasts. Plant Physiol., 107: 737-75.
[27] Mhamdi, A., G. Queval, S. Chaouch, S. Vanderauwera, F.V. Breusegem and G. Noctor, 2010. Catalase function in plants: a focus on Arabidopsis mutants' asstress-mimicmodels. J.Exp.Bot., 6: 4197-4220.
[28] Shigeoka, S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta, *et al*., 2002. Regulation and function of ascorbate peroxidase iso-enzymes. J. Exper. Bot., 53: 1305-1319.
[29] Caverzan, A., G.P assaia, S.B. Rosa, C.W. Ribeiro, F. Lazzarotto and M. Margis Pinheiro, 2012. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection.Genet.Mol.Biol., 35: 1011-1019.
[30] Seckin, B., I. Turkan, A.H. Sekmen and C. Ozdan, 2010. Theroleofantioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (Sea barley grass) and *Hordeum vulgare* L. (cultivated barley).Environ.Exp.Bot., 69: 76-85.
[31] Ellouzi, H., K.B. Hamed, J. Cela, S. Munne-Bosch and C. Abdelly, 2011. Early effects of salt stress on the physiological and oxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte).Physiol.Plant., 142: 128-143.
[32] Srivastava, A.K., S. Srivastava, V.H. Lokhande, S.F. D'Souza and P. Suprasanna, 2015. Salt stress reveals differential antioxidant and energetics responses in glycophyte(*Brassica juncea* L.) and halophyte (*Sesuvium portulacastrum* L.). Front.Environ.Sci., 3: 19.
[33] Yu, J., S.C hen, Q.Z hao, T. Wang, C. Yang, C. Diaz, *et al*., 2011. Physiological and proteomic analysis of salinity tolerance in *Puccinellia tenuiflora*. J.Proteome Res., 10: 3852-3870.
[34] Nazar, R., N. Iqbal, S. Syeed and N.A. Khan, 2011. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mung bean cultivars. J.Plant Physiol., 168: 807-815.
[35] Shobbar, M., O. Azhari, Z.S. Shobbar, V. Niknam, H. Askari, M. Pessarakli, *et al*., 2012. Comparative analysis of some physiological responses of rice seedlings to cold, salt, and drought stresses. J. Plant Nutr., 35: 1037-1052.
[36] Shaheen, S., S. Naseer, M. Ashraf and N.A. Akram, 2013. Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in *Solanum melongena* L. J.Plant Interact., 8: 85-96.
[37] Ben Rejeb, K., M. Benzartia, A. Debez, C. Bailly, A. Savouré and C. Abdelly, 2015. NADPH oxidase-dependent H_2O_2 production is required for salt induced antioxidant defense in *Arabidopsis thaliana*. J.Plant Physiol., 174: 5-15.
[38] Passardi, F., C. Cosio, C. Penel and C. Dunand, 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep., 2: 255-265.
[39] Benzarti, M., K.B. Rejeb, A. Debez, D. Messedi and C. Abdelly, 2012. Photosynthetic activity and leaf antioxidative responses of *Atriplex portulacoides* subjected to extreme salinity. Acta Physiol. Plant., 34: 1679-1688.
[40] Amjad, M., S.S. Akhtar, A. Yang, J. Akhtar and S.E. Jacobsen, 2015. Antioxidative response of quinoa exposed to iso-osmotic. Ionic and Non Ionic Salt Stress. J. Agro. Crop. Sci., 201: 452-460.
[41] Pompeu, G.B., V.A. Gratao, R.A. Vitorello and Azevedo, 2008. Antioxidant isoenzyme responses to nickel-induced stress in tobacco cell suspension culture. Sci. agric., 65(5): 548-552.
[42] Abedi, T and H. Pakniyat, 2010. Antioxidant Enzyme Changes in Response to Drought Stress in Ten Cultivars of Oilseed Rape (*Brassica napus* L.), Czech J. Genet. Plant Breed., 46(1): 27-34.