Abiotic stress triggers mitochondrial defense system: A comprehensive review

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Abstract

Mitochondria are small organelles widely distributed within the cells of living organisms. Their main function is the oxidative phosphorylation-coupled ATP synthesis. Under abiotic stress conditions mitochondrial function is significantly affected triggering the mitochondrial stress response. However, the stressful conditions could increase the chance of ROS generation in various cellular organelles. Plant cells struggle ROS generation through the induction of specific enzymatic and non-enzymatic defense systems. Nevertheless, the excessive accumulation of ROS within mitochondria induces mitochondrial signaling and the cellular responses to mitochondrial dysfunction in a process known as mitochondrial retrograde regulation. The exclusive accumulation of ROS causes severe disorders in ROS homoeostasis that stimulate programmed cell death. The inner mitochondrial membrane has uncoupling mitochondrial proteins (UMP) to keep mitochondrial integrity and function at both normal and stress conditions. Mitochondria can perform nonphosphorylating respiration under stress conditions, like the presence of respiration inhibitors, by the help of specific proteins called alternative oxidases (AOX). The expression of AOX or its mRNA has been reported to be induced by various abiotic stress conditions. The expression of AOX plays an important role in acclimation to many stress conditions like salinity, temperature and drought in various plant species.

Keywords: abiotic stress; alternative oxidases; mitochondria; mitochondrial retrograde; uncoupling proteins

Abbreviations

AA: Antimycin A; ABI4: Abscisic acid insensitive4; AOX: Mitochondrial alternative oxidase; CAREs: Cis-acting regulatory elements; Complex I: NADH–coenzyme Q reductase; Complex II: Succinate/ubiquinone oxidoreductase; Complex III: UQ-cytochrome c oxidoreductase; Complex IV: cytochrome c oxidase; COX: Cytochrome oxidase; Drp1: Dynamin-related protein 1; Fis1: Mitochondrial fission protein 1; HIF-1α: Hypoxia-inducible factor-1α; Mfn1: Mitofusin-1; Mfn2: Mitofusin-2; MRR: Mitochondrial retrograde regulation; mtETC: Mitochondrial electron transport chain; RNS: Reactive nitrogen species; UCP: Plant mitochondrial uncoupling protein; UQ: Ubiquinone

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Introduction

Mitochondria are organelles found nearly in all eukaryotic cells with varying sizes (0.5-10 µm) and numbers (1- over 1000 per cell). The most fundamental role exhibited by mitochondria in cells is the production of adenosine triphosphate (ATP) during the respiration process, which is utilized as a source of cellular energy during growth, development, and diverse metabolic

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pathways. Mitochondria encompass their specific genetic material, which is analogous to bacterial genome (Lang et al., 1997). Along with energy production, mitochondria consider the key mediators in Ca^{2+} transport, as Ca^{2+} carry out fundamental metabolic processes within mitochondria (Rizzuto et al., 2000). Additionally, Ca^{2+} is a secondary messenger playing significant roles in genome transcription and cellular propagation (Csordás et al., 1999). Green and Reed (1998) reported that mitochondria have an axial role in cell life, especially the programmed cell death (apoptosis). The mechanism of controlling cell death by mitochondria might be mediated via perturbation of oxidative phosphorylation and energy generation, catalytic activation of cysteine proteases (caspases), as well as modification of cellular redox homeostasis (Green and Reed, 1998). Plant mitochondria are dually involved in ATP biosynthesis and effusion of ATP to the cytosol because, aside from containing complexes collectively known as NADH dehydrogenase (cI), succinate dehydrogenase (cII), ubiquinol-cytochrome c reductase (cIII), and cytochrome c oxidase (cIV), the majority of plant mitochondria as well encompass an externally located non-proton pumping NAD(P)H dehydrogenase that bypasses complex I. This NAD(P)H dehydrogenase was identified as rotenone-insensitive and to varying degrees a cyanide and antimycin-insensitive (Moore and Siedow, 1991). The exact internal structure of mitochondria has become clear after the studies done by the electron microscope at the mid-twentieth century and these studies allowed the intensive understanding of the secrets of this fundamental organelle.

Mitochondrial structure

Mitochondria are cytoplasmic organelles with about 0.5 to 1.0 mm diameter and up to 7.0 mm length. Mitochondria take different shapes and their number per cell varies according to the function and type of the owning tissue. Their general architectures are alike, though the shape varies from globular to filamentous. Cellular energy demand determines the number of mitochondria they own; highly active metabolic tissues as heart muscle and kidney already have abundant number of mitochondria. Each mitochondrion is enclosed with double-layered membrane, each consisting of a phospholipid bilayer. The physio-biochemical function of each membrane is distinct although each membrane has its specific architecture and appearance. The inner membrane is highly folded comprising a great number of cristae for increasing its surface area. The number and surface area of cristae is greatly variable depending on the type and metabolic activity of the cell or the organelle (Smith and Ord, 1983). The internal mitochondrial membrane of the mitochondrion encapsulates a central stodgy filling known as the matrix. This membrane is impermeable to polar and ionic substances; however, the outer membrane is far more permeable to these substances. Additionally, this membrane is enriched in cardiolipin (diphosphatidylglycerol lipid) and in proteins compared to the outer membrane. The high level of proteins in the folded mitochondrial membrane makes it much appropriate for a multitude of biochemical pathways within the organelle. The difference in permeability between the two membranes, as the inner one is far less impermeable compared to the outer membrane, develops an intermembrane space, a cytoplasm-like environment with high specificity towards larger proteins that have a mitochondrial role. Accordingly, the inner membrane comprises a family of mitochondrial carriers to enable exchange between the inter-membrane space and the matrix (Kunji, 2004). In addition to carrier proteins, the folded membrane contains protein complexes and redox cofactors relatable to electron transfer and ATP synthesis (Fig. I).

The mitochondrial matrix encompasses a variety of enzymes responsible for all aspects of metabolism, together with the mitochondrial genome (encoding 39 genes in mammalian mitochondria). The tissue identification is mainly based on the internal membrane or cristae (Sjöstrand, 1953). The concentration of cristae on the internal membrane is correlated with the number of cytochromes and other electron carriers. For example, the mitochondria in hepatic

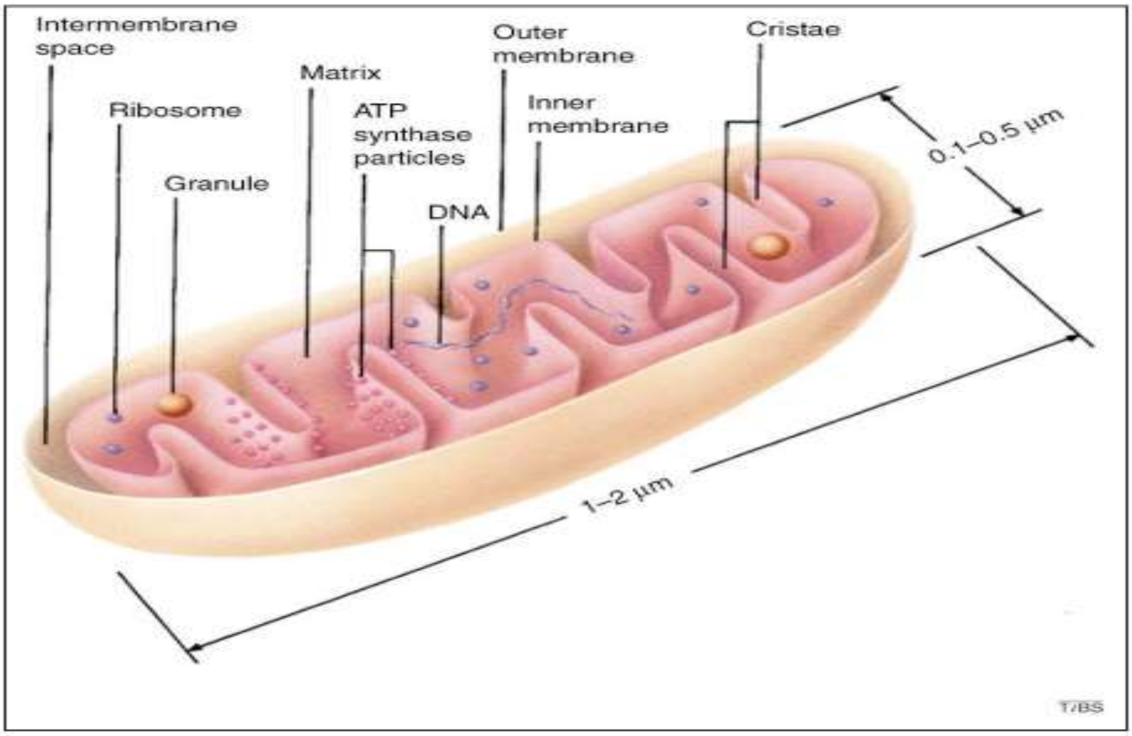

Fig. I. The common structure of mitochondria (adopted from Lodish et al., 1995)

cells carry out numerous auxiliary functions other than energy production and have to a great extent less number of cristea with large space in between; however, cardiac mitochondria which is principally concerned with ATP synthesis are heavily crowded with cristae with little space in between. The presence of cristae on the inner membrane provides large surface area for oxidative phosphorylation and maintenance of the generated electrochemical gradient. It is clearly understood now that the shape and complication of mitochondria differ according to tissue and even cell type, depending on the developmental stage and the physiological state (Mannella, 2006).

Mitochondrial dynamics

Mitochondrial dynamics is a description of continual changes in mitochondrial position, size, and, shape within the cells. Eukaryotic mitochondria possess various shapes, ranging from tubular to spherical (Bereiter-Hahn and Vöth, 1994). This is a controlled process in plants, fundamental for the exchange of metabolic, hereditary, and protein contents and to modulate mitochondrial bioenergetics, ATP biosynthesis, autophagy, plant cell death (PCD), and associations with the cell cycle (Westermann, 2010).

The principal objective of mitochondrial dynamics would be to optimize mitochondrial work according to the exact energetic requirements of the cell. As specified, one of the principle confirmations connecting dynamics to function was known in plant photosynthetic tissues throughout the observation of the co-existence or proximity of mitochondria and chloroplasts (Logan and Leaver, 2000). Recent reports demonstrated a significant correlation between mitochondrial and chloroplast movements as affected by light regimes. In dark, an arbitrary allocation of mitochondria was observed in the mesophyll cells of chloroplasts. Nevertheless, mitochondria moved coordinately with chloroplasts under various light intensities, indicating that mitochondria either follow a particular signal or are physically connected with chloroplasts through the cytoskeleton. In spite of the fact that there is no particular proof on this, it is expected that this nearby association sustains the gases and metabolites exchange for the maintenance of effective photosynthesis (Islam and Takagi, 2010).

In addition to the changes in the whole mitochondrial shape, recent comprehensive studies revealed that the inner mitochondrial membrane is also dynamic. The elasticity and re-organization of this membrane has been elucidated using the tridimensional imaging of cryopreserved samples (Mannella, 2006). As a characteristic feature of the inner membrane, it is distinguished into three regions; the inner boundary membrane, the cristae membrane and the cristae junctions. The inner membrane regions are easily distinguished morphologically and their function appears to be totally different. Furthermore, the metabolic status of the mitochondria has a direct effect on the inner membrane structure. When the separated mitochondria was placed in a medium with low ADP concentration it showed less respiration rate with change in structure, as it contained few cristae with less junctions in the normal shape. Conversely, in the high concentration of ADP, isolated mitochondria showed higher respiration rates and the morphology was greatly changed. The inner membrane possessed large cristae and numerous junctions giving it the condensed morphology (Mannella, 2006). These findings point out that morphology of the inner membrane is closely correlated to metabolic status and bioenergetics of the mitochondria.

Mitochondrial fission and fusion proteins

Mitochondria have their own genetic material; therefore, their replication is dependent on cellular division. The existing mitochondrion undergoes binary fission, like that of bacterial cells, producing two equivalent organelles. Conversely, one or more organelles can fuse together producing single mitochondrion. Additionally, mitochondria have the ability to change their architecture and distribution within the cytosol to be malleable with their function. In mammalian and yeast cells, the cells with low fission to fusion ratio possess few numbers of mitochondria which are elongated and greatly interconnected. while those with high fission to fusion ratio (Bleazard et al., 1999; Smirnova et al., 2001). On the other hand, cells with a high fission to fusion ratio encompass abundant mitochondria, which are small in size and their shape might be spherical or rod-shaped, in addition to their fragmented nature. These dynamics of mitochondria throughout development significantly influence their morphology. Drosophila is an ideal example, where during melanogaster spermatogenesis, a synchronous fusion of many mitochondria take place as a requirement for the production of a structure called the Nebenkern, which is essential for sperm motility (Hales and Fuller, 1997). Mitochondrial fission and fusion is basically dominated by many factors. Mitochondria have membrane-bound proteins called Mitofusins (Mfn1 and Mfn2) which play a critical role in mitochondrial fusion. Chen et al. (2003) reported that mitochondria deficient in Mfn1 or Mfn2 have a decreased fusion rate. This could be attributed to the loss of mitochondrial tubules and dysfunction (Chen et al., 2005). Mitochondrial tubules fission has been identified in many studies to be controlled by two proteins, dynamin-related protein 1 (Drp1) and mitochondrial fission protein 1 (Fis1) (Smirnova et al., 2001; James et al., 2003). Mitochondrial fusion efficiency is critically affected by the inner membrane electrical gradient, cytoplasmic protein synthesis, microtubules and kinesin (energy-dependent motor proteins) (Sheahan et al., 2005). However, fission process in plant mitochondria requires dynamin-related proteins (DRP); the model plant *Arabidopsis thaliana* has a fission proteins system called DRP3A/B. In yeast, mitochondrial fission requires an orthologue of Fis1 called BIGYIN and a plant-specific factor of unknown function called NETWORK1/ELONGATE MITOCHONDRIA1 (Logan, 2010). A diverse range of other proteins including MIRO1 GTPase, the phosphatidylethanolamine biosynthesis enzyme PECT-1 and myosin XI-K also influence either the morphology or distribution of mitochondria.

Plant respiration and ETC

Photosynthesis and respiration are the main metabolic pathways of carbon dioxide assimilation and energy production in plants. In photosynthesis, sunlight, CO_2 and H_2O are used to produce carbohydrates and release O_2. The

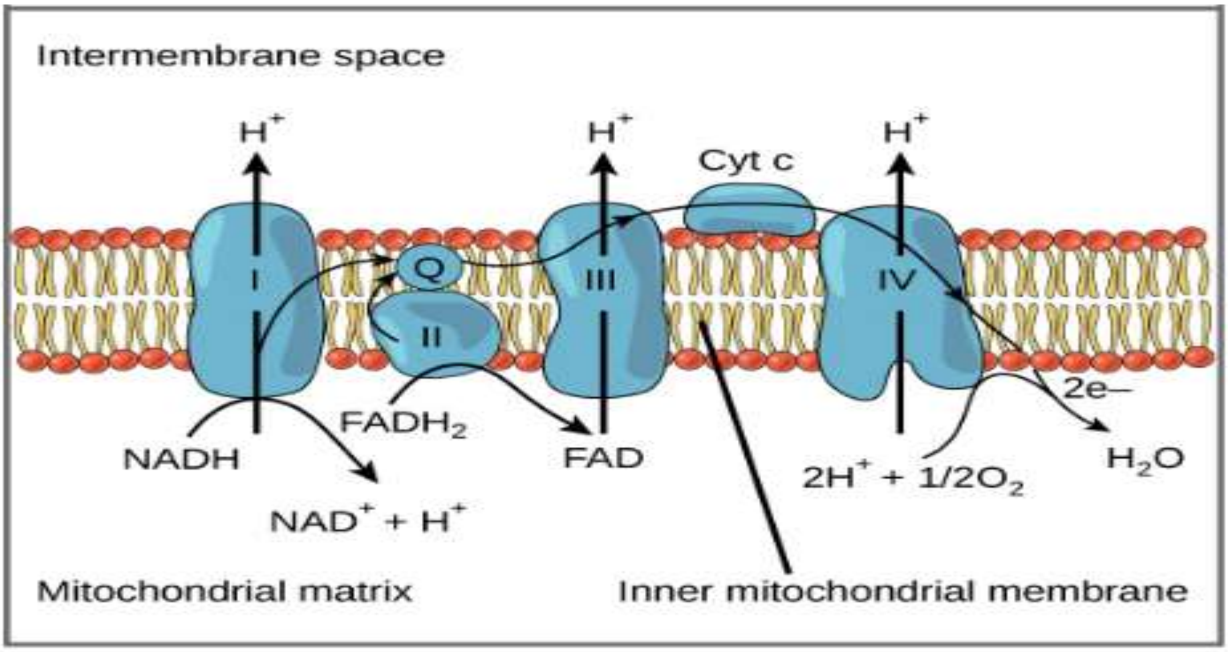

Fig. II. The inner mitochondrial membrane protein complexes

produced carbohydrates in photosynthesis are consumed during respiration to support growth and maintenance through the offering of carbon intermediates, reducing equivalents and ATP. Respiration, in turn, liberates CO_2 and converts O_2 back to H_2O. Mitochondrial matrix encompasses the dicarboxylic acid cycle enzymes, which produce energy through the reduction of electron carriers, NADH and $FADH_2$. Pyruvate crosses mitochondria directly from the cytosol via pyruvate translocator, but it can also be generated in the mitochondrial matrix from malate by malate dehydrogenase (Tronconi et al., 2008). Pyruvate dehydrogenase oxidize pyruvate forming acetyl CoA which is the starting point for TCA cycle. The conservative pathway for electron transport in mitochondria comprises electrons passage from TCA cycle by means of four inner membrane protein complexes to oxygen (Fig. II). This is in a consort with protons translocation to the inter-membrane space generating an electrochemical potential gradient, which is used to drive ATP synthesis. Many attempts have been made for investigating the biochemical composition and mechanisms of the mitochondrial electron transport chain (Huang et al., 2008; Lee et al., 2008; Ito et al., 2009) with a great focus on the functional associations (Braun and Zabaleta, 2007) and the supercomplexes (Eubel, 2003).

Complex I (NADH–coenzyme Q reductase) function is to convey electrons to the respiratory chain and quinol in the membrane through oxidizing the soluble carrier molecule NADH. Oxidation on NADH releases amount of energy harnessed in pumping 4 protons from the matrix into the cristae lumen. Complex I consists of about 49 subunits, the largest complex among mitochondrial complexes, and is specialized in reducing ubiquinone by oxidizing NADH.

$$\underset{\text{(Reduced)}}{\text{NADH}} + \underset{\text{(Oxidized)}}{\text{CoQ}} + 2H^+ \longrightarrow \underset{\text{(Oxidized)}}{\text{NAD}^+} + H^+ \ \underset{\text{(Reduced)}}{\text{CoQH}_2}$$

Complex II (Succinate/ubiquinone oxidoreductase) is an enzyme complex responsible for oxidizing succinate to fumarate in TCA cycle. The released electrons are exported to the ETC via the reduction of ubiquinone.

$$\underset{\text{(Reduced)}}{\text{Succinate}} + \underset{\text{(Oxidized)}}{\text{CoQ}} \longrightarrow \underset{\text{(Oxidized)}}{\text{Fumarate}} + \underset{\text{(Reduced)}}{\text{CoQH}_2}$$

Electron exchange through complex II is independent of proton translocation. It has been shown that this complex is the smallest complex in the ETC and is the only complex completely encoded by the nuclear genome. Electron flow through this complex is not associated with proton translocation (Cecchini, 2003).

Complex III (UQ-cytochrome c oxidoreductase) is made up of 10 subunits. It reduces the soluble electron carrier "cytochrome c" by transferring electrons from the reduced quinol. This process is accompanied by pumping one proton. Polyacrylamide gel electrophoresis (PAGE) technique has been used in identifying most of the subunits in the mitochondria of *Arabidopsis* and has been found to be belonged to a set of mainly single-copy genes in the nuclear genome (Meyer et al., 2008).

$$CoQH_2 + 2Cyt\ c^{3+} \longrightarrow CoQ + 2H + 2Cyt\ c^{2+}$$

(Reduced)(Oxidized) (Oxidized)(Reduced)

Complex IV (cytochrome c oxidase) is a 14-subunit complex that catalyzes the reduction of oxygen into water (the final step of electron transport). Electron transport is coupled with proton translocation at complexes I, III, and IV, generating a pH and electrochemical gradient.

$$2Cyt\ c^{2+} + 2H + \frac{1}{2}O_2 \longrightarrow 2\ Cyt\ c^{3+} + H_2O$$

(Reduced) (Oxidized)

Complex V is responsible for the last step in the oxidative phosphorylation as it catalyzes the conversion of the electrochemical gradient across the inner membrane into ATP, providing cellular energy for biosynthetic processes. This complex is a dimer of two functional domains, F1 and F0 (Fig. III). The general configuration of complex V, in both prokaryotic and eukaryotic organisms, is highly conservative. In plants, F1 domain in mitochondrial ATP synthase consists of 5 subunits (α, β, γ, δ, and ε). While α-subunit is the only subunit encoded in the plant mitochondrial genome, the other subunits are encoded in the nuclear genome (Unseld et al., 1997). This domain projects into the matrix associated with the F0 stalk (composed of 14 subunits) integrated into the inner membrane. Some reports presume a prominent role for complex V in the inner mitochondrial membrane folding (Dudkina et al., 2006).

$$ADP + P_i \longrightarrow ATP + H_2O$$

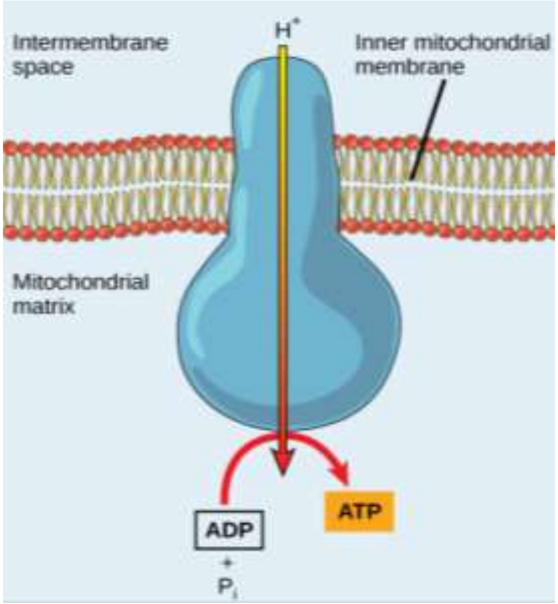

Fig. III. Complex V in the inner mitochondrial membrane

Mitochondrial response to stresses

Mitochondria are principle actors in respiratory metabolism. Therefore, it is necessary to investigate the influence of various stressors on its function, for understanding the manner of cellular adaptation with stress situations to keep mitochondria well functionalized. Recent noteworthy developments have been made to determine cellular responses to mitochondrial dysfunctions. Partially, retrogressive response characterization of the mitochondrion functional status that controls the expression of nuclear genes encoding mitochondrial proteins have been used (Rhoads, 2011). For example, microarray studies on the transcriptional response have demonstrated dysfunction of complex I in response to rotenone inhibitor (Garmier et al., 2008), complex III in response to antimycin A (AA) inhibitor (Umbach et al., 2012), aconitase in response to monofluoroacetate (Umbach et al., 2012), and ATP synthase in response to mutation (Geisler et al., 2012). As an overall conclusion, these studies established that abiotic and biotic stresses caused a considerable overlap with transcriptional responses (Van Aken et al., 2009b; Umbach et al., 2012). This means that mitochondrial function is significantly affected by stress conditions triggering the mitochondria stress response.

Mitochondrial ROS

Reactive oxygen species (ROS) are a group of free radicals, reactive molecules, and ions that are derived from molecular oxygen. It has been assessed that around 1% of O_2 used by plants is harnessed in ROS production (Asada, 1987). The common forms of ROS are hydrogen peroxide (H_2O_2), superoxide ($O2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen ($^1O_2$) (Puntarulo et al., 1988). Recently, it has been established that ROS have their cons and pros, as they are assessed as toxic molecules as well as main controllers of several biological processes like growth, cell cycle, apoptosis, signaling, biotic and abiotic cell responses, and development (Mittler et al., 2004; Fujita et al., 2006). The imbalance between ROS production and scavenging could be induced by various stress conditions like salinity, drought, temperature, heavy metals, nutrient deficiency, air pollution, herbicides and pathogen invasions. These abiotic and biotic factors result in rapid accumulation of ROS in various organelles causing enormous injury to cell structures (Bhattacharjee, 2005). Mitochondrial ROS at high concentrations bring about impairment in their function through damaging biomolecules, but they operate as secondary messenger molecules for signaling cascades at low concentrations leading to a number of responses in plant cells.

Source of ROS in mitochondria

Since plant mitochondria are a major source of energy, they are accepted to be a primary site for the production of ROS, in addition to being attacked by ROS (Rasmusson et al., 2004). Aerobic respiration in mitochondria ends with the reduction of O_2 into water through the passage of energy-rich electrons by means of a series of electron carriers during mitochondrial ETC, which is an irresistible source of ROS. Although mitochondrial ROS generation occurs normally at respiration process, the stressful conditions could increase the chance of ROS generation in various cellular organelles. In the mitochondrial ETC, the most common sites for O_2 production are complexes I and III. Oxygen is relatively reactive in aqueous media; however, SOD could reduce it into H_2O_2 (Sweetlove and Foyer, 2004). There is a certain assumption that about 5% of oxygen consumed by mitochondria result in H_2O_2 generation (Møller, 2001). The resultant H_2O_2 can react with highly reactive ions like Fe^{2+} and Cu^{+} producing highly toxic uncharged hydroxyl radical (OH). The OH radical is highly mobile and can go through mitochondrial membrane diffusing into other organelles (Sweetlove and Foyer, 2004; Rhoads et al., 2006). ROS can be produced in the mitochondrial matrix enzymatically. Some enzymes are involved directly in ROS production like aconitase. Meanwhile some enzymes are indirectly involved in ROS production through feeding electrons into ETC like 1-galactono-γ-lactone dehydrogenase (GLDH) (Andreyev et al., 2005; Rasmusson et al., 2008). Superoxide radical ($O2^{\bullet-}$) is the primary ROS formed during ETC through monovalent reduction. However, its conversion into the most stable and membrane permeable H_2O_2 takes place rapidly either by MnSOD or ascorbate peroxidase (APX).

ROS involvement in plant defense

Exposure of plants to any stressful condition results in activation of ROS generation and/or inactivation of antioxidant defense system, specially the enzymatic one. The perception of oxidative stress signal stimulates the plant defense system to form a suitable acclimation mechanism. The availability and reduction status of the antioxidant molecules like glutathione, ascorbate, thioredoxin, and NADPH determine the appropriate cellular response against the mitochondrial ROS. For example, the presence of glutathione and ascorbate direct the cell into the expression of some defense genes and generation of signals for redox homeostasis and programmed cell death (apoptosis) (Foyer et al., 1997; Pinto et al., 2002). The rate of ROS generation in other organelles can be modified according to the events occurring within the mitochondria. The mitochondria ETC is directly connected with the excessively produced reductants during the light-dependent reactions of photosynthesis. In case reductants produced in the chloroplast exceed the mitochondrial ETC processing ability, in addition to increased probability of ROS generation in the mitochondria, the reduced activity in photosynthetic reaction system could increase

ROS production within the chloroplast (Fernie et al., 2004). Recently, ROS has been found to positively contribute to the defense responses against various stresses through signal transduction pathways and affecting the expression of defense genes (Dalton et al., 1999).

ROS regulation in mitochondria during abiotic stresses

Generation of mitochondrial ROS has been reported to increase upon the exposure of plants to abiotic stress, in particular drought and salinity (Pastore et al., 2007). The role of ROS during abiotic stresses seems to be completely different from their role during pathogen invasion. Under abiotic stress conditions, plants stimulate the expression of ROS scavenging enzymes and metabolites. Several plant species were reported to increase the activity of their enzymatic antioxidant defense systems during their exposure to the water stress (Chool Boo and Jung, 1999; Sharma and Dubey, 2005). Comparative evaluation of the antioxidant defense system in water-deficiency tolerant and water-deficiency sensitive plant species revealed elevated antioxidant capacity in tolerant species. In wheat, water-deficiency tolerant genotype "C306" showed higher ascorbate peroxidase (APX) and catalase (CAT) activities, along with higher ascorbate (AsA) content and lower H_2O_2 and MDA contents, compared to the water-deficiency susceptible genotype "HD2329" (Sairam et al., 1998). Moreover, rice plants showed a significant increase in the *de novo* synthesis of AsA through the increased activities of monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) as an adaptive mechanism in response to water deficit-induced oxidative damage (Chool Boo and Jung, 1999; Sharma and Dubey, 2005). The key enzymes controlling this pathway of signaling were detected to be the mitochondrial alternative oxidase (AOX) and manganese SOD (Mn-SOD) (Foyer, 2005). The role of AOX is to sustain the reduction state of the ubiquinone (UQ) pool and decrease the generation of ROS within the mitochondria whereas Mn-SOD stimulates the initial step in ROS detoxification by converting the superoxide radical (O_2^-) into H_2O_2 (Møller, 2001; Rhoads et al., 2006). A current study on *Arabidopsis thaliana* mutants without the mitochondrial AOX1a gene showed that these mutants are susceptible to water deficiency and light stress combinations, but they showed an increased expression of new specific genes involved in antioxidant defense mechanisms within the chloroplast and the mitochondria (Giraud et al., 2008).

Exposure of plants to excessive amounts of salts stimulates the production of ROS within the different plant tissues and organelles. Salt stress stimulates the overproduction of ROS ($^1O_2$, $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2) through impairing the cellular electron transport in the different organelles like chloroplasts, peroxisomes, and mitochondria, together with the initiation of specific metabolic pathways like photorespiration. The salt-induced ROS cause the impairment of normal metabolism by increasing the rate of lipid peroxidation, protein misfolding, and nucleic acids denaturation in many plant species (Karray-Bouraoui et al., 2011). In a study conducted by Mishra et al. (2013), the salt-sensitive seedlings of indica rice exposed to salt stress showed a considerable increase in the rate of $O2^{\bullet-}$, H_2O_2 and MDA production. However, these seedlings showed lower contents of thiol, AsA and reduced glutathione (GSH) and lower activity of antioxidant enzymes, in comparison with the salt-tolerant cultivar. Their study proposed that the higher contents of antioxidant molecules AsA and GSH, in a coordinated higher activity of the antioxidant enzymes SOD, CAT, GPX, APX, and GR are the main clues representing the salt tolerance in indica rice seedlings. The argument that ROS could travel for long distances within the plant is still huge due to the high reactivity of ROS and their immediate scavenging in the apoplast by the antioxidant systems. More work is required on the plants with varying mechanisms and levels of ROS scavenging and/or ROS production mechanisms could answer this question.

ROS and redox signaling in mitochondria during stresses

Mitochondrial retrograde signaling

Mitochondrial retrograde regulation (MRR) is a term expressing the mitochondrial signaling, and the cellular responses to mitochondrial dysfunction in plants. This issue has been studied principally in the cases of male infertility or embryo mortality resulting from mitochondrial malfunction (Rhoads et al., 2006; Rhoads and Subbaiah, 2007). The increased rate of ROS production and accumulation seems to be closely involved in MRR (Rhoads et al., 2006; Rhoads and Subbaiah, 2007). Studies on ROS generation from nitric oxides (NOs) and the interactions of NO with biological systems have been launched using the concept of redox signaling in biology (Finkel, 2011).

The use of mitochondrial electron transport chain (mtETC) inhibitors drive the expression of nuclear-encoded alternative oxidase 1 (AOX1); the major marker for retrograde responses in plants resulted in studying the mitochondrial redox and ROS signaling. The most commonly used mtETC inhibitors were antimycin A (AA; Complex II), rotenone (complex I – NADPH dehydrogenase), and monofluoroacetate (MFA; TCA cycle), that enhance AOX expression or increase the concentration of the antioxidants that prevent AOX stimulation (Vanlerberghe et al., 2002). After that, the importance of mitochondria in redox signaling has been evolved in many biological areas. The most significant role of mitochondrial ROS is their role in oxygen sensing during hypoxia (Patten et al., 2010). The formation of superoxide radical (O_2^{-}) during mtETC was found to be stimulated to higher levels in hypoxia conditions (Guzy et al., 2005). The higher levels of mitochondrial O_2^{-} stimulate superoxide dismutase to convert it into H_2O_2 in the mitochondrial matrix. The formed H_2O_2 then diffuses into the cytosol causing the stabilization of hypoxia-inducible factor-1α (HIF-1α), which stimulates the transcription of specific genes activating cellular defense against hypoxia (Sanjuán-Pla et al., 2005). Mitochondrial ROS contribution in redox signaling is associated with different biologically significant processes, like the determination of yeast chronological life-time (the period in which the cell in a culture at the stationary phase keep on viable) (Pan et al., 2011), and mitochondrial homeostasis (St-Pierre et al., 2006). *Arabidopsis* mutants deficient in MRR were found to be ineffective in inducing luciferase activity (regulated by the AOX1a promoter) following antimycin A(AA) treatment; though, some of these mutants maintained its response to monofluoroacetate (MFA) (Zarkovic et al., 2005). The studies on AOX1a promoter sequence in *Arabidopsis* identified a repressor B cis-acting element which is a target for the transcription factor ABI4 (abscisic acid insensitive 4), and AOX1a promoter activity was fully de-repressed in ABI4 (Giraud et al., 2009).

ROS modulates Programmed Cell Death

Programmed cell death (PCD) is a kind of responsive mechanism to severe disorders in ROS homoeostasis. PCD is a specific term that refers to the controlled death of cells that occurs during defense (hypersensitive response) and development (e.g., in the suspensor during the last stages of embryogenesis) (Lombardi et al., 2007). In plants, many types of PCD are found; however, the most common form is apoptotic-like PCD. In apoptotic-like PCD the mitochondria incorporate the stress and developmental signals (Reape and McCabe, 2010). Apoptotic-like PCD comprises many procedures beginning with Ca^{2+} release to the cytosol, mitochondria permeabilization through activation of a permeability transition pore (PTP), following the release of apoptogenic proteins (cytochrome c) and ending with the activation of metacaspases (cysteine proteases).

In several cases of plant-pathogen interactions, PCD is a desirable option for the host plant. For example, plant invasion with nectrophic fungi kill the host tissues by secreting specific mycotoxins which stimulate the production of ROS causing eventual programmed cell death, enabling them to feed on the host-dead tissue (Stone et al., 2000). Moreover, exposure of transgenic tobacco plants to TMV invasion caused the expression of *p35* gene of baculovirus, that stimulates the PCD in tobacco plants as a functionally-significant adaptive strategy for viral invasion (Del Pozo and Lam, 1998). In normal conditions, tobacco cultivars carrying the *N* gene possess a potent resistance against TMV virus, and the viral replication just followed by small hypersensitive response (HR) lesions. On the other hand, in transgenic plants containing the *p35* gene, the virus could spread systematically away from the infection site due to the delay in cell death. Even though HR occurs immediately in these plants following viral attack, the slowness of this process hinders the viral movement to the neighboring cells (Lam et al., 2001).

Recently, it has been revealed that PCD plays a significant role in allelopathic plant-plant interactions, besides its role in plant-pathogen interactions (Bais et al., 2003). For example, the root system of *Centaurea maculosa* secretes a flavonoidal phytotoxin called catechin, which restrains the growth of the contiguous plants by inducing ROS accumulation in their root meristems leading to Ca^{2+}-release dependent cell death. This allelopathic mechanism allows the dispersal of *Centaurea maculosa* eliminating the other species from their habitat.

Uncoupling proteins

Biotic and abiotic stress tolerance involves a variety of physiological and biochemical mechanisms. Different approaches have been applied to increase crop plants tolerance, in addition to transgenic plants which became an influential and potential technique in this field. A main issue in approximately all abiotic stresses is the generation of secondary (oxidative) stress at the cellular level. The objective of enhancing the antioxidant potential of plants for increasing tolerance to abiotic stresses became a priority for many scientists. In this context, plant mitochondria, like mammalian mitochondrial, possess uncoupling proteins in their inner membrane known as "plant uncoupling mitochondrial proteins" (Zhu et al., 2011). Plant mitochondrial uncoupling proteins (pUCPs) are a class of mitochondrial anion carriers integrated in the inner mitochondrial membrane (Picault et al., 2004). Many types of abiotic stresses induce the genes encoding pUCPs (Kreps, 2002; Seki et al., 2002). A lot of evidence emphasize the importance of UCP in sustaining mitochondrial function under both normal and stress conditions. Previous reports had shown that the plant UCP in the isolated mitochondria transport protons by the mechanism of a fatty acid cycling, like those of mammalian UCP (Jevzek et al., 1996). Fatty acids are not directly involved in the activation of UCP. It has been reported that exogenously produced superoxide activates UCPs in both mammalian and plant mitochondria, in the presence of fatty acids (Echtay et al., 2002). The activation of UCP by superoxide requires its presence in the mitochondrial matrix, thus the exogenously applied superoxide must cross the inner mitochondrial membrane (Echtay et al., 2002). In *Arabidopsis*, the insertion of AtUCP1 (one of UCP genes) caused a localized oxidative stress but did not disrupt the plant ability to resist the various abiotic stresses. Nevertheless, lack of UCP1 gene caused restriction of photorespiration, due to the decline in the rate of glycine oxidation in the mitochondrion (photorespiratory intermediate) and a decreased photosynthetic carbon fixation in the chloroplast (Sweetlove et al., 2006). In tobacco plants, the overexpression of AtUCP1 enabled the oxidative stress tolerance (Brandalise et al., 2003). Furthermore, overexpression of AtUCP1 improved seed germination, improved performance, and increased rates of photosynthesis under both control and stressful conditions (Begcy et al., 2011). Thus, overexpression of UCP proposed that these proteins could modify mitochondrial and cytosolic metabolism.

Alternative oxidases

Plant mitochondria have the potential to perform respiration process in the presence of some respiratory inhibitors such as cyanide (cytochrome c oxidase inhibitor) and rotenone (cytochrome c1 inhibitor), along with the conventional ETC, through nonphosphorylating pathway (non-energy conserving). In the presence of cyanide, quinol oxidase [specific alternative oxidase (AOX)], which diverges from the respiratory chain at ubiquinone (UQ), catalyzes the oxidation of UQH_2 and the reduction of oxygen into water without the concomitant transfer of protons (cyanide-insensitive respiration) (Considine et al., 2002). Up to now, the role of AOX in respiration is still being studied. Previous reports indicated that AOX allows the overflow of electrons from UQH_2 to oxygen after the COX reaches its full capacity (Lambers, 1982). But, the use of oxygen isotopes in differentiating AOX from COX demonstrated that COX can counterbalance the inhibition in AOX activity, indicating that AOX and COX are competitors for electrons (Ribas-Carbo et al., 1995). Many trials have been emerged for explaining the role for AOX, but most of these explanations affirmed that AOX reduces the oxidative stress through buffering respiratory fluctuations (Rasmusson et al., 2009).

A main distinction between the role of AOX in metabolic homeostasis and their role in signaling homeostasis is essentially recognized. Dissimilar to metabolic pathways, secondary messengers are frequently integrated in signals amplification during the signaling pathways. Accordingly, the changes in AOX activity for providing signaling homeostasis are insignificant in comparison with the changes in AOX activity during metabolic homeostasis. For instance, during mitochondrial ETC, the minute changes in the rate of electron transport or in the potential of the membrane can have a large impact on ROS generation, consequently ROS signaling is activated (Echtay, 2007). Several studies have demonstrated that ROS homeostasis is disturbed by several biotic and abiotic stressors, such disturbances activate the alternative ETC components, including AOXs, for mitigating ROS production and keeping ROS homeostasis (Polidoros et al., 2009). AOX seems to be involved in the antioxidant system in plant mitochondria and is encoded by many genes in most plants that can be classified as either AOX1 or AOX2 types (Considine et al., 2002). The pattern of AOX expression varies with the plant organ, the developmental stage and the stress type (Whelan et al., 1996; Considine et al., 2002; Thirkettle-Watts et al., 2003). The evidence supporting the role of AOX in stress response came from:

1. The lack of AOX results in disturbance in stress tolerance. The AOX mutant tobacco lines were more vulnerable to the initiation of programmed cell death, hence AOX in these plants were called "survival protein" (Robson and Vanlerberghe, 2002). Additionally, antioxidant enzymes like catalase and glutathione peroxidase were overexpressed in cell suspensions and leaf discs of plant mutants lacking AOX gene, with respect to wild type (Amirsadeghi et al., 2006).
2. Various signals induce the expression of AOX, referring to its role as a common response to different stresses for example, analysis of the cis-acting regulatory elements (CAREs) regulating AOX expression. In *Arabidopsis*, the analysis of the CAREs in the promoter of AOX1a, showed that there are several sequences that can bind transcription factors regulating the expression of stress-responsive gene (Van Aken et al., 2009a). A region of 93 bp in the promoter of AOX1a was found to be important for induction by antimycin A and monofluoroacetate, containing WRKY and Dof-binding motifs (Dojcinovic et al., 2005).
3. AOX regulates the induction of programmed cell death (Van Aken et al., 2009a). The hypersensitive response (HR) is an example of localized PCD in response to pathogen infection in plants and acts to restrict further infection of surrounding tissues (Reape and McCabe, 2008). Thus, AOX raises the cell's threshold for the execution stage of cell death. Furthermore, although AOX is unlikely to play any specific role in viral resistance, it was shown that increased AOX expression leads to reduced lesion size in the HR (Ordog et al., 2002), again consistent with a role in suppressing PCD.

Disparity in AOX genes expression under abiotic stresses

Many previous studies showed that different environmental stresses like drought, salinity, chilling, light, temperature, heavy metals, and minerals deficiency are able to induce the expression of AOX or its mRNA. For example, exposure of *Arabidopsis thaliana* to chilling had induced the expression of AOX1 in its leaves, but AOX1b, AOX1c, and AOX2 genes were not affected by chilling although AOX1d expression decreased following the exposure to chilling stress (Borecký et al., 2006). Furthermore, the roots and leaves of rice seedlings exposed to drought, chilling, and salinity showed a significant induction of AOX1a and AOX1b expression; however, the gene of AOX1c non-significantly affected by these stress factors (Christ et al., 2002; Li et al., 2013). Most of the studies focused on AOX1a because it is the most stress-responsive gene. Most environmental stresses (water stress, chilling, salinity, light, and nutrient deficiency) were reported to induce the expression of AOX1a gene in *Arabidopsis* (Watanabe et al., 2008, 2010; Wang et al., 2010; Zhang et al., 2010).

Additionally, different mechanisms have evolved elucidating the role of AOX in abiotic stresses tolerance. These mechanisms include:

(i) AOX possesses the ability to reduce the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and sustain redox homoeostasis within plant cells (Maxwell et al., 1999; Giraud et al., 2008; Cvetkovska and Vanlerberghe, 2012);

(ii) (ii) AOX can amend the impact of abiotic stresses on photosynthetic machinery (Bartoli et al., 2005); and

(iii) (iii) AOX modulates carbon fixation efficacy and the carbon/nitrogen, the NAD(P)H/ATP, and the ATP/ADP ratios (Parsons et al., 1999; Watanabe et al., 2008; Rasmusson et al., 2009; Kornfeld et al., 2013).

Other AOX genes other than AOX1a were found to be expressed under abiotic stresses. For instance, AOX1b transcription increased in *Arabidopsis*, like AOX1a, under elevated light intensity. Additionally, AOX1a and AOX1b-deficient mutants of *Arabidopsis* showed severe photodamage in the plant leaves under high light conditions, demonstrating that both genes play a significant role in adaptation to high light stress. The AOX1a mRNA level in *Arabidopsis* leaves was found to be higher than that of AOX1b at high light stress. Also, AOX1a-deficient mutants showed severe photodamage symptoms compared to those of AOX1b-deficient mutants (Zhang et al., 2010). Apart from light stress, AOX was shown to play an important role in acclimation to drought stress in wheat leaves (Bartoli et al., 2005; Vassileva et al., 2009), but no significant increase in AOX level was observed in soybean leaves (Ribas-carbo et al., 2005). Nevertheless, *Medicago* leaves showed a severe decline in the AOX level under drought (Filippou et al., 2011). Salt stress was reported to cause harmful effects on the mitochondrial electron transport chain, leading to increased ROS accumulation and membrane deteriorations, as well as inducing the antioxidant system within mitochondria (Chen et al., 2009; Zsigmond et al., 2012). In pea, long-term exposure to salt stress considerably decreased the normal respiratory pathway (cyt pathway) whilst AOX respiratory pathway was retained and represented about 50% of the whole electron flow (Martí et al., 2011). As a concluding remark, scientific studies on different AOX genes expression under various stress factors showed that these proteins differentially contribute to abiotic stress tolerance, depending on stress type and plant species.

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