Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2015 Vol. 4 (4) October-December, pp. 134-138/Malik

Review Article

SINGLET OXYGEN GENERATION AND OXIDATIVE STRESS IN PLANTS

***Punjab Singh Malik**
Department of Botany, DAV College, Muzaffarnagar-251001, India.
**Author for Correspondence*

ABSTRACT

Metabolic processes such as photosynthesis and respiration are mostly responsible for the generation of reactive oxygen species (ROS) including singlet oxygen ($^1O_2$) in chloroplasts, mitochondria and other sites of the plant cell. Imbalance between ROS generation and their detoxification by antioxidant enzymes results in higher net ROS formation, which ultimately causes oxidative damage and cell death in plants. In plants, $^1O_2$ is mainly produced inside chloroplasts and mitochondria. Chlorophyll (Chl) and its tetrapyrrole metabolic intermediates, which are synthesized inside the chloroplasts, in the presence of light get excited and transfer the excitation energy to molecular oxygen resulting in $^1O_2$ production. $^1O_2$ is highly reactive and causes necrotic spots and cell death by destroying the plasma membrane. Genetic mutants that are deficient in Chl biosynthetic enzymes or regulatory proteins accumulate excess tetrapyrroles leading to excess $^1O_2$ generation and cell death. Similarly, plants that are genetically deficient in Chl degradation enzymes accumulate excess Chl catabolic products that generate $^1O_2$ via photosensitization reactions and cause cell death. As there is no enzymatic means available to detoxify $^1O_2$, it is essential to minimize its production rather than detoxifying it after it is generated. In this review, the mechanisms of generation of $^1O_2$, its detoxification, its mode of cellular damage and ways to minimize its destructive potential and programmed cell death are discussed.

Keywords: *Reactive Oxygen Species, Singlet Oxygen, Biotic and Abiotic Stresses*

INTRODUCTION

The activation or reduction of oxygen gives rise to reactive oxygen species (ROS) that includes the singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$). Plants and other living organisms in the oxidizing environment constantly produce ROS in different organelles because of their metabolic processes such as photosynthesis and respiration. The generation of ROS in plants is triggered by both biotic and abiotic stresses, such as high light, high or low temperature, salinity, drought and pathogen attack. Plants have evolved a set of anti-oxidative enzymes and other small molecules to harmlessly dissipate ROS. Imbalance between ROS production and their detoxification by enzymatic and non-enzymatic reactions causes oxidative stress. As a result of higher net ROS formation, there is photo oxidative damage to DNA, Proteins and lipids and ultimately cell death. Recent studies also indicate that ROS can act as signaling molecules that regulate different sets of gene expression (Apel and Hirt, 2004).

Generation of Singlet Oxygen in Plants

The ground state molecular oxygen is a biradical, as it has two unpaired electrons. Its two unpaired electrons have parallel spins that do not allow them to react with most molecules. However, if the molecular oxygen absorbs sufficient energy, the spin of one of its unpaired electrons is reversed. As a result there is generation of singlet oxygen ($^1O_2$), whose outermost pair of electrons has antiparallel spins. In plants, $^1O_2$ is mainly produced by the chlorophyll (Chl) and its tetrapyrrole metabolic intermediates in the presence of light. Inefficient transfer of energy results in the generation of triplet state Chl that reacts with triplet oxygen to produce the highly reactive $^1O_2$. Singlet oxygen ($^1O_2$) is also produced near the reaction centers of the photosystems. With increase in light intensity, light absorption by leaves increases almost linearly. However, the rate of photosynthesis reaches its maximum value before the linear increase in light absorption ceases. Therefore, plants end up absorbing more light than they could utilize in photosynthesis. This results in the over-excitation of the photosynthetic apparatus. In the presence of excess light energy, the QA and QB, the first and second plastoquinone electron acceptors of Photosystem

II (PS II) in the electron transport chain, are over reduced (Barber and Andersson, 1992) and because of that, charge separation cannot be completed between P680 and pheophytin. As a result the triplet state of the reaction center Chl P680 (3P680) is favored (Aro *et al.*, 1993) leading to the formation of $^1O_2$ (Foote *et al.*, 1984). Normally when excess light is absorbed, an alternative dissipating pathway is activated that safely returns 1Chl* to its ground state before it is converted to 3Chl*. The excitation energy of excess 1Chl* is dissipated by zeaxanthin or other binding protein complexes (Baroli and Niyogi, 2000; Pogson and Rissler, 2000). The carotenoids, which quench the excited state of Chl, must be in close proximity with triplet Chl. In the reaction center, the distance between Chl and carotenoid is too large to allow triplet quenching. $^1O_2$, produced in the reaction center, directly reacts with carotenoids. The release of $^1O_2$ is also detected in isolated PS II particles (Macpherson *et al.*, 1993) and in thylakoids (Chakraborty and Tripathy, 1991). $^1O_2$ is also generated from the cytochrome b6f complex (Suh *et al.*, 2000).

Singlet Oxygen-Induced Oxidative Damage in Plants

Chlorophyll Biosynthesis Pathway Mutants Show Cell Death Phenotypes

*Chl*orophyll biosynthesis pathway intermediates such as uroporphyrin, coproporphyrin, Protoporphyrin IX, Protochlorophylide and chlorophyllide are photodynamic in nature and generates $^1O_2$ in presence of light. The etiolated PORA and PORB mutant seedlings accumulate significant amounts of non-phototransformable Pchlide in darkness and upon light exposure they show bleaching effect and germination defect (Armstrong *et al.*, 1995). The isolation and studies on Arabidopsis *flu* mutant by Klaus Apel's group confirm the role of Pchlide in $^1O_2$ generation; it leads to oxidative damage. In *flu* mutant there is a massive accumulation of Pchilde if those plants are grown under constant dark/light cycle and there is growth arrest and cell death because of generation of $^1O_2$. Lee *et al.*, (2003) have revealed that the *TIGRINA d* gene of barley is an ortholog of the *FLU* gene of *Arabidopsis thaliana*. Pchlide-mediated $^1O_2$ formation leads to the induction of the early stress-responsive gene (Op den Camp *et al.*, 2003). There is no change in amounts of other photosensitizers i.e, Proto IX, Mg -proto IX and MPE in the *flu* mutant. Oxygenation derivatives of linolenic acid, by far the most prominent polyunsaturated fatty acid of chloroplast membrane lipids, start to accumulate rapidly in the *flu* mutant after the dark/ light shift. Application of vitamin B6, an inhibitor of $^1O_2$, was able to protect *flu* protoplasts from cell death (Danon *et al.*, 2005). Similarly, application of ALA to plants in dark resulted in massive accumulation of Pchlide in dark and upon transfer to light the plants show necrotic spots that bacuase of $^1O_2$-mediated photo-oxidative damage (Chakraborty and Tripathy, 1991).

Apart from Pchlide, early intermediates i.e., coproporphyrin and protoporphyrin also act as a photosensitizer (Ishikawa *et al.*, 2001; Kruse *et al.*, 1995). The antisense coproporphyrinogen oxidase (that converts coproporphyrinogen III to protoporphyrinogen IX) in tobacco plants, have an excessive amount of coproporphyrin. This oxidized porphyrin gives rise to photodynamic reactions, which affect cellular processes resulting in retarded growth and necrotic leaves (Kruse *et al.*, 1995). The Arabidopsis coproporphyrinogen oxidase mutants (*lin2*; lesion initiation 2) had pale leaves and developed lesions on the young leaf (Ishikawa *et al.*, 2001). 3, 3-Diamino benzidine and trypan blue staining of the mutant leaves shows H_2O_2 accumulation and cell death. Seedlings homozygous for a null mutation in the cpx1 gene of maize completely lack chlorophyll and develop necrotic lesions in the light (Williams *et al.*, 2006). The accumulation of uroporphyrin I in the uroporphyrinogen III cosynthase antisense barley plants results in necrotic leaves and ultimately cell death because of accumulation of ROS (Ayliffe *et al.*, 2009). Like uroporphyrin I, uroporphyrin III, an oxidized derivative of uroporphyrinogen III, an intermediate of the chlorophyll biosynthesis pathway, also acts as a photosensitizer. Accumulation of Uroporphyrin III leads to light-dependent necrosis in tobacco (Mock and Grimm, 1997) and in maize (Hu *et al.*, 1998). Antisense tobacco plants of Uroporphyrinogen decarboxylase have stunted growth with necrotic leaves and high PR1 gene expression. The maize lesion mimic mutant, coding for uroporphyrinogen decarboxylase, that has necrotic spots in the leaves. Inhibition of protox in Arabidopsis leads to production of lesion-mimic phenotype, high endogenous level of salicylic acid and PR1 gene expression (Molina *et al.*, 1999). Overexpression of plastidic protox leads to resistance to the DPE herbicide acifluorfen. The overexpressed plants did not show any necrotic leaves (Lermontova and Grimm, 2000).

Tobacco plants having reduced ferrochelatse activity also show necrotic leaves in a light intensity dependent manner (Papenbrock *et al.*, 2001).

Chlorophyll Degradation Pathway Mutants Show Cell Death Phenotypes

Intermediates involved in the Chl degradation pathway also produce ROS. Expression of the citrus chlorophyllase protein in Squash plants display a lesion-mimic phenotype. The phenotype is caused by the accumulation of chlorophyllide (Harpaz-Saad *et al.*, 2007). The Arabidopsis Pheophorbide a oxygenase (PAO, also called *acd1*, accelerated cell death 1) mutant shows a cell death phenotype because of the accumulation of the Chl degradation intermediate pheophorbide a. The lesions in *acd1* mutant leaves start mostly at the tip of the leaf and subsequently run down the leaf blade (Pruzinska *et al.*, 2003). Hirashima *et al.*, (2009), also observed that the accumulation of Pheophorbide a in dark grown *acd1* antisense plants caused cell death. The maize *lls1* mutant formed lesions when grown in the light (Gray *et al.*, 1997). Similarly, the Arabidopsis Red chlorophyll catabolite reductase (RCCR, also called *acd2*, Accelearated cell deah 2) mutant showed lesion formation in leaves and spontaneous cell death phenotype (Mach *et al.*, 2001). It is observed that the accumulation of H_2O_2 and $^1O_2$ in the *acd2* mitochondria is causal for its cell death phenotype (Yao and Greenberg, 2006, Pattanayak *et al.*, 2012). The lesion formation in *acd2* is caused by the accumulation of red chlorophyll catabolite (RCC) in darkness that generates $^1O_2$ in the presence of light (Pruzinska *et al.*, 2007).

Future Prospects

Acclimation to $^1O_2$ has been shown in the green alga *Chlamydomonas reinhardtii* (Ledford *et al.*, 2007). This approach could be further exploited to generate plants that could tolerate higher doses of $^1O_2$. As there is no enzymatic means available to detoxify $^1O_2$, it is essential to minimize its production rather than detoxifying it after it is generated. Overexpression of PORC that enzymatically converts Pchilde to chlorophyllide makes plants resistant to $^1O_2$-mediated cell death (Pattanayak *et al.*, 2011). Microarray experiments show generation of $^1O_2$ alters genome wide transcription rate that ultimately leads to apoptosis. There is bound to be cross-talk between $^1O_2$-mediated and other ROS-mediated signaling events leading to cell death. Therefore, a better knowledge of the plant responses to $^1O_2$ could have important implications not only for the understanding of how plants can adapt to changing and unfavorable climatic environments, but also for the development of plants tolerant to various types of stresses, including biotic stresses.

REFERENCES

Apel K and Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55** 373–399.

Armstrong GA, Runge S, Frick G, Sperling U and Apel K (1995). Identification of NADPH: protochlorophyllide oxidoreductases A and B: a branched pathway for light dependent chlorophyll biosynthesis in Arabidopsis thaliana. *Plant Physiology* **108**(4) 1505–1517.

Aro EM, Virgin I and Andersson B (1993). Photoinhibition of photosystem II: inactivation, protein damage and turnover. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1143**(2) 113–134.

Ayliffe MA, Agostino A, Clarke BC, Furbank R, von Caemmerer S and Pryor AJ (2009). Suppression of the Barley uroporphyrinogen III synthase gene by a Ds activation tagging element generates developmental photosensitivity. *Plant Cell* **21**(3) 814–831.

Barber J and Andersson B (1992). Too much of a good thing: Light can be bad for photosynthesis. *Trends in Biochemical Sciences* **17**(2) 61–66.

Baroli I and Niyogi KK (2000). Molecular genetics of xanthophyll- dependent photoprotection in green algae and plants. *Philosophical Transactions of the Royal Society B: Biological* **355**(1402) 1385–1394.

Chakraborty N and Tripathy BC (1991). 5-Aminolevulinic acid induced photodynamic damage of the photosynthetic electron transport chain of cucumber (*Cucumis sativus* L.) Cotyledons. *Plant Physiology* **96**(3) 761–767.

Danon A, Miersch O, Felix G, Camp RG and Apel K (2005). Concurrent activation of cell death-regulating signaling pathways by singlet oxygen in Arabidopsis thaliana. *Plant Journal* **41**(1) 68–80.

Foote CS, Shook FC and Abakerli RB (1984). Characterization of singlet oxygen. *Methods in Enzymology* **105** 36–47.

Gray J, Close PS, Briggs SP and Johal GS (1997). A novel suppressor of cell death in plants encoded by the Lls1 gene of maize. *Cell* **89**(1) 25–31.

Harpaz-Saad S, Azoulay T, Arazi T, Ben-Yaakov E, Mett A, Shiboleth Y M, Hörtensteiner S, Gidoni D, Gal-On A, Goldschmidt EE and Eyal Y (2007). Chlorophyllase is a rate-limiting enzyme in chlorophyll catabolism and is posttranslationally regulated. *Plant Cell* **19**(3) 1007–1022.

Hirashima M, Tanaka R and Tanaka A (2009). Light-independent cell death induced by accumulation of pheophorbide a in Arabidopsis thaliana. *Plant Cell Physiology* **50**(4) 719–729.

Hu G, Yalpani N, Briggs SP and Johal GS (1998). A porphyrin pathway impairment is responsible for the phenotype of a dominant disease lesion mimic mutant of maize. *Plant Cell* **10**(7) 1095–1105.

Ishikawa A, Okamoto H, Iwasaki Y and Asahi T (2001). A deficiency of coproporphyrinogen III oxidase causes lesion formation in Arabidopsis. *Plant Journal* **27**(2) 89–99.

Kruse E, Mock HP and Grimm B (1995). Reduction of coproporphyrinogen oxidase level by antisense RNA synthesis leads to deregulated gene expression of plastid proteins and affects the oxidative defense system. *European Molecular Biology Organization Journal* **14**(15) 3712–3720.

Ledford HK, Chin BL and Niyogi KK (2007). Acclimation to singlet oxygen stress in Chlamydomonas reinhardtii. *Eukaryotic Cell* **6**(6) 919–930.

Lee KP, Kim C, Lee DW and Apel K (2003). TIGRINA d, required for regulating the biosynthesis of tetrapyrroles in barley, is an ortholog of the FLU gene of Arabidopsis thaliana. *FEBS Letters* **553**(1-2) 119–124.

Lermontova I and Grimm B (2000). Overexpression of plastidic protoporphyrinogen IX oxidase leads to resistance to the diphenyl-ether herbicide acifluorfen. *Plant Physiology* **122**(1) 75–84.

Mach JM, Castillo AR, Hoogstraten R and Greenberg JT (2001). The Arabidopsis-accelerated cell death gene ACD2 encodes red chlorophyll catabolite reductase and suppresses the spread of disease symptoms. *Proceedings of the National Academy of Sciences USA* **98**(2) 771–776.

Macpherson AN, Telfer A, Barber J and Truscott TG (1993). Direct detection of singlet oxygen from isolated photosystem II reaction centres. *Biochimica et Biophysica Acta* **1143**(3) 301–309.

Mock HP and Grimm B (1997). Reduction of uroporphyrinogen decarboxylase by antisense RNA expression affects activities of other enzymes involved in tetrapyrrole biosynthesis and leads to light dependent necrosis. *Plant Physiology* **113**(4) 1101–1112.

Molina A, Volrath S, Guyer D, Maleck K, Ryals J and Ward E (1999). Inhibition of protoporphyrinogen oxidase expression in Arabidopsis causes a lesion-mimic phenotype that induces systemic acquired resistance. *Plant Journal* **17**(6) 667–678.

Op den Camp RG, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Göbel C, Feussner I, Nater M and Apel K (2003). Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis. *Plant Cell* **15**(10) 2320–2332.

Papenbrock J, Mishra S, Mock HP, Kruse E, Schmidt EK, Petersmann A, Braun HP and Grimm B (2001). Impaired expression of the plastidic ferrochelatase by antisense RNA synthesis leads to a necrotic phenotype of transformed tobacco plants. *Plant Journal* **28**(1) 41–50.

Pattanayak GK and Tripathy BC (2011). Overexpression of protochlorophyllide oxidoreductase C regulates oxidative stress in Arabidopsis. *PLoS One* **6**(10) e26532.

Pattanayak GK, Venkataramani S, Hortensteiner S, Kunz L, Christ B, Moulin M, Smith AG, Okamoto Y, Tamiaki H, Sugishima M and Greenberg JT (2012). Accelerated cell death 2 suppresses mitochondrial oxidative bursts and modulates cell death in Arabidopsis. *The Plant Journal* **69**(4) 589-600.

Pogson BJ and Rissler HM (2000). Genetic manipulation of carotenoid biosynthesis and photoprotection. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **355**(1402) 1395–1403.

Pruzinská A, Anders I, Aubry S, Schenk N, Tapernoux- Lüthi E, Müller T, Kräutler B and Hörtensteiner S (2007). In vivo participation of red chlorophyll catabolite reductase in chlorophyll breakdown. *Plant Cell* **19**(1) 369–387.

Pruzinska A, Tanner G, Anders I, Roca M and Hortensteiner S (2003). Chlorophyll breakdown: pheophorbide a oxygenase is a Rieske-type iron-sulfur protein, encoded by the accelerated cell death 1 gene. *Proceedings of the National Academy of Sciences USA* **100**(25) 15259–15264.

Suh HJ, Kim CS and Jung J (2000). Cytochrome b6/f complex as an indigenous photodynamic generator of singlet oxygen in thylakoid membranes. *Photochemistry and Photobiology* **71**(1) 103–109.

Williams P, Hardeman K, Fowler J and Rivin C (2006). Divergence of duplicated genes in maize: evolution of contrasting targeting information for enzymes in the porphyrin pathway. *Plant Journal* **45**(5) 727–739.

Yao N and Greenberg JT (2006). Arabidopsis ACCELERATED CELL DEATH2 modulates programmed cell death. *Plant Cell* **18**(2) 397–411.