ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

DESICCATION- INDUCED CHANGES IN LIPID PEROXIDATION SUPEROXIDE LEVEL AND CELL MEMBRANE H^+ATPASE ACTIVITY IN FORKED FERN –*DICRANOPTERIS LINEARIS* (BURM.F.) UNDERW.

Kavitha C H and Murugan K*

Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum, 695 034, Kerala, India.

Manuscript Info

Manuscript History:

Received: 14 December 2015
Final Accepted: 16 January 2015
Published Online: February 2016

Key words:
Dicranopteris linearis, desiccation, oxidative stress, superoxide radical, hydrogen peroxide, Lipid peroxidation.

Corresponding Author

Murugan K.

Abstract

Desiccating environments pose physiological challenges to plants, especially those experiencing them on recurring basis. Many desiccation-tolerant organisms are known to withstand loss of body water in response to seasonal dehydration events through adaptations that facilitate their survival in the dry state followed by recurring back upon favourable conditions. Free radical-induced non-enzymatic peroxidation leads to damage membranes, enzymes and nucleic acids and it is likely to be the major causes of injury during desiccation of plants. Protective mechanisms to scavenge the peroxidatively-produced free radicals and peroxides have evolved within plants, keeping these reactive molecules to minimum. The extent of drought tolerance varies from species to species. The formation of superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH·), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are common physiological events noticed with desiccation. In this juncture, the objective of this study is to analyze the desiccation stress induced features from 2nd day to 10th day of the terrestrial forked fern –*Dicranopteris linearis*. The hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$), showed a remarkable increase in the treated groups when compared to control revealing its role as signaling cascade. Peroxidation of membrane lipids is a sensitive diagnostic index of oxidative stress. Lipid peroxidation levels in the analysis showed an initial rise and subsequently decreased at par with the control indicating the active anti-oxidative machinery operating in the plant. The H^+ATPase in the plasma membrane showed an increased activity throughout the treatment period. The enhanced H^+ATPase may be due to the plant's adaptation in maintaining transportation normal in cells. Abrupt changes in the lipid peroxidation levels due dehydration rehydration treatments may also induce the increase in the plasmalemma H^+ATPase activity. Thus, the present results indicate the oxidative stress happened in the plant due to induced ROSs synthesis. Maintenance of lipid peroxidation and increased membrane bound H^+ATPase activity throws light into the desiccation adaptation of the plant.

Introduction:-

In plant tissues, the role of water is complex and varied. Marginal water loss can lead disruption of the mechanical and metabolic stability, and desiccation tolerant plants appear to have unique potentialities to minimize such disruption. Loss of water interferes with metabolic processes and, at the extreme level, results in loss of membrane structure and causes metabolic destabilization within the cellular system. There is increased aggregation of ROSs results in the disintegration of organelles due to oxidative peroxidation (Varghese and Naithani, 2002). Further,

overproduction of ROS causes damage to macromolecules and subcellular components by reacting with proteins and lipids.

Desiccation tolerant plants appear to protect against the potential damage of desiccation-induced ROS production by minimizing the formation of photosynthesis-associated ROS, more effective use of housekeeping antioxidants and production of novel antioxidants in response to drying. Most common aspect of adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Van Breusegem *et al*., 2001). ROS can occur as by-products of regular cellular metabolism such as in photosynthesis and respiration. However, under stress, their formation is usually exacerbated. Drought stress leads to the disruption of electron transport systems and thus under water deficit conditions the main sites of ROS production in the plant cell are organelles with highly oxidizing metabolic activities or with sustained electron flows: chloroplasts, mitochondria and microbodies. Within the photosynthetic apparatus, photosystem II (PS II) is affected most by drought stress, particularly within the oxygen-evolving complex and the reaction centres (Toivonen and Vidaver, 1988; He *et al*., 1995).

In addition to physical stresses that desiccation-sensitive tissues incur as a consequence of dehydration, they are subject to metabolic damage. It has been argued that desiccation differentially affects the activities of various enzymes and hence results in metabolic imbalance (Ntuli *et al.*, 2011). Of particular interest in this regard is respiratory metabolism. For instance, it has been shown that phosphofructokinase (PFK) and malate dehydrogenase (MDH) were slightly and mildly affected by dehydration, respectively, while the glucose-6-phosphate dehydrogenase (G6PDH) and NADH dehydrogenase of the NADH-ubiquinone (coenzyme Q) reductase (complex I) were extremely sensitive to desiccation in germinating maize (Leprince *et al.*, 1994). In contrast, Carpenter *et al.* (1987) observed that PFK was highly sensitive to *in vitro* desiccation. In this regard, it is noteworthy that the cause of the differences in sensitivity among enzymes is unknown. More recently, there are reports of decline in the activity of malate dehydrogenase in recalcitrant *Antiaris toxicaría* seeds and orthodox *Zea mays* embryos during dehydration. In general, ROS (particularly superoxide and hydroxyl radicals) are damaging to essential cellular components such as DNA, proteins and lipids. The initial response to ROS production is at the level of plasma membrane. Lipid peroxidation disrupts the membrane integrity of the plant cell. Activation of peroxidation of lipids is one of the earliest response of plants against stress particularly drought stress. H^+ adenosine triphosphatases (H^+ ATPases) located on the plasma membrane is largely affected by the changes in the membrane lipids.These pumps establish an H^+ electrochemical potential across their respective membranes, which can be used as the driving force for secondary active transport. These enzymes maintain homeostasis inside the cell and thus help the cell to compact stress (Małgorzata Janicka-Russak, 2011). In this scenario, the aim of the present study is to evaluate the oxidative stress felt by the terrestrial fern forked fern –*Dicranopteris linearis* in response to dehydration rehydration stress, followed by determining the stress induced membrane lipid peroxidation and changes in the activity of plasma membrane bound H^+ ATPases enzyme.

Materials and methods:-

Plant material:-

Dicranopteris linearis (Burm.f.)Underw. was collected from Ponmudi Hills, Thiruvananthapuram, Kerala. The identification was confirmed by referring with the floras and authenticated by comparing with the voucher sample at Department of Botany, University of Calicut.

Dicranopteris linearis had characteristic forking stem that grows horizontally at ground level with stalked compound fronds. Main rachis was dichotomously branched to forms two secondary rachis, which also subsequently forks. At the end of the upward-growing, forked frond, there are two ultimate leafy branches of equal lengths which are deeply, pinnately lobed and comb-like. The sporangia, produced along the underside of the lobes of the ultimate two branches. The sporangia are non-indusiate with trilete spores. The fern was distributed all over the tropical and subtropical part of the world.

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. After the desiccation exposure a set of desiccated samples were subjected to rehydration for 30 min. The samples

were divided into two groups: desiccated and desiccated subsequently rehydrated. Control plants were maintained in an optimal water conditions in each case during the whole experimental period.

Quantification of superoxide anions ($O_2^{\cdot-}$):-
Superoxide anions were quantified, following the method of Doke (1983). 1 g tissue of the plant body was homogenized in a pre-chilled mortar using 50 mM sodium acetate buffer containing 10 mMNaCl (pH 6.5). The assay mixture was incubated for 30 min and the initial absorbance at 580 nm was taken in a spectrophotometer. Final absorbance was taken after heating the mixture at 85^{o}C for 15 min.

Quantification of hydrogen peroxide (H_2O_2):-
H_2O_2 concentration of the experimental tissues was estimated as per the procedure of Bellincampi *et al*., (2000) with some modifications. The assay mixture was incubated for 45 min and the absorbance of the Fe^{3+} xylenol orange complex at 560 nm was observed. Control was performed by eliminating the H_2O_2 in the reaction mixture without extract.

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). 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}.

Determination of H^+ATPase activity:-
Plasma membrane H^+ATPase was isolated from the leaf samples of *Dicranopteris linearis* under desiccation rehydration stress according to the protocol of Gallaghard and Leonard (1987). The released inorganic phosphate (Pi) was determined according to the method of Fiske and Subbarow (1925) .After termination the reactive solution was mixed with 0.1 ml of 1-amino1-naphthol-sulphonic acid(0.125% in 15% $NaHSO_3$ 1% Na_2SO_3). This mixture was shaken at 25^{o}C for 30min.The absorbance was recorded at 750 nm. The protein content of the enzyme was estimated by Bradford method (1976).

Results and Discussion:-

Accumulation of ROS in response to dehydration rehydration stress:-
Elevated production of ROS is one of the most deleterious effects of drought stress in plants. The measurement of ROS like H_2O_2, $O_2^{\cdot-}$ and lipid peroxidation are proven parameters of oxidative stress. Desiccation rehydration stress showed significant variation in their values in the plant at different periods of stress which indicates the extent to which desiccation affects the cellular metabolism. Polle, (2001) predicted a model of reactive oxygen production in the chloroplast i.e., H_2O_2 concentration increased linearly with increasing superoxide production in the photosynthetic electron transport chain. Similarly in *D.linearis* an increased concentration of both H_2O_2, $O_2^{\bullet-}$ in response to desiccation stress has been noticed. H_2O_2 content in *D. linearis* showed increase up to 10 d of desiccation when compared with control. H_2O_2 peak reached maximum at the 10 d (Table 1). This increase in H_2O_2 may be due to enhanced activities of NADPH oxidase and superoxide dismutase enzyme systems in the cells induced by desiccation stress (Oboznyi *et al*, 2013). Desiccation followed by rehydration of the samples also showed an increase in H_2O_2 content and interestingly the content was greater than respective period of desiccation (Table 1). The enhanced level reflects the severe stress felt by the plant when rehydrated after desiccation. Jin-Fen Wen *et al*, (2012) also reported H_2O_2 accumulation in cultured tobacco cells subjected to heavy metal cadmium stress.

Table. 1. Effect of desiccation stress on amount of H_2O_2 (µmol g^{-1} FW) in *D.linearis*
Abbrevations: D-Desiccated; R- Desiccated and subsequently rehydrated; H_2O_2 – hydrogen peroxide.
$P< 0.01$.

	Control	2 day D	2 day R	4 day D	4 day R	6day D	6 day R	8 day D	8 day R	10 day D	10 day R
H_2O_2	7.9± 0.02	8.46± 0.18	9.41± 0.52	10.42± 0.27	11.69± 0.09	15.39± 0.04	27.2± 0.81	31.86± 0.55	37.18± 0.34	45.22± 0.76	48.22± 0.07

Superoxide anion, a free radical released from molecular oxygen enzymically under desiccation stress condition was quantified spectrophotometrically by Nitro blue tetrazolium (NBT) method. The results showed a higher concentration of $O_2^{\cdot-}$ from the initial day of desiccation treatment up to the 10^{th} day when compared to the control (Table-2). Rehydration after respective period of desiccation showed higher concentration on superoxide radical indicating the oxidative stress in the plant due to desiccation treatment.

The active accumulation of $O_2^{\cdot-}$in the desiccated tissues provides a clear indication of abiotic stress (Torres *et al*, 2006). The observations suggest that $O_2^{\cdot-}$is not only a free radical, but also a signaling molecule. The rapid accumulation of $O_2^{\cdot-}$ in the tissue system is an indicator of physiological events, known as 'Oxidative burst'. Therefore, such plants, that express a tendency to produce $O_2^{\cdot-}$ at higher level should maintain an antioxidant system for reducing its content to a tolerable level.

Table.2. Effect of desiccation stress on amount of O_2.-(µg/g−1 FW) in D.linearis
Abbrevations: D-Desiccated; R- Desiccated and subsequently rehydrated; $O_2^{\cdot-}$ – superoxide radical.
$P < 0.01$.

	Control	2 day D	2 day R	4 day D	4 day R	6day D	6 day R	8 day D	8 day R	10 day D	10 day R
$O_2^{\cdot-}$	1.38± 0.07	2.05± 0.02	2.24± 0.08	5.68± 0.01	7.09 ± 0.05	6.73 ± 0.02	5.51 ± 0.04	8.89 ± 0.05	9.64± 0.03	10.43± 0.06	9.1± 0.01

Lipid Peroxidation :-

Lipid peroxidation was estimated by measuring the contents of malodialdehyde (MDA). Peroxidation of membrane in terms of polyunsaturated fatty acids is a sensitive diagnostic index of oxidative stress (Lin and Kao, 2000). Peroxidation of lipids in plant cells appears to be initiated by ROSs. The rate of lipid peroxidation in terms of MDA can be used a marker to evaluate the tolerance of plants to oxidative stress as well as the sensitivity of plants to stress.

The rate of lipid peroxidation was found to increase two fold during the initial stages i.e., 2 days of desiccation as indicated by the increased rate of MDA accumulation (Table-3). But, subsequently, a reduction in the MDA content was seen and it is maintained at par with the control plant up to the final day of desiccation. Rehydration after each period of desiccation showed values in correspondence with respective period of desiccations. The initial increase in peroxidation of lipids is due to the oxidation burst inside the plants induced as a result of immediate dehydration treatment. The decrease in lipid peroxidation during prolonged desiccation treatment indicates an effective anti-oxidative mechanism operating in the plant.

Table.3. Effect of desiccation stress on amount of lipid peroxidation (µmolg^{-1} FW) in *D. linearis*
Abbreviations: D-Desiccated; R- Desiccated and subsequently rehydrated. $P < 0.01$.

	Control	2 day D	2 day R	4 day D	4 day R	6day D	6 day R	8 day D	8 day R	10 day D	10 day R
MDA content	24.9± 0.17	45.2±0.23	51.8±0.12	23.9±0.36	24.8±0.07	17.6±0.22	19.9±0.16	21.09± 0.19	17.48±0.37	13.29±0.11	20.3±0.32

Regulatory effect of lipid peroxidation on H^+ATPase activity of plasma membrane:-

Plasma membrane bound H^+ATPase is a tightly bound integral membrane protein which helps to generate electro chemical proton gradient across the membrane. Thus, this enzyme play critical role in several physiological processes inside the plant and more over it help to resist stress under extreme conditions of stress. Here a regulation in H^+ATPase activity due to abrupt changes in the lipid peroxidation rate was noticed related with stress. The initial phases of desiccation up to 4 days showed an increased activity when compared with the control(Table-4). Prolonged desiccation resulted in almost a constant H^+ATPase activity at par with the control indicating the normal functioning of the plasma membrane even in the stressful conditions. The results suggest the presence of active anti-oxidative machinery helping the plant to tolerate the induced stress.

Table.4. Effect of desiccation stress on H^+ATPase activity (U/mg protein) in *D.linearis*
Abbreviations: D-Desiccated; R- Desiccated and subsequently rehydrated. $P < 0.01$.

	control	2 day R	4 day D	4 day R	6day D	6 day R	8 day D	8 day R	10 day D	10 day R	2 day D
H^+ATPase	0.131±0.09	0.346±0.12	0.25±0.07	0.228±0.02	0.197±0.11	0.174±0.13	0.198±0.11	0.155±0.09	0.157±0.08	0.124±0.17	0.128±0.12

The accumulation of superoxide anion, lipid peroxidation and differential expression of superoxide dismutase and catalase/peroxides activities in response to desiccation has been reported in neem seeds. (Varghese and Naithani, 2002).Desiccation tolerance studies in the recalcitrant seeds of *Trichilia dregeana* (Song-Quan *et al*, 2004) showed a correlation between increase in activities of antioxidant enzymes and decrease in lipid peroxidation rate. The production of ROS during desiccation has also been reported in the alga *Scytosiphon arbuscula* (Burritt *et al.*, 2002) and in the moss *Atrichum androgynum* (Mayaba *et al.*, 2002). Contreras-Porcia *et al*, 2011 studying the relationship between desiccation and the cascade of events involving oxidative stress, cellular changes, and physiological and biochemical responses in red alga *Poyphyra columbina* reported high levels of ROS quickly induced during desiccation and its rapid return to the basal level during rehydration. High rate of lipid peroxidation along with high H+ATPase activity has been observed in pea leaves under stress conditions suggesting a regulatory role of peroxidation of lipid membranes on plasmalemmal H+ATPase enzyme.(Veselov *et al*,2002).

Conclusion:-

Results obtained in this study confirmed the occurrence of oxidative stress in the fern due dehydration and subsequent rehydration treatments. Increased lipid peroxidation levels during the initial stages and its subsequent reduction during the final stages of treatment indicate the presence of multi-level ROS scavenging machinery in the plant system. More over the enhanced activity of plasma membrane bound H^+ATPase enzyme clearly indicate the adjustments made by the plant to maintain an equilibrium condition inside the cellular environment. The results obtained regarding the LPX and H^+ATPase enzyme activity provides an insight into the desiccation tolerance of the plant by minimizing the injury due to oxidative burst.

Acknowledgement:-

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. **B Varghese , S C Naithani**. Desiccation-induced changes in lipid peroxidation, superoxide level and antioxidant enzymes activity in neem (*Azadirachta indica* A. Juss) seeds Acta Physiologiae Plantarum2002; 24(1): 79-87.
2. **Bellincampi D, Dipierro N, Salvi G, Cervone F, De Lorenzo G**. Extracellular H_2O_2 induced by oligogalactouronides is not involved in the inhibition of the auxin regulated ro 1B gene expression in tobacco leaf plants.Plant Physiology 2000;122:1379-1385
3. **Bradford M A** .Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemisty1976;72 :248-254.
4. **Burritt D J, Larkindale J, Hurd K**. Antioxidant metabolism in the intertidal red seaweed *Stictosiphonia arbuscula* following desiccation. Planta 2002; 215:829–838. [PubMed]
5. **Carpenter J F, Crowe L M, Crowe, J H.** Stabilization of phosphofructokinase with sugars during freeze-drying: characterization of enhanced protection in the presence of divalent cations. Biochem. Biophys. Acta1987; 923:109-115.
6. **Doke N**. Involvement of super oxide anion generation in the hypersensitive response of potato tuber tissue to infection with an incompatible race of *Phytophthora infestance* and to the hyphal wall components.Physiological Plant pathology1983; 23 : 345 – 357.
7. **Fiske C , Subbarow Y J**. The colorimetric determination of phosphorus. Biol. Chem1925; 66: 375-400.
8. **Gallagher S R, Leonard R T**. Electrophoretic characterization of a detergent-induced plasma membrane fraction from maize roots. Plant Physiology 1987; 83: 265-271.
9. **He J X, Wang J, Liang H G**. Effects of water stress on photochemical functions and protein metabolism of photosystem II in wheat leaves. Physiol. Plant1995; 93:771-777.

10. **Jin-Fen Wen, Ming-hua Deng and Ming Gong**. Cd^{2+} stress induces two waves of H_2O_2 accumulation associated with ROS-generating system and ROS-scavenging system in cultured tobacco cells. Australian Journal of Crop Science 2012; 6(5):846-853.
11. **Leprince O, Vertucci C W, Hendry Gaf, Atherton N M.** The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature. Physiol. Plant. 1995; 94: 233-240.
12. **Lin CC and Kao CH** .Effect of NaCl stress on H2O2 metabolism in rice leaves. Plant Growth Regulation 2000;30:151-155.
13. **Loretto Contreras-Porcia, Daniela Thomas, Verónica Flores, Juan A. Correa**.Tolerance to oxidative stress induced by desiccation in *Porphyra columbina*(Bangiales, Rhodophyta). J Exp Bot. 2011; 62(6): 1815–1829.
14. **Małgorzata Janicka-Russak**.Plant Plasma Membrane H+-ATPase in Adaptation of Plants to Abiotic Stresses, Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives, In Tech, 2011.
15. **Mayaba N, Minibayeva F, Beckett R P**. An oxidative burst of hydrogen peroxide during rehydration following desiccation in the moss. *Atrichum androgynum*. New Phytology.2002;155:275–283.
16. **Ntuli T M, Finch-Savage W E, Berjak P, Pammenter N W**. Increased drying rate lowers the critical water content for survival in embryonic radicles of English oak (*Quercus robur* L. J. Integr. Plant Biol. 2011; 53: 270-280.
17. **Oboznyi A I, KolupaevYu E, Vayner A A, Yastreb T O**. The Role of Superoxide Dismutase in Inducing of Wheat Seedlings Tolerance to Osmotic Shock. Journal of Stress Physiology & Biochemistry 2013; 9(3): 251-261
18. **Polle A**. Dissecting the superoxide dismutase–ascorbate–glutathione pathway in chloroplasts by metabolic modelling. Computer simulations as a first step towards flux analysis. Plant Physiology 2001;126:445–462.
19. **Toivonen P, Vidaver W** .Variable chlorophyll a fluorescence and CO_2 uptake in water stresses white spruce seedlings. Physiol. Plant.1988; 86:744-748.
20. **Torres M A, Jones J D, Dangl J L**. Reactive oxygen species signalling in response to pathogens. Plant Physiology 2006; 141 : 373–378.
21. **Van Breusegem F, Vranová E, Dat JF, Inzé Dirk** .The role of active oxygen species in plant signal transduction. Plant Sci. 2001;161:403-414.
22. **Veselov A P,Kurganova L N,Likhacheva A V,Sushkova A.** Possible regulatory effect of lipid peroxidation on H^+ ATP ase activity of plasmalemma under stress conditions. Russian Journal of plant physiology. 2002; 49(3):344-348.
23. **Zhang J X, Kirkham M B**. Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. Plant Science 1996; 113: 139-147.