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Role of Oxidative Burst in Plant-Pathogen Interaction and Antioxidants Defenses

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The oxidative burst is a rapid and transitory process that is production of enormous amounts of reactive oxygen species (ROS). ROS is one of the earliest noticeable aspects of a plant's defense approach. First this Appraisal describes the chemistry of ROS (superoxide radical, hydrogen peroxide and hydroxyl radical). The oxidative burst is a process that plant's show early response to a pathogen attack and is characterized by the rapid production of reactive oxygen species (ROS). This response is one of the most well-characterized ways that plants defend themselves against pathogens. The high concentrations of ROS can overthrow a plant's antioxidant defenses and damage the pathogens. The oxidative burst can trigger a number of defense responses, including the production of phytoalexins, the induction of defense-related genes and the hypersensitive response (HR). An oxidative burst is an early response of plants to various biotic/abiotic stresses. In plant-microbe interactions, the plant body can induce oxidative burst to activate various defense mechanisms to combat phytopathogens. A localized oxidative burst is also one of the typical behaviors during hypersensitive response (HR) caused by gene-for-gene interaction. The oxidative burst can cross-link cell wall proteins, which can help build up the plant's defense. The oxidative burst can trigger programmed cell death in tested cells. The oxidative burst can signal adjacent cells to produce cellular protectants.

Keywords: Oxidative burst, ROS, antioxidant defenses

Introduction

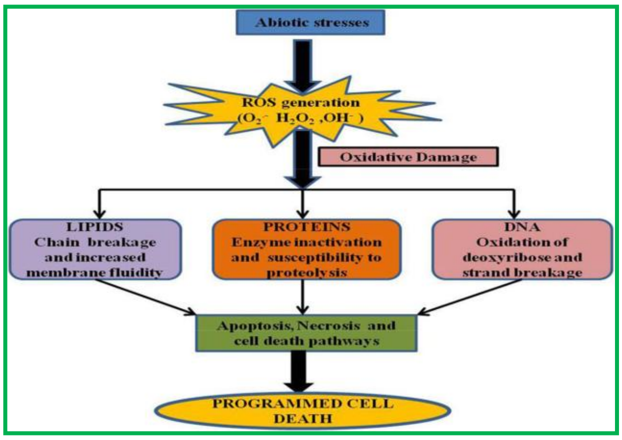
Oxidative burst, also known as respiratory burst, is the rapid production of reactive oxygen species (ROS) in response to external stimuli. It's an important part of the host defense system against pathogens. Oxidative burst is an early defence response in plants to pathogens. It involves the production of ROS, such as hydrogen peroxide, superoxide radical, and hydroxyl radical. These ROS can have both beneficial and harmful effects, such as regulating metabolism and signalling, and inducing hypersensitive responses.

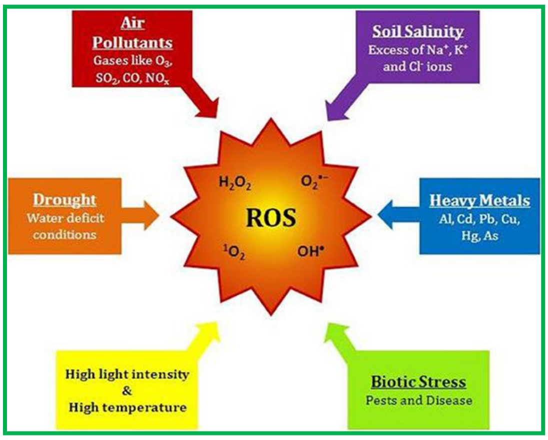
What is Oxidative Burst

The rapid release of reactive oxygen species (ROS) by cells in response to external stimuli is known as oxidative burst. This reaction includes the production at the cell surface of different molecule such as: hydrogen peroxide (H_2O_2), superoxide (O_2), singlet oxygen (O_2) and hydroxyl radical (OH^-). The production of reactive oxygen species (ROS) *via* consumption of oxygen in a so-called oxidative burst is a hallmark of successful recognition of infection and activation of plant defenses. ROS are not only toxic by-products of aerobic metabolism, but are also signaling molecules involved in several developmental processes in all organisms. Specifically, against microorganisms a sophisticated sensory system enables them to perceive chemical signals from potential pathogens and to translate them into appropriate biochemical responses. The oxidative burst has been known for more than 30 years in mammals from studies on the 'respiratory burst' in phagocytes (Wientjes and Segal, 1995), however, in plants the phenomenon was demonstrated much later (Doke, 1983). In biological systems 'oxidative stress' results from the presence of elevated levels of oxidizing agents that are able to abstract electrons from essential organic molecules and disturb cellular functions. Under normal conditions ROS appear in cells as unwelcome harmful by-products formed as a result of successive one-electron reductions of molecular oxygen (Ryter *et al.*, 2007). As a consequence of disturbances in the normal redox state of the cell ROS molecules are produced, which have a toxic effect on the cells. Most plant cells possess facing an even greater burden of ROS has the ability to detoxify it and also acquired the relevant protective mechanism to maintain the lowest possible levels of ROS inside. To these protective mechanisms belong some antioxidant molecules (átocopherol, ascorbate (ASC), glutathione (GSH), proline, betaine and carotenoids) and antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Torres et al., 2006).

Role and Mechanism of Oxidative Burst (Respiratory Burst)

Oxidative burst acts as a resistance mechanism to the pathogen infection in plants. This is seen post PAMPs detection by cell-surface located receptors (e.g. FLS2 or EFR) (Jabs T *et al.* 1997). In plant immunity, the NADPH oxidase subunits RbohD and RbohF have corresponding functions are conveyed in different tissues and at different levels (Morales *et al.*, 2016). Consequently, generated ROS bear additional effects alongside pathogen toxicity. Hydrogen peroxide induces oxidative cross-linking of the plant's cell wall glycoproteins (Wojtaszek et al., 1995). This reduces susceptibility to enzymatic degradation by pathogens. Systemic acquired resistance, which is analogous to innate immunity in animals, is also induced in the exposed plant cells. Hydrogen peroxide exposure may also result in hypersensitive response, which is the death of a small number of host cells at the site of infection, for the purpose of limiting pathogenic infection (Levine *et al.*, 1994). ROS production in plants can be used as a readout for successful pathogen recognition via a luminol-peroxidase based assay. Throughout their life cycle, plants have to react to various threats coming from the outside environment. That is why they have developed a broad range of strategies, collectively known as 'defense' or 'stress' responses, to protect themselves against abiotic (temperature, drought, etc.) and biotic (pathogenic fungi, bacteria and viruses) factors (Breusegem and Dat, 2006). To cope with this stress response plants, possess physical barriers, such as the cuticle, the cell walls and a number of biological and molecular mechanisms to counteract this effect, which includes the synthesis ROS, namely the oxidative burst.





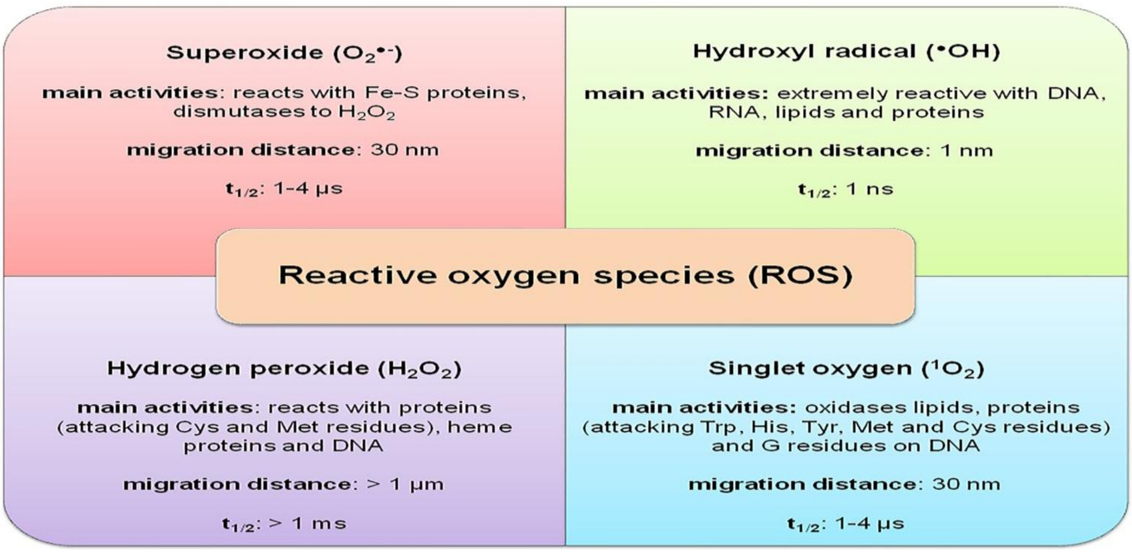
ROS Species

In the plant cell the term ROS is used to describe the products of the sequential reduction of molecular oxygen. They are produced at a low level in non-stressed cells in different organelles such as: chloroplasts, mitochondria, micro bodies, peroxisomes, being in chloroplasts the Mahler reaction, the primary source of it. The main characteristics of the different ROS molecules are:

H_2O_2 = 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^- can be converted to hydroxyl radicals (which are very strong oxidants) by the Haber-Weiss reaction.

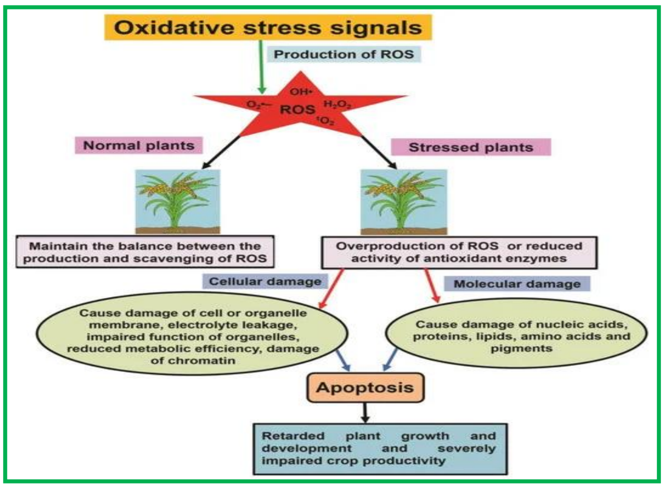
O_2^- = in living cells exists in equilibrium with its protonated form, the hydroperoxyl radical (O_2H-). At a physiological pH is not very reactive against major macromolecular components of the cell.

OH^- = is the most reactive specie that could be formed directly through Haber-Weiss reaction. Although significant levels could be produced through the cycle of reactions that involve oxidation of transition metals such as Fe^{+2} and Cu^+. It is believed that it is the major ROS responsible for the irreversible modifications of cellular macromolecules and damage of organelles (Wojtaszek, 1997).



ROS Function in Plant Defense

- Plant organisms possess a complex set of defense mechanisms that are responsible for preventing unfavorable interactions with other living organisms. Besides, constitutive physical or chemical barriers, plants have developed an array of inducible, local and systemic responses to defend themselves against pathogen attack.
- In resistant plants, specific recognition of a pathogen, an early response occurs immediately, the hypersensitive response (HR), that is notably associated with the



generation of ROS in and around the infected cell and afterwards a late response, usually transcription and translation dependent responses that take part in minimizing the long-term effects of the infection and in preventing further infections.

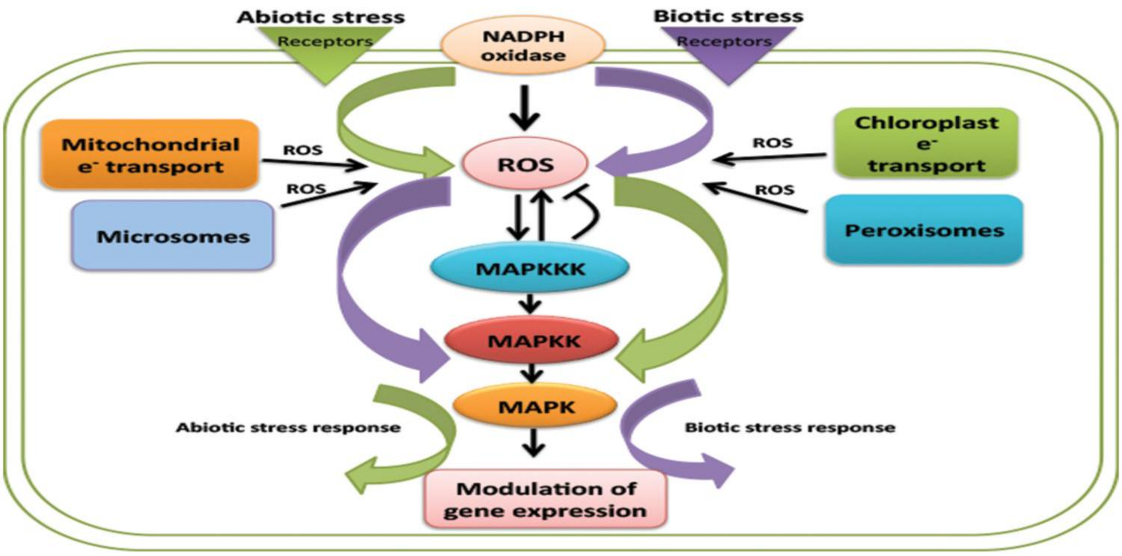
- These rapid events are transcription independent and they cause morphological and physiological changes in the infected cells and their surroundings. ROS enhancement under stress functions as an alarm signal that triggers acclamatory/defense responses by specific signal transduction pathways that involve H2O2 as secondary messenger (Zaninotto *et al*., 2006).
- ROS could contribute to the activation of plant defenses by inducing changes in gene expression. The rapidity of its production and the potential for H2O2 to freely diffuse across membranes suggested that, ROS could exert this function either directly through redox regulation of transcription factors or indirectly by interacting with other signaling components like phosphorylation cascades (Mou *et al*., 2003).

The first line of defense in plant

- In resistant plants when a pathogen is detected the first responses occur at the site of infection within minutes of invasion. These rapid events are transcription-independent, cause morphological and physiological changes in the infected cells and their surroundings and dependent on allosteric changes of several enzymes and fast chemical reactions.
- A massive synthesis of ROS is produced mainly in the apoplast, although it can be produce in other cellular compartments. At the same time ion fluxes, cytoskeletal rearrangements, protein phosphorylation/dephosphorylation, nitric oxide (NO) synthesis, transcriptional and post-translational activation of transcription factors takes place.
- All these events act as the first line of defense, slowing down the pathogen's spread and initiating a signaling mechanism that leads to more fundamental changes in the metabolism of the infected plant

ROS scavenging machinery in plant cells

- Like most aerobic organisms, plants possess the ability to produce and detoxify ROS.
- In plants, under normal physiological conditions, ROS are produced during the process of molecular oxygen assimilation and under stress a rapid, intensive production of ROS is done. This excess leads to cellular damage and ultimately to cell death, primarily through damage to the photosystem II reaction center and to membrane lipids.
- That is why, plants have evolved an elaborate enzymatic and non-enzymatic antioxidant mechanisms to scavenge excess ROS to prevent cellular damage, including up-regulation of antioxidant defense mechanisms, such as small antioxidant molecules like átocopherol, ASC, GSH, proline, betaine, carotenoids and antioxidant enzymes such as SOD, APX and CAT (Shao *et al*., 2008).
- Higher plants can sense, transduce and translate the ROS signals into appropriate cellular responses, the process of which requires the presence of redox-sensitive proteins that can undergo reversible oxidation/ reduction and may switch 'on' and 'off 'depending upon the cellular redox state.
- In many ways, ROS are ideally suited to be signaling molecules: they are small, and can diffuse short distances; there are several mechanisms for their production, some of which are rapid and controllable and there are numerous mechanisms for their rapid removal. ROS, in particular hydrogen peroxide, are now recognized as important signaling molecules in both the animal and plant kingdoms, being able to cross cellular membranes, is also a diffusible signal for the activation of defense genes and systemic acquired resistance (Van Breusegem *et al*., 2008).



Example of ROS

1. Superoxide Anion Radical ($O2^{-}$)
2. Hydrogen Peroxide (H_2O_2)
3. Hydroperoxy Radical (HOO)
4. Hydroxyl Radical (OH)
5. Lipid Peroxide Radical (ROO)
6. Singlet Oxygen ($1O_2$)

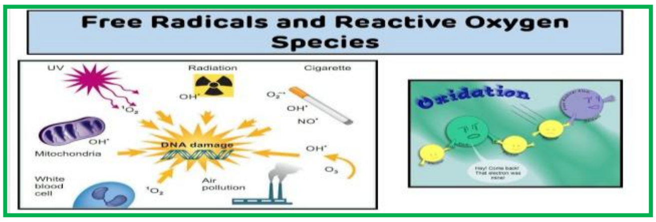
Examples of RNS

1. Nitric Oxide (NO)
2. Peroxy Nitrite (ONOO-)˚

NOTE: Hydrogen Peroxide (H_2O_2) and Singlet Oxygen ($1O_2$) are not classified as free radicals, but due to their Reactivity, they are included in the group of ROS

Features of ROS

1. Extreme Reactivity
2. Short Half Life
3. Generation of new ROS by Chain Reaction
4. Damage to various tissues

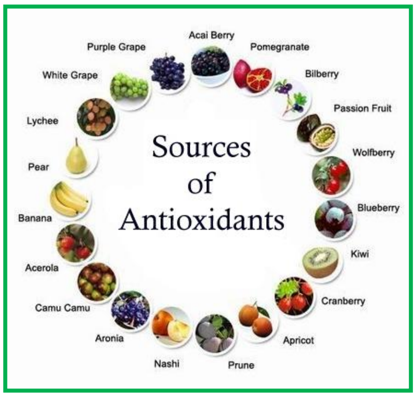


S. NO.	Endogenous ROS	Exogenous ROS
1	ETC	Ionizing radiation
2	Phagocytosis	Uvlight
3	Reaction with metal iron/transition metal	Cigarette smoke
4	Xanthine oxidase	Carbon tetrachloride
5	Arachidonate pathway	Pollutants
6	Lipoxygenase in platelets and WBC	Drugs
7	Peroxisomes	Pesticides
8	Exercise	
9	Inflammation	

Antioxidants defenses

Antioxidants Compounds that dispose of the Reactive Oxygen Species by Scavenging, Suppressing their formation, Conflicting their actions. Antioxidants are substances that help protect cells from damage caused by free radicals, which are unstable molecules that can be harmful to cells. Antioxidants can be found in many foods, including fruits and vegetables, as well as in dietary supplements.

Here are some examples of antioxidants



- ✓ Vitamins: A, C, and E
- ✓ Minerals: Copper, zinc, and selenium
- ✓ Phytochemicals: Lycopenes in tomatoes and anthocyanins in cranberries
- ✓ Foods: Broccoli, spinach, carrots, potatoes, artichokes, cabbage, asparagus, avocados, beetroot, radish, lettuce, sweet potatoes, squash, pumpkin, collard greens, and kale
- ✓ Nuts and seeds: Walnuts, pecans, and sunflower seeds are all high in antioxidants
- ✓ Grains: Buckwheat, millet, and barley are good sources of antioxidants when made into flour

Free Radical Scavengers

1. Superoxide Dismutase
2. Catalase
3. Glutathione Peroxidase
4. Glutathione Reductase
5. Ferricytochrome
6. Endogenous Ceruloplasmin

Superoxide Dismutase: Superoxide dismutase (SOD) is an enzyme that protects cells from damage by free radicals by converting superoxide radicals into hydrogen peroxide and oxygen:

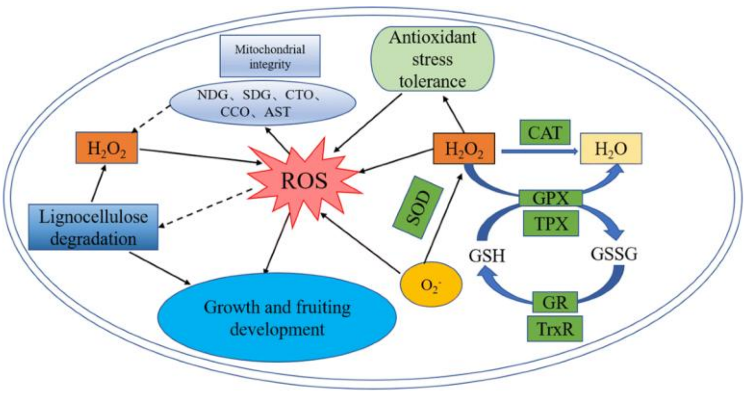
SOD is present in almost all aerobic organisms, including plants, animals, and trypanosomatids. In humans, there are three forms of SOD:

Present in both Cytosol and Mitochondria –

- Mitochondrial SOD - Manganese (Mn) dependent
- Cytosolic SOD - Copper-zinc (Cu-Zn) dependent
- Reaction Catalyzed: $2(O_2\ -)^{o} + 2\ (H^{+}) \rightarrow H_2O_2 + O_2$

Glutathione peroxidase (GP) and glutathione reductase (GR): - GP and GR are enzymes that work together to protect cells from oxidative stress and damage caused by free radicals: -

- **Glutathione peroxidase: -** A primary antioxidant enzyme that scavenges free radicals and decontaminates hydrogen peroxide into alcohols or water. GP combines with selenium and to protect cells.
- **Glutathione reductase: -** GR is a secondary antioxidant enzyme that reduces oxidized glutathione to its reduced form, recycling glutathione for GP activity. GR also neutralizes anions, peroxides, and hydroxyl radicals.



Catalase: Liver peroxisomes When H_2O_2 is generated in large amounts ◊ catalase destroys it to O2 Reaction catalyzed: $H_2O_2 \rightarrow 2H_2O + O_2$

Antioxidants-Classification

In relation to Lipid Peroxidation:

1. Preventive Antioxidants: Inhibit the initial production of free radicals. Eg: catalase, glutathione peroxidase, and ethylene diamine tetra-acetate (EDTA).

2. Chain Breaking Antioxidants: Inhibit the propagative phase. Eg: superoxide dismutase, uric acid and vitamin E.

On the basis of Site of Action:

1. **Cell membrane**: Vitamin E, β-Carotene
2. **Cytosol:** Vitamin C, Enzymes
3. **Plasma:** Uric Acid, Bilirubin, Ceruloplasmin, Albumin, Transferrin

On the basis of Nature

1. Artificial: Propyl Gallate, Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT)

2. Natural

- **Lipid Soluble:** Vitamin E (Tocopherol), β-Carotene (An Antioxidant at low pO2), Lycopene
- **Water Soluble:** Vitamin C (Ascorbic Acid), Urates

Antioxidants as Pro-oxidants: - Pro-oxidants: Compounds capable of generating Free Radicals or ROS (particularly)

1. Vitamin C
2. Beta-Carotene (β-carotene)
3. Vitamin E

1. Vitamin C: Can also be a source of Superoxide Radicals by reaction with Oxygen, and Hydroxyl Radicals by reaction with Cu2+ ions

Vitamin C: As an Antioxidant: -

Ascorbate + $O_2 \rightarrow H_2O_2$ + Monodehydroascorbate

Ascorbate + OH• → H2O + Monodehydroascorbate

Catalase and Peroxidases catalyze the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$

2. Beta-Carotene (β-carotene): A Radical-trapping Antioxidant under conditions of Low Partial Pressure of Oxygen In Tissues like lungs (with high pO2 and especially in high concentrations) ◊ Autocatalytic Pro-oxidant◊ Can initiate damage to Lipids and Proteins

3. Vitamin E Chain breaking antioxidants may have pro oxidant role. Some research has shown high dose vitamin E supplement may have pro oxidant role. The major Lipid-soluble Antioxidant in Cell Membranes and Plasma Lipoproteins Chain-breaking and Free-radical trapping Antioxidant Reacts with the Lipid Peroxide Radicals (formed by Peroxidation of Polyunsaturated Fatty Acids)

References

1. Breusegem, F.V., Dat, J.F. 2006. Reactive oxygen species in plant cell death. *Plant Physiology*, **141**:384-390.
2. Del, F.C., Prati, D. 2015. The relative importance of immediate allelopathy and allelopathic legacy in invasive plant species. *Basic and Applied Ecology*, **16**(1):28–35.
3. Doke, N. 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiologial Plant Pathology*, **23**(3): 345-357.
4. Jabs, T., Tschope, M., Colling, C., Hahlbrock, K. and Scheel, D. 1997. "Elicitor-stimulated ion fluxes and O2- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley". *Proceedings of the National Academy of Sciences of the United States of America*. **94** (9): 4800–4805.

5. Morales, J., Kadota, Y., Zipfel, C., Molina, A. and Torres, M.A. 2016. "The Arabidopsis NADPH oxidases RbohD and RbohF display differential expression patterns and contributions during plant immunity". *Journal of Experimental Botany*, **67**(6):1663–1676.
6. Wojtaszek, P., Trethowan, J. and Bolwell, G.P. 1997. "Specificity in the immobilization of cell wall proteins in response to different elicitor molecules in suspension-cultured cells of French bean (*Phaseolus vulgaris* L.)". *Plant Molecular Biology*, **28** (6): 1075–1087.
7. Ryter, S.W., Hong, P.K. Kim, A.H., Jeong W.P., Kiichi N., Xue Wang and Augustine M.K. 2007. Mechanisms of cell death in oxidative stress. *National Centre for Biotechnology Information*, **9**(1):49-89.
8. Torres, A.N., Baccaccini, M.T. and Millar, H.I. 2006. Perceptions of the Validity and Utility of Criminal Profiling Among Forensic Psychologists and Psychiatrists. *Professional Psychology Research and Practice*, 37(1).
9. Federica, Z., Sylvain, L.C., Annalisa P. and Massimo D. 2006. Cross Talk between Reactive Nitrogen and Oxygen Species during the Hypersensitive Disease Resistance Response. *Plant Physiology*, **141**:379–383,
10. Zhonglin, M., Weihua, F. and Xinnian, D. 2003. Inducers of Plant Systemic Acquired Resistance Regulate NPR1 Function through Redox Changes. *Cell*, Vol. 113, 935–944,
11. Hong-bo Shao, Li-ye Chu, Ming-a Shao, Cheruth Abdul Jaleel, and Mi Hong-mei. 2008. Higher plant antioxidants and redox signaling under environmental stresses, *Biologies*, **331**(6):433-441.