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RESEARCH ARTICLE

REACTIVE OXYGEN OR NITROGEN SPECIES (ROS/ RNS) AND RESPONSE OF ANTIOXIDANTS AS SCAVENGERS DURING BIOTIC STRESS IN PLANTS: AN OVERVIEW

Lubaina, A.S.[1] and K. Murugan[2*]
[1]Department of Botany, Christian College Kattakada.
[2]Plant Biochemistry and Molecular Biology Laboratory, Department of Botany,
University College, Thiruvananthapuram, Kerala 695 034, India.

ABSTRACT

Phytopathogens have evolved diverse independent and complex modes of penetrating and accessing plant cellular contents. The synthesis of reactive oxygen or nitrogen species (ROS/RNS) by the utilization of molecular oxygen during plant–pathogen interactions results in to oxidative burst, a signaling cascade to defense. ROS array includes singlet oxygen, the hydroxyperoxyl radical, the superoxide anion, hydrogen peroxide, the hydroxyl radical and the closely related reactive nitrogen species, nitric oxide. ROS acts synergistically directs to signal amplification to drive the hypersensitive reaction (HR) and initiates systemic defenses. The role of ROS in successful pathogenesis, it is ideal to inhibit the cell death machinery selectively and simultaneously to monitor other defense and pathogenesis-related processes. With the understanding of the interplay underlying the localized activation of the oxidative burst following perception of pathogen avirulence signals and key downstream responses including gene activation, cell death, and long-distance signaling, novel strategies will be developed for engineering enhanced protection against pathogens by manipulation of the oxidative burst and oxidant-mediated signal pathways. In this over review, it is reported the different roles of ROS/RNS in host–pathogen interactions with example on *Alternaria- Sesamum* interaction.

Keywords: Antioxidants, ROS, RNS, plant, pathogen, defense, oxidative burst.

1. INTRODUCTION

Plant diseases caused by biotic stresses such as bacteria, fungi, viruses, or nematodes forms the major concern in agriculture leads to economic loss of crop productivity. The increasing human population needs an increase in agricultural production. This challenge is made difficult by the fact that changes in the environmental conditions under which the crops grown have resulted in the appearance of diseases. Similarly, drastic genetic changes within the pathogen have resulted in the emergence of new plant diseases. To meet this challenge, study of plant-pathogen interactions in terms of physiology, biochemistry and molecular level is a pre-requisite to uncover the mechanisms by which disease resistance is achieved. Plant pathogens include a wide array of organisms such as fungi, bacteria, oomycetes and viruses. Pathogens have evolved different strategies to invade a host, as well as to feed on and multiply in the plant. Biotrophic pathogens need living tissue for growth and multiplication, in course of time, the tissue will die so that the pathogen becomes hemi-biotrophic. On the other hand, necrotrophic pathogens kill the host tissue at the initial stage of the infection and utilize the dead tissue. Bacteria and fungi show both bio-trophic as well as necrotrophic strategies. The jasmonate/ethylene pathway is more important in defending necrotrophic pathogens while salicylic acid dependent responses are more effective against biotrophic pathogens (1).

Exploring the interplay between plants and pathogens can lead to develop strategies to control or minimize the impact of pathogenicity on the host. Pathogen infection leads to alter the primary and secondary metabolism which in turn tunes defense strategies as well as growth and development of the host plant. The regulation of defense responses has been intensively studied for decades but less is known about the interplay between the pathogen infection and reactive oxygen and nitrogen cycles. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are linked to signaling in both developmental and stress responses. Currently, interest in this research area has been growing in terms of biochemistry of antioxidant machinery and source-sink regulation in different types of plant-pathogen interactions. Further, the plant pathogen studies started analyzing the physiological status of the infected tissues to elucidate the infection mechanisms.

Plant-pathogen interaction is a multifaceted event, regulates pathogen ingress and establishment of disease. Studies revealed that plants and

*Correspondence: K. Murugan, Associate Professor and Head, Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Thiruvananthapuram, Keral. E-mail.: harimurugan@gmail.com

pathogens communicate with each other in a conversation through reactive oxygen species (ROS) and reactive nitrogen species (RNS) signalling network. Induction of ROS and RNS are the earliest observable manifestations of a plant defence strategy orchestrated by ROS and RNS gene network. The interplay between ROS and RNS production and their scavenging during plant pathogen interaction appears to be more and more complex. In this scenario, the ROS and RNS cycle seems to have an increasing importance. Despite this significance, there is still a lacuna to dissect the identities, activities and relative importance of the ROS and RNS generating system in host pathogen interaction.

The level of ROS and RNS are controlled by both production and removal through various scavengers including ascorbic acid and glutathione. Ascorbic acid and glutathione are central components in regulating the redox balance of the plant cell. It has become increasingly clear that signalling pathways in plants are not organized into linear pathways; instead, as a web of interactions. Not even individual ROS and RNS give uniform responses; instead, separate molecules (hydrogen peroxide, superoxide, singlet oxygen, and nitric oxide), acting at different subcellular locations give rise to unique changes in gene expression (2). ROS and RNS production and their scavenging are intimately linked, and the balance between them will determine defence signalling output as well as damage and cell-death responses. An active phase of enzymatic ROS and RNS scavengers (including catalase, superoxide dismutase, and ascorbate peroxidase) and low-molecular-weight non enzymatic scavengers (ascorbate, glutathione, and α-tocopherol) protect plants from excessive ROS and RNS production. Ascorbate and glutathione are connected through the ascorbic acid-glutathione cycle (3) and are essential for plants. The ROS and RNS -mediated plant response is variable and depends on the pathogen life style (biotrophy versus necrotrophy), the type of plant–pathogen interaction (compatibility versus incompatibility) and the stage of plant development. Thus, the tightly regulated balance between ROS and RNS production and its removal at the cellular and subcellular levels seems to be of primary importance for fulfilling the multiple functions of ROS and RNS controlling redox homeostasis.

Analysis of the host parasite relationships reveals the pattern of pathogenesis and the defence mechanisms exhibited by the plants challenged by the pathogen. The induction of defence mechanism may be either specific or non-specific. Perception of a pathogen by a plant triggers rapid defense responses via multiple signalling pathways that lead to the induced expression of genes encoding pathogenesis-related (PR) proteins and enzymes involved in the production of secondary metabolites and hormones (4). In many host-parasite interactions, substances such as phenolic compounds, phytoalexins, tannins and some fatty acid like compounds, which are potent inhibitors of many hydrolytic enzymes form the basis of resistance. Plant cells contain variable amounts of hydrolytic enzymes such as glucanases and chitinases that cause breakdown of cell wall components of the pathogen.

Biotic elicitors induced several dynamic defense mechanisms that act as physical and chemical barriers, which prevent further colonization or spread of the pathogen. The receptor-proteins located in cell membrane detect the pathogen or the factor translocated by pathogens. Plants have evolved sophisticated biochemical mechanisms to exert self-defense against pathogen infections. The rapid and transient production of ROS and RNS induce oxidative burst is one of the earliest plant cell responses following pathogen recognition and is involved in cell wall strengthening via cross-linking of glycoproteins and induction of the hypersensitive response. The predominant types of ROS and RNS detected in plant pathogen interaction include $O_2^{\bullet -}$, H_2O_2, OH^- and NO (5). Enzymes such as NADPH oxidase and superoxide dismutase are responsible for the formation of reactive oxygen species. Increased synthesis and activity of phenylalanine ammonia lyase (PAL) has been reported in the plants against fungal and bacterial pathogens as active defense response. PAL plays key role in the synthesis of phenols, phytoalexins and lignin (6). The effectiveness of resistance depends on speed and amount of synthesized products and their movements to neighbouring healthy tissues to create defensive barriers. Due to the entry of the pathogen, a rapid and temporary increase in cellular metabolic activities capable of triggering hypersensitive cell death. The induced resistance offered by biochemical changes in host plants is the last line of host defence, which includes activation of lignin synthesis, enhanced activity of several antioxidant enzymes and suitable changes in plant metabolism.

2. *Antioxidant systems vis-a-vis reactive oxygen species (ROS) and reactive nitrogen species (RNS) during plant - pathogen interaction*

Plant resistance to pathogens requires the induction of complex metabolic pathways in the infected tissues, focused at recognizing pathogen and inhibiting its multiplication within the host plant tissues. In spite of compatible and incompatible

reactions induce alterations in plant metabolism, only incompatible reactions in the plant is able to efficiently inhibit pathogen invasion without substantial damage. The common incompatible responses is hypersensitive response (HR), in which cells surrounding the pathogen invasion switch on genes encoding for phenyl propanoid metabolism and other pathogenesis related proteins before activating programmed cell death (PCD) (3).

In plants, ROS continuously produced as by-products of different metabolic pathways compartmentalized in different cellular organelles. Plants also produce ROS, by inducing various oxidases and peroxidases, which in turn produce ROS in response to environmental challenges. The major sites of ROS production in cell systems include chloroplast, mitochondrion, peroxisome, endoplasmic reticulum, plasma membrane and cell wall.

2.1. Singlet Oxygen ($^1O_2$)

One of the most reactive forms of oxygen, the singlet oxygen generate by an input of energy that rearranges the electrons in the molecule. In singlet oxygen, the electron spins are opposed in a higher energy state and is many times more reactive than triplet oxygen. Singlet oxygen is the common name used for the two metastable states of molecular oxygen (O_2), with higher energy than the ground state. In both forms of singlet oxygen, if the spin restriction removed then the oxidizing ability is greatly increased. Singlet oxygen can directly oxidize biomolecules like DNA and protein.

2.2. Superoxide radical or Superoxide radical anion ($O_2^{\bullet-}$)

Supplying of single electron to O_2, it enters one of the (π_x^*) antibonding orbitals leading to the formation of superoxide radical. Membrane bound NAD(P)H oxidase (NOX) that generate the super oxide anions ($O_2^{\bullet-}$) using NADPH as the electron donor.

$$O_2 + NADPH \rightarrow O_2^{\bullet-} + NADP^+ + H^+$$

2.3. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is a relatively stable ROS being not very reactive and electrically neutral, is able to pass through cell membranes and reach cell locations remote from the site of its formation. Together with $O_2^{\bullet-}$ it can be converted to hydroxyl radicals by the Haber-Weiss reaction. Superoxide dismutase (SOD) enzymes are responsible for H_2O_2 production by dismutation reaction of $O_2^{\bullet-}$.

Free radicals / ROSs have signalling function mediating defence gene activation and establishment of additional defences, by redox control of transcription factors or by interaction with other signalling components like phosphorylation cascades. ROS can generate lipid derivatives by non-enzymatic oxygenation that can produce membrane damage or function as signalling molecules like cyclic oxylipins of the jasmonate type. Similarly, these molecules can activate the generation of phytoalexins or other secondary metabolites that arrest pathogen growth and also in terms of lignification (7).

Moreover, H_2O_2 also block pathogen invasion in plant cells because it contributes to wall strengthening by activating peroxidase reactions via intra- and inter-molecular cross-links between cell wall components and lignin polymerization. As H_2O_2 is a diffusible molecule in biological membranes, it also acts as intracellular signal regulate ion flux across the membrane (Ca^{2+} influx and K^+, Cl^- efflux) as well as changes in *p*H and plasma membrane depolarization.

2.4. Nitric oxide (NO)

Under biotic stress, plant cells exhibit a rapid synthesis and accumulation of reactive nitrogen species known as nitric oxide and these response trigger a programmed cell death (PCD) process leading to an intrinsic execution in plant cells. PCD is an integrated cellular process occurring in plant defense responses to facilitate normal growth and development and better survival against various stresses as a whole. Both NO and ROS play key roles in PCD. These redox active small molecules can trigger cell death either independently or synergistically. Nitric oxide and H_2O_2 reciprocally enhanced the production of each other whereas NO and $O_2^{-\bullet}$ showed reciprocal suppression on each other's production. It was the interaction between NO and $O_2^{-\bullet}$but not between NO and H_2O_2 that induced PCD, probably through peroxynitrite ($ONOO^-$). Nitric oxide and reactive oxygen species are major players of biotic interactions in plants and are crucial components of plant immunity. Besides their role in plant defence response they have been demonstrated to be involved in symbiotic interactions between plants and microorganisms (8). In plants, nitric oxide could also function as a signal molecule in the transduction pathway leading to the induction of defence responses against pathogens and in damage leading to cell death.

2.5. Antioxidant (AOX) machinery and defence against phytopathogens

In plant cells, enzymes and redox metabolites act in synergy to detoxify ROSs. SOD dismutates $O_2^{\bullet-}$ to H_2O_2, catalase (CAT)

peroxidatively cleaves H_2O_2 to oxygen and water, and ascorbate peroxidase (APX) reduces H_2O_2 to water by utilizing ascorbate (ASC) as electron donor. These are the major enzymatic AOX systems for protecting cells against oxidative burst (Fig. 1). Most of the AOX enzymes are isoenzymatic and their expression is genetically regulated by developmental and environmental stimuli, accordingly ROS cycle will be regulated in the cells (7).

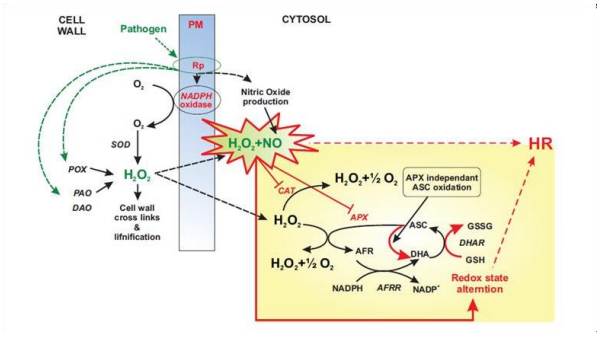

Fig. 1. ROS cycle induced during pathogen stress producing reactive oxygen and/or nitrogen species leading to oxidative burst and induction of antioxidant system

APX- ascorbate peroxidase, ASC- ascorbate, AFR- ascorbate free radical, AFRR- ascorbate free radical reductase, CAT- catalase, DAO- aiamine oxidase, DHA- dehydro ascorbic acid, DHAR- dehydro ascorbic acid reductase, GSH- glutathione, GSSG- glutathione disulfide, HR- hypersensitive response, NO- nitric oxide, PAO-polyamine oxidase, PM- plasma membrane, POX- peroxidase, Rp- receptor proteins, SOD- superoxide dismutase.

Glutathione (GSH) forms the ubiquitous antioxidants present in plant cells and play pivotal role in plant defence activation. Depletion of GSH or increase in its oxidised form, glutathione disulfide (GSSG), induces accumulation of phytoalexins (3). Up regulation of GSH content has been reported in leaves attacked by avirulent biotrophic pathogens. It is also reported that an increase in the expression of glutathione 5-transferase and glutathione peroxidase in the cells near to hypersensitive cell death region induced by an avirulent phytopathogen (9). H_2O_2 accumulation and GSH oxidation occur in different sites and with different timing in leaves of resistant barley line attacked by powdery mildew (10). Decline in GSH content was noticed in tomato infected with the *Botrytis cinerea* (11) and in *Avena sativa* infected with virulent necrotrophic fungus (12). Both these cases, the decline in antioxidant defence could increase necrotic spots that facilitate the penetration of necrotrophic phytopathogens. Cotyledons of tomato carrying Avr genes and injected with race-specific elicitors of *Cladosporium fulvum,* showed an enhanced profile of GSH pool in the oxidised form (13). GSH responsive elements have been reported as promoters of phenylalanine ammonia lyase (PAL) and chalcone synthase (7). GSH acts in synergy with other signals in the induction of defence strategies has been reviewed on phytopathogen induced diseases in *Arabidopsis* mutants having GSH levels 70% lower than the wild type parental ecotype. The infection of the *Arabidopsis* mutant with either virulent or avirulent fungal and bacterial pathogens gives the same responses with wild type (14). A probable explanation of these findings could be GSH compensation with other antioxidant molecules and enzymes. Indeed, the ASC levels are higher in the mutant than in the wild type. Compensation between GSH and thioredoxin-glutaredoxin systems has also been reported in lower organisms also (15).

In the ascorbate-glutathione system suppression of APX is required in tissues undergoing HR. Similarly decrease in CAT activity has also been reported in cells undergoing HR. However, the mode of suppression of these scavenging enzymes is dissimilar (3). CAT is down-regulated at the transcription level, whereas APX regulation in HR involves changes both at the transcription and translation or post-translation levels. Tobacco mosaic virus (TMV) infection of tobacco enhance mRNA expression of APX probably as an antioxidant response triggered by increasing of H_2O_2 within cells similar to that activated under other environmental constraints (16). In spite of the increase in its expression, the APX activity is strongly suppressed in the TMV-infected tissues by a mechanism that acts at the transcriptional or post-transcriptional level. The transcriptional / post-transcrip-tional regulation of APX is unique to HR-related incompatible response and indicates the necessity of accumulating ROS during this defence process. Meanwhile, it has been also reported that in a compatible reaction between barley and powdery mildew the cytosolic APX isoenzyme is up-regulated in epidermal and mesophyll cells. In spite of this, APXs are unable to block the pathogens, its increase limits the propagation of oxidative processes permitting cells to maintain their viability, a condition required for the penetration of biotrophic pathogens in to the plant tissues. This up-regulation of APX confirms previous results of enhancement in APX activity during successful infection of barley leaves by biotrophic compatible pathogens and in leaves of susceptible apricot infected by plum pox virus (17).

Ascorbate-glutathione redox enzymes and ASC/DHA levels play significant roles against plant pathogens but still the available data are

fragmentary. However, an increase in ascorbate free radical reductase, the enzyme responsible for the reduction of ascorbate oxidation, seems to occur in the compatible reactions, thus mimicking the behaviour of APX (17).

Similarly, the activities of ascorbate recycling enzymes such as dehydro ascorbate reductase, that reduces dehydroascorbate to ascorbate using GSH as reductant, and the enzyme GSSG reductase not clearly indicative of resistance or susceptibility in the host cells (3). This is probably due to multiple intrinsic factors such as plant species specific sensitivity, plant pathogen interactions, the suppression or strengthening of ROS detoxification that regulate ascorbate-glutathione interplay and the corresponding redox state (biosynthesis, oxidation pathways and recycling enzymes).

Further, increasing focus has also been given to reactive nitrogen species (RNS) such as nitric oxide (NO) as a signal molecule involved in plant pathogen interaction (18). Results obtained with cultured tobacco cells, in which hypersensitive programmed cell death (PCD) is induced by simultaneous treatments with NO and H_2O_2 generators, indicate that suppression of APX and decrease in the ascorbate (ASC + DHA) and glutathione (GSH + GSSG) pools, the key events in PCD. Moreover, during the HR process, redox balances of ASC and GSH are strongly shifted towards the oxidized forms (Fig. 1). These changes in the cellular antioxidant systems are not only because of NO and H_2O_2, but also due to other intrinsic factors (19). Similarly, when programmed cell death was blocked by treatment with protein synthesis inhibitors in the tissues leads to the induction of HR with simultaneous generation of NO and H_2O_2. Parallely, the inhibition of APX and the decrease in ASC and GSH pools are also reversed (19). These results suggests that changes in the cellular redox balance are not as a consequence of disease impacts but part of the transduction signalling cascade that induces defense responses under the opportunate stimuli. Further, like GSH/GSSG pair, the pool and redox state of ascorbate play regulatory role in plant metabolism, both acting at the level of gene expression and also with enzymatic pathways (18). Thus, ROSs are secondary messenger cascades in stress responses and their level or redox status of the cell dictates the response types i.e., high level leads to cell death whereas low initiate defence genes and ends in adaptive responses. ROS sources and complex regulatory antioxidant systems provide the flexibility necessary to allow the dual role of ROS.

Free radicals like superoxide anions, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), RNS like NO are potential reactive molecule produced in the cell as by-products of normal cellular metabolism or under biotic stress. During plant-pathogen interactions ROS and RNS are generally involved in stimulating plant defense genes encoding pathogenesis-related (PR) proteins or regulating synthesis of secondary metabolites or/and genes encoding ROS and RNS scavenging enzymes.

ROS and RNS regulatory mechanisms at the biochemical level especially the communication and interplay across cellular organelles are still poorly documented. Due to the central role of ROS and RNS as signalling cascades, it is essential to obtain a comprehensive knowledge of the synthesis and regulation of ROS and RNS during plant-pathogen interaction. This adds additional information towards physiological, molecular and evolutionary research perspectives in phytopathology.

In this scenario, the present study aims to unravel the biochemical mechanism of ROS cycle in *Sesamum orientale* L. against *Alternaria* leaf spot disease in sesame. As an initial part conidium germination, inoculation, penetration and colonization of the pathogen on the plant surfaces were studied using scanning electron microscopy. Transmission electron microscopy studies showed structural changes in the chloroplast and mitochondria of diseased plants. Changes in different biochemical parameters in the diseased sesame plants were compared to control. Meanwhile the activity of different antioxidant enzymes such as catalase, peroxidase polyphenol oxidase and superoxide dismutase in diseased plants showed remarkable levels compared to control. Due to the infection, chlorophyll content, carbohydrates and total soluble protein decreased whereas free amino acid, proline, phenols and disease-related proteins increased in the host plants. Lubaina and Murugan, (20) reported the ultra-structural changes and oxidative stress markers appeared in *S. orientale* cultivar – Thilarani following *Alternaria sesami* infection. Subsequently Lubaina and Murugan (21) published the biochemical changes during oxidative burst in this particular plant pathogen interaction. To date no information is available on the involvement of the AsA-GSH cycle in *Sesamum orientale* L. against *Alternaria sesami*. The implication of ROS cycle and ascorbate–glutathione interplay in signaling and stress responses of the oxidative stress in sesame - Alternaria interactions was also well documented (22). The ROS such as H_2O_2 formed can be detoxified via the ascorbate–glutathione cycle. Increases in ascorbate peroxidase, and glutathione reductase activities concomitant with ascorbate (AsA) and glutathione interplay, as well as AsA regeneration ability, function to keep the

balance of cellular H_2O_2 under pathogenicity. Dehydroascorbate reductase and monodehydroascorbate reductase are responsible for AsA regeneration. Oxidative damage in Thilarani is attributed by a lower induction of the ascorbate – glutathione cycle as an antioxidant defense system and were not sufficient to protect the ultrastructural damage of chloroplasts and mitochondria. Overall, the availability of antioxidants and the induction of antioxidant enzyme activities for detoxifying reactive oxygen species (ROS) are not regulated effectively in sesame against *A.sesami* induced oxidative stress. The experiments using ROS scavengers demonstrate that the antioxidant defense system is modulated by $O_2^{\bullet-}$ or H_2O_2 signals.

REFERENCES

1. Thomma, B.P.H.J., I.A. Penninckx, W.F. Broekaert and B.P. Cammue, (2001). The complexity of disease signalling in Arabidopsis. *Curr. Opin. Immunol*. **13**: 63-68.
2. Wrzaczek, M., M. Brosche, J. Salojarvi, S. Kangasjarvi, N. Idanheimo, M. Mersmann, *et al.* (2010). Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in *Arabidopsis*. *BMC Plant Biol*. **10**: 95-99.
3. Bhattacharjee, S. (2012). The language of reactive oxygen species signalling in plants. *J. Bot.* **6**: 1-22.
4. Leon, I.P.D. and M. Montesano, (2013). Activation of defense mechanisms against pathogens in mosses and flowering plants. *Int. J. Mol. Sci.***14**: 3178-3200.
5. Torres, M.A., J.D. Jones and J.L. Dangl, (2006). Reactive oxygen species signalling in response to pathogens. *Plant Physiol*. **141**: 373-378.
6. Dixon, R.A. and N.L. Paiva, (1995). Stress-induced phenyl propanoid metabolism. *Plant Cell.* **7**: 1085-1097.
7. De Gara, L., M.C. de Pinto and F. Tommasi, (2003). The antioxidant systems vis-a-vis reactive oxygen species during plant-pathogen interaction. *Plant Physiol. Biochem*. **41**: 863-870.
8. Wang, Y., G.J. Loake and C. Chu, (2013). Cross-talk of nitric oxide and reactive oxygen species in plant programed cell death. *Front Plant Sci*. **4**: 314.
9. Kaur, P., R. Jost, K.P. Sivasithamparam and M.J. Barbetti, (2011). Proteome analysis of the *Albugo candida-Brassica juncea* pathosystem reveals that the timing of the expression of defence-related genes is a crucial determinant of pathogenesis. *J. Expt. Bot.* **62**: 1285-1298.
10. Vanacker, H., T.L.W. Carver and C.H. Foyer, (2000). Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during hypersensitive response in the barley-powdery mildew interaction. *Plant Physiol*. **123**: 1289-1300.
11. Kuzniak, E. and M. Sklodowska, (2005). Compartment-specific role of the ascorbate-glutathione cycle in the response of tomato leaf cells to *Botrytis cinerea* infection. *J. Expt. Bot.* **56**: 921-933.
12. Gonnen, M.V. and E. Schlosser, (1993). Oxidative stress in interaction between *Avena sativa* L. and *Drechlera* spp. *Physiol. Mol. Plant Pathol*. **42**: 221-234.
13. May, M.J., K.E. Hammond-Kosack and J.D.G. Jones, (1996). Involvement of reactive oxygen species, glutathione metabolism, and lipid peroxidation in the Cf-gene dependent defence response of tomato cotyledons induced by race specific elicitors of *Cladosporium fulvum*. *Plant Physiol*. **110**: 1367-1379.
14. May, M.J., J.E. Parker, M.J. Daniels, C.J. Leaver and S. Christopher, (1996). An *Arabidopsis* mutant depleted in glutathione shows unaltered responses to fungal and bacterial pathogens. *Mol. Plant Microb. Int*. **9**: 349-356.
15. Lemaire, S.D. and M.M. Maslow, (2004). The thioredoxin superfamily in *Chlamydomonas reinhardtii. Photosynth. Res*. **82**: 203-220.
16. Mittler, R., E. Lam, V. Shulaev and M. Cohen, (1999). Signal controlling the expression of cytosolic ascorbate peroxidase during pathogen-induced programmed cell death in tobacco. *Plant Mol. Biol.* **39**: 1025-1035.
17. Hernandez, J.A., J.M. Talavera, P. Martinez-Gomez, F. Dicenta and F. Sevilla, (2001). Response of antioxidative enzymes to plum pox virus in two apricot varieties. *Physiol. Plant.* **111**: 313-321.
18. Mendoza, M. (2011). Oxidative burst in plant-pathogen interaction. *Biotechnol. Vegetal*. **11**(2): 67-75.
19. De Pinto, M.C., F. Tommasi and L. De Gara, (2002). Changes in the antioxidant systems as part of the signalling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco bright-yellow 2 cells. *Plant Physiol*. **130**: 698-708.
20. Lubaina, A.S. and K. Murugan, (2013). Ultrastructural changes and oxidative stress markers in wild and cultivar *Sesamum orientale* L. following *Alternaria sesami* (Kawamura) Mohanty and Behera. Inoculation. *Indian J. Expt. Biol*. **51**: 670-680.
21. Lubaina, A.S. and K. Murugan, (2013). Biochemical characterization of oxidative burst during interaction between sesame (*Sesamum indicum* L.) in response to *Alternaria sesami*. Prospects in Biosci. Addressing the Issues, Springer, **28**: 243-250.
22. Lubaina, A.S. and K. Murugan, (2016). Reactive oxygen species and ascorbate-glutathione interplay in signaling and stress responses in *Sesamum orientale* L. against *Alternaria sesami* (Kawamura) Mohanty and Behera. *J. Saudi Soc. Agri. Sci.* **15**: 48-56.