ABSTRACT

Metabolic processes such as photosynthesis and respiration are mostly responsible for the generation of reactive oxygen species (ROS) including singlet oxygen ($^1$O$_2$) 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, $^1$O$_2$ 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 $^1$O$_2$ production. $^1$O$_2$ 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 $^1$O$_2$ generation and cell death. Similarly, plants that are genetically deficient in Chl degradation enzymes accumulate excess Chl catabolic products that generate $^1$O$_2$ via photosensitization reactions and cause cell death. As there is no enzymatic means available to detoxify $^1$O$_2$, it is essential to minimize its production rather than detoxifying it after it is generated. In this review, the mechanisms of generation of $^1$O$_2$, 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 ($^1$O$_2$), superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (HO$^-$). 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 ($^1$O$_2$), whose outermost pair of electrons has antiparallel spins. In plants, $^1$O$_2$ 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 $^1$O$_2$. Singlet oxygen ($^1$O$_2$) 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 1O2 (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. 1O2, produced in the reaction center, directly reacts with carotenoids. The release of 1O2 is also detected in isolated PS II particles (Macpherson et al., 1993) and in thylakoids (Chakraborty and Tripathy, 1991). 1O2 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

Chlorophyll biosynthesis pathway intermediates such as uroporphyrin, coproporphyrin, Protoporphyrin IX, Protoprochlorphylide and chlorophyllide are photodynamic in nature and generates 1O2 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 1O2 generation; it leads to oxidative damage. In flu mutant there is a massive accumulation of Pchlide if those plants are grown under constant dark/light cycle and there is growth arrest and cell death because of generation of 1O2. 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 1O2 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 1O2, 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 bacause of 1O2-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 H2O2 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).
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Tobacco plants having reduced ferrochelatase activity also show necrotic leaves in a light intensity dependent manner (Pappenbrock 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 gene 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, Accelerated cell death 2) mutant showed lesion formation in leaves and spontaneous cell death phenotype (Mach et al., 2001). It is observed that the accumulation of H2O2 and 1O2 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 1O2 in the presence of light (Pruzinska et al., 2007).

**Future Prospects**

Acclimation to 1O2 has been shown in the green alga Chlamydomonas reinhardti (Ledford et al., 2007). This approach could be further exploited to generate plants that could tolerate higher doses of 1O2. As there is no enzymatic means available to detoxify 1O2, 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 1O2-mediated cell death (Pattanayak et al., 2011). Microarray experiments show generation of 1O2 alters genome wide transcription rate that ultimately leads to apoptosis. There is bound to be cross-talk between 1O2-mediated and other ROS-mediated signaling events leading to cell death. Therefore, a better knowledge of the plant responses to 1O2 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**


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