Chapter 4

# Catalase

## A Versatile Antioxidant in Plants

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### 4.1 INTRODUCTION

Catalase (CAT, 1.11.1.6) is present in all living beings, ranging from unicellular prokaryotes to multicellular eukaryotes. The evolutionary design of catalytic enzymes started about 3.5 billion years ago when the ancestral planctonic bacteria began aerobic respiration (Lenton, 2003). Thereafter, the catalase evolution proceeded with development of the aerobic biosphere on Earth (Drews, 2011). All aerobic organisms performed both photosynthesis and respiration within cells with generation of harmful reactive oxygen species (ROS), namely superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide ($H_2O_2$) and singlet oxygen ($^1O_2$). The enhanced production of ROS can pose oxidative stress to cells by causing peroxidation of lipids, oxidation of proteins, and damage to nucleic acids, enzyme inhibition, activation of the programmed cell death (PCD) pathway and ultimately leading to cell death (Ahmad et al., 2010a,b, 2011; Ahmad and Umar, 2011; Koyro et al., 2012; Sharma, 2012). Further, the enzymatic and nonenzymatic antioxidant defense system had developed for detoxification of ROS in aerobic organisms. As an enzymatic antioxidant, catalase has a key role to prevent cellular oxidative damage by degrading hydrogen peroxide ($H_2O_2$) into water and oxygen with high efficiency (Ahmad et al., 2011). It is a predominant peroxisomal enzyme, but it also exists in the mitochondria and cytoplasm of cells. In plants, catalase scavenges $H_2O_2$ generated during mitochondrial electron transport, oxidation of the fatty acids, and most importantly, photorespiratory oxidation during normal and stress conditions. Catalase always draws the attention of researchers due to its efficient catalytic and regulatory properties among all antioxidant enzymes of the plant system. Thus, it has been purified and extensively characterized at the genetic, biochemical, and molecular level of plants. Dounce (1983) proposed the monofunctional and

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bifunctional mechanism for the catalytic action of catalase. Recently it has been established that catalase is present as multiple isoforms (CAT1, CAT2, CAT3) encoded by multiple genes (*Cat1*, *Cat2*, *Cat3*) expressed in organelle, temporal and stress specific manners. Specific evolutionary events including horizontal gene transfer, paralog formation and gene fusion have been revealed by phylogenetic study of the catalase isozymes (Zamocky et al., 2012). Although much effort has been made to understand catalase among prokaryotes and animals, very little information is available on the versatility of catalase in plants. The chapter presents a review of the overall aspects of catalase in plants.

## 4.2 OXIDATIVE STRESS

Atomic oxygen is the most abundant element in aerobic organisms. The oxygen molecule has the potential to be partially reduced and form reactive oxygen species through physical or chemical activation (Halliwell and Gutteridge, 1992). Physical activation occurs mainly by transfer of excitation energy from photo-activated pigments, such as the excited chlorophyll molecule to dioxygen, which absorbs sufficient energy and, as a result, the spin of one $e^-$ is inverted and the singlet state of oxygen ($^1O_2$) is formed (Perl et al., 1992). Chemical activation is the other mechanism to circumvent spin restriction. It occurs when molecular oxygen absorbs activation energy of 22 Kcal/mol to rise from its ground state and by a chain of reduction reactions various stable intermediates are formed in a stepwise manner. Four $e^-$ (and 4 protons) are required for the full reduction of dioxygen to water; all the intermediates of univalent reduction of oxygen, namely superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide ($H_2O_2$), are known as reactive oxygen species (ROS) (Yu et al., 1992). In a biological system reactive oxygen species are mainly generated in chloroplast, mitochondria and peroxisomes during respiration, photosynthesis and metabolic reactions (Ahmad et al., 2011).

## 4.3 $H_2O_2$ AN EFFECTIVE ROS PRODUCED IN PLANTS: PRODUCTION AND TOXICITY

Though all reactive oxygen species are extremely reactive and cytotoxic, $H_2O_2$ is unique among them. $H_2O_2$ is formed during reactions like dismutation of $O_2^{\bullet -}$ by superoxide dismutase (SOD) activity, photorespiration, β-oxidation of fatty acids and proton-induced decomposition of $O_2^{\bullet -}$ and defense against pathogens (Scandalios, 1994; Sagi and Fluhr, 2006; Andre et al., 2013). It is produced in various subcellular organelles and can diffuse through aquaporins of intracellular and intercellular membranes of cells (Bienert et al., 2007).

In peroxisomes $H_2O_2$ can be formed by glycolate oxidase during the photorespiratory glyoxylate cycle (Foyer et al., 2009) and by xanthine

oxidase with the superoxide dismutase enzyme system (Corpas et al., 2008). Moreover, chloroplast also generates ample amounts of $H_2O_2$ by electron leakage during electron flow through the photosystem (I), which results in production of the superoxide radical. Further, $O_2^{\bullet -}$ reduces to form $H_2O_2$ by the action of superoxide dismutase (SOD). Superoxide radical can also react with NAD(P)H molecules to produce $H_2O_2$. $H_2O_2$ may also be generated in chloroplast during synthesis of glycolate from 3-Phosphoglycolate and conversion of glycolate back to 3-Phosphoglycolate in peroxisome.

In mitochondria the rate of electron transfer is directly proportional to $H_2O_2$ production during respiration. The electron-rich form of ubiquinon, ubisemiquinon, can reduce molecular oxygen to form superoxide radical. The radical, being charged, accumulates in the mitochondria and SOD degrades it to $H_2O_2$ and $O_2$ (Fridovich, 1975).

Another source of $H_2O_2$ production is the conversion of ribonucleotide diphospates to deoxyribonucleotide diphosphates through riboncleotide reductase. An enzyme, namely NAD (P)H:flavin oxidoreductase, produces tyrosyl free radical for the action of ribonucleotide reductase. During production of tyrosyl free radical, the enzyme also liberates superoxide ion which readily changes to $H_2O_2$ via superoxide dismutase.

$$2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

Chance et al. (1979) also reported the production of $H_2O_2$ in cytosol, endoplasmic reticulum (ER) and nucleus during different metabolic processes.

$H_2O_2$ is a potentially dangerous by-product of oxygen metabolism, a process that occurs in most living things, from aerobic bacteria to plants and animals. $H_2O_2$ is quite reactive with molecules containing $Fe^{2+}$ or other transition metals through the Fenton reaction, which results in homolysis of $H_2O_2$ to two harmful $^{\bullet}OH$ radicals (Becana et al., 1998; Sharma, 2013a). Moreover, direct reaction of $H_2O_2$ with the $-SH$ groups inactivates enzymes, e.g. fructose bisphosphatase (Charles and Halliwell, 1981), sedoheptulose bisphosphatase, and glyceraldehyde 3-phosphate dehydrogenase. $H_2O_2$ can react with metalloproteins, leading to dissociation of their metal ion and hindering their biological activity.

In addition, $H_2O_2$ has an important role in processes from signal transduction, cell wall lignification, plant growth, development of root hair, and xylem differentiation, to root shoot coordination and stomatal control (Cheesman, 2007).

## 4.4 ANTIOXIDANT SYSTEM AGAINST $H_2O_2$

A well-maintained balance between $H_2O_2$ production and its removal is essential for the survival of plants. To minimize the damaging effects of $H_2O_2$, plant cells evolved both enzymatic and nonenzymatic defenses.

Nonenzymatic consist of glutathione, ascorbate and flavonoids. The enzymatic defense system includes the ascorbate glutathione cycle (i.e. ascorbate peroxidase (APX), ascorbate reductase, glutathione reductase) and catalase (Ahmad et al., 2010a,b, 2011; Ahmad and Umar, 2011; Singh et al., 2012; Koyro et al., 2012). Among these, catalase is the most active catalyst produced in all aerobic organisms. It decomposes $H_2O_2$ at an enormously fast rate with an energy-efficient approach.

## 4.5 CATALASE: A POTENT ANTIOXIDANT IN PLANTS

Catalase plays a central role in maintaining balance of cellular hydrogen peroxide in plants. It was the first antioxidant enzyme documented and it appears in all prokaryotes and eukaryotes. Degradation of hydrogen peroxide ($H_2O_2$) through catalase was first noticed by Loew (1901). Then Warburg (1923) proposed that catalase is inhibited by cyanide so it may be an iron-containing enzyme. Next, Zeile and Hellstrom (1930) discovered that catalase has a hematin prosthetic group. Catalase was initially isolated and crystallized from beef extract by Sumner and Dounce (1937). Thereafter, a great deal of information on catalase has been generated and, recently, Ray et al. (2012) have used *E. coli* as a heterologous expression system and have isolated a soluble bioactive recombinant catalase-A in rice plant. The new protein has molecular and biochemical features of a typical monofunctional plant catalase. Today, isozymes of catalase and phylogenic relationships among various species of catalase have been identified by researchers.

The significance of catalases in the antioxidant defense system of plants has been proven by various studies (Shim et al., 1999; Sharma et al., 2007; Beulah and Ramana, 2013). Willekens et al. (1997) observed that CAT-deficient mutants are more prone to oxidative stress as compared to wild plants treated with salt, ozone, paraquat and $H_2O_2$. Increased CAT activity is crucial for survival of plants under moderate metal stress, while severe metal stress leads to irreversible damage of the catalase enzyme (Youssef and Azoo, 2013; Bocova et al., 2012; Sharma and Travlos, 2012).

### 4.5.1 Location in Cellular Environment

Location and import of catalase in peroxisomes has been confirmed by studies of subcellular fractionation and in-situ activity staining in different plant species (Mullen et al., 1997). Catalase activity was also detected in isolated chloroplasts. However, no catalase has been found in the stroma of chloroplast, but in lumen a PSII membrane associated heme catalase was reported by Sheptovitsky and Brudig (1996). Catalase activity in the cytosol and mitochondria has also been reported in photosynthetic organisms (Kato et al., 1997; Spanou et al., 2012; Gu et al., 2013).

### 4.5.2 Structure of Catalase

Catalase is a heme-containing redox enzyme present in all organisms exposed to oxygen. The enzyme has a dumbbell-shaped tetrameric structure with four identical monomer subunits of 220,000 to 350,000 kD. The heme group is the key component for enzymatic activity. The heme prosthetic group of each monomer unit consists of a central iron atom attached with a protoporphyrin ring (Fig. 4.1A,B). The protoporphyrin ring has four pyrrole rings linked by methene bridges. Side chains made up of four methyl, two vinyl, and two propionate are also attached. The central iron atom of the prosthetic group may present in ferrous ($Fe^{+2}$) or the ferric ($Fe^{+3}$) valance state. A specific nonpolypeptide unit is also bound at the catalytic center of some plant catalase. In certain species catalase monomer also contains one tightly bound NADP (nicotinamide adenine dinucleotide phosphate) for each subunit. The NADP acts as the main electron carrier in reductive biosynthesis and shields the enzyme oxidation by its $H_2O_2$ substrate (Regelsberger et al., 2002).

Catalase has the highest turnover rates among all antioxidant enzymes. It has been reported that one molecule of catalase can convert 6 million molecules of $H_2O_2$ into $H_2O$ and $O_2$ per minute.

On the basis of subunit sizes, quaternary structures, prosthetic groups, and sequences, catalase can be divided into three main groups (I - Monofunctional catalases, II - Catalase peroxidases and III - Nonheme catalases) (Loewen et al., 2002). Most of the plant species have I - Monofunctional catalase, which executes peroxidase activity on small organic substrates.

### 4.5.3 Molecular Isoforms of Catalase

In recent years, the role of catalases in plant metabolism, antioxidant defense system, and signaling has been reported by various studies. Multiple molecular forms of catalase have been reported in different plant species, e.g. *Nicotiana tobacco* (Havir and McHale, 1987), cotton (Ni et al., 1990), *Nicotiana plumbaginlfolia* (Willekens et al., 1994b), *Arabidopsis thaliana* (Zhong et al., 1994), *Pinus taeda* (Mullen and Gifford, 1993), sunflower (Eising et al., 1989), pumpkin (Yamaguchi et al., 1986) and tomato (Gianinetti et al., 1993). Catalase nomenclature is also based on its isoforms in different plant species. According to a classification suggested by Willekens et al. (1995), Class I, Class II and Class III catalases are specifically expressed in photosynthetic tissues, vascular tissues and reproductive tissues, respectively.

The presence of multiple catalase isozymes suggests structural and functional versatility of catalases in a variety of plant species. The cDNA of various catalases has been isolated and characterized from different plant species to understand genes and their regulatory components (Scandalios, 1992). The

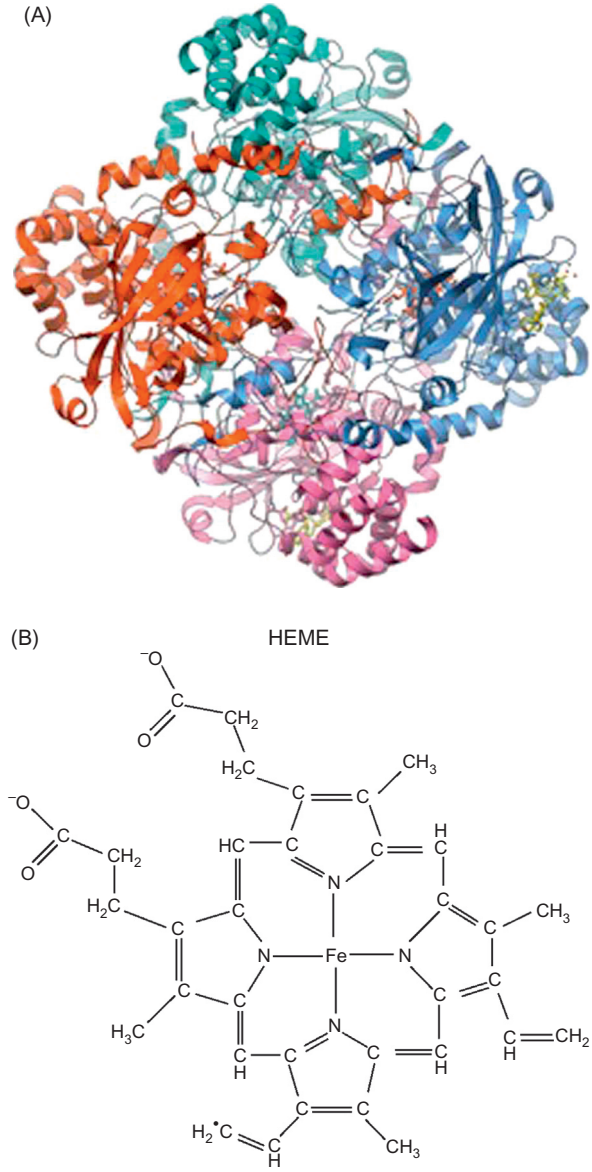


**FIGURE 4.1** (A) Three dimensional structure (PDB picture based on 1dgb). (B) Single heme group of catalase.

isozymes of catalase exhibit developmental stage and organ specificity in plants (Table 4.1).

On the basis of unrooted parsimony and codon analysis of plant catalase sequences, Guan et al. (1996) have established the level of phylogenetic and

**TABLE 4.1** Site and Tissue Specific Gene Expressions of *Cat* Gene

| S. No. | Developmental Stage of Plant | Plant Organ | Catalase Isozyme Expressed | Reference |
|---|---|---|---|---|
| **1.** | Anthesis | Pollen | CAT-1 | Scandalios et al., 1984 |
| **2.** | Ovule development and early postpollination kernel development | Pericarp | CAT-3 and CAT-1 | |
| **3.** | Postpollination | Scutellum | CAT-1 | |
| **4.** | Early kernel development | Mature pollen<br>Milky endosperm<br>Aleurone<br>Scutellum | CAT- 1 | Scandalios, 1983; Wadsworth and Scandalios 1989; Acevedo and Scandalios, 1990 |
| **5.** | Late kernel development | Aleurone | CAT-1 and CAT 2 | Scandalios, 1974 |
| **6.** | Post imbibitions | Seeds | CAT-2 | |
| **7.** | Dark grown tissues | Leaf | CAT-1 and CAT-3 | |
| **8.** | Light grown tissues | Green leaf | CAT-2 | |
| **9.** | Early sporophytic development | Scutellum<br>Cleoptile | CAT-2<br>CAT-1 and CAT-3 | |
| **10.** | Mature green leaves | Mesophyll cells<br>Bundle sheath cells | CAT- 1 and CAT-3<br>CAT-2 | Tsaftaris et al., 1983 |
| **11.** | Root development<br>Shoot development | Young roots<br>Mature stem | CAT-1 and CAT-3<br>CAT-3 | |

evolutionary relations among the various plant catalases. These workers divided plant catalase into three groups. Group I includes the monocot maize CAT-1 and most of the dicot catalases, while group II includes *N. plumbaginifolia* CAT-3 and tomato catalase, and group III contains the monocot specific catalases maize CAT-3, rice CAT-A, and barley CAT-2. Similarly, the three catalase genes of monocot plants have specific nucleotide preferences at the third codon position (Redinbaugh et al., 1990). Frugoli et al. (2012) reported

that CAT1, CAT2 and CAT3 map to chromosome 1, 3 and 1, respectively. Phylogenetic studies reveal that the majority of the dicot catalases and several monocot catalases are closely linked to each other.

### 4.5.4 Biochemistry of Catalase (Antioxidant Activity of Catalase)

Catalase is an excellent $H_2O_2$-degrading antioxidant enzyme that can reduce $H_2O_2$ at a rapid rate and without consuming cellular energy. The enzymatic efficiency (frequency of enzyme substrate collision) of catalase ($k_{cat}/K_M$ = $4.0 \times 10^8\ M^{-1}\ s^{-1}$) has a very high value. The enzymatic efficiency of catalase is at its diffusion limit, and therefore catalase shows catalytic excellence. A detailed mechanism for catalatic action has been proposed by Boon et al. (2007) who showed interaction of $H_2O_2$ with amino acids on heme active sites leads to a proton (hydrogen ion) transfer between the oxygen atoms. The free oxygen atom coordinates release the newly formed water molecule and E-$Fe^{+4}$ = O (Compound I). Newly formed E-$Fe^{+4}$ = O reacts with a second $H_2O_2$ molecule to reform E-$Fe^{+3-}$E and produce water and oxygen. In the fifth iron ligand a phenolate ligand of Tyr357 has been observed, which helps in conversion of $Fe^{+3}$ to $Fe^{+4}$ and improves the reaction efficiency of iron center. Spectrophotometric and kinetic evidence suggests that, depending on the concentration of $H_2O_2$, catalase can function in the peroxidatic or catalytic mode of activities (Deisseroth and Dounce, 1970).

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Catalase can either catalyze the direct conversion of $H_2O_2$ into $H_2O$ and $O_2$ (catalatic mode) or oxidize substrates (such as methanol, ethanol, formaldehyde, formate, or nitrite) by using hydrogen peroxide (peroxidatic mode). The ratio of enzymatic reactions is calculated as Rpic = (mU peroxidatic/U catalatic) × 10 (Havir and McHale, 1987).

Initially, the interaction of substrate $H_2O_2$ and the catalase iron center leads to production of oxygen-rich iron peroxide.

$$\text{E-Fe-OH} + H_2O_2 \rightarrow \underset{\text{(intermediate)}}{\text{E-Fe-OOH}} + H_2O$$

**(I) Peroxidatic mode of action:** (At low substrate concentrations)

At low concentration of $H_2O_2$ ($<10-6\ mol\ L^{-1}$), catalase reacts in the peroxidatic mode and various hydrogen donors (e.g. methanol, ethanol, phenols, formaldehyde, formate, nitrite and ascorbic acid) can be oxidized in the following mode.

$$RH2 + H_2O_2 \rightarrow R + 2H_2O$$

$$\text{CAT-Fe-OOH} + C_2H_5OH \rightarrow \text{CAT-Fe-OH} + H_2O + \text{CH3CHO (Peroxidatic Rx)}$$

At low substrate concentrations E-Fe-OOH can be reduced by hydrogen donors (e.g. ethanol including phenols, formic acid, formaldehyde and alcohols) peroxidatically. The enzyme produces useful products and water from toxins and hydrogen peroxide, respectively.

**(II) Catalatic mode of action:** (At high substrate concentrations)

At high concentrations of the substrate, $H_2O_2$ acts as both acceptor and donor of hydrogen molecules for an extremely rapid catalatic reaction.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

$$\text{CAT-Fe-OOH} + H_2O_2 \rightarrow \text{CAT-Fe-OH} + H_2O + O_2 \text{ (Catalatic Rx)}$$

Intermediate compound CAT-Fe-OOH reacts with $H_2O_2$ to produce water and molecular oxygen (Scandalios, 1994). Catalase has a very high Michaelis constant, so the enzyme activity increases linearly with $H_2O_2$ concentrations and maintains a balanced intracellular $H_2O_2$ concentration. Finally, catalase certainly consumes hydrogen peroxide during execution of catalytic and peroxidative modes of reaction in a biological system.

On the basis of reaction pattern, catalase isozymes fall into two main classes:

(i) HPI Catalase: This isoenzyme is bifunctional and has both catalytic and peroxidative activities.
(ii) HPII Catalase: Catalase catalyzes only disproportionation of $H_2O_2$ by peroxidative reactions.

Many reports are available that show a prominent role of catalase as an antioxidant in plants exposed to different environmental stresses (Isah and Mujib, 2012). Xu et al. (2013a) have reported that coexpression of MeCu-ZnSOD and MeCAT1 in cassava could dramatically improve ROS scavenging ability, leading to reduced $H_2O_2$ accumulation, improved abiotic stress resistance and delayed post-harvest physiological deterioration in cassava storage roots. Table 4.2 illustrates the biochemical and physical factors affecting the catalytic activity of catalase. Moreover, Xu et al. (2013a,b) has also reported that implementation of Cu/Zn superoxide dismutase (MeCu/ZnSOD) and catalase (MeCAT1) leads to extending the life of plant storage-roots by the stabilization of reactive oxygen species (ROS) homeostasis subsequent to harvest.

### 4.5.5 Factors Affecting Catalase Activities

The expression of the catalase gene is subject to various exogenous environmental factors. These physical and chemical factors are also known to cause

**TABLE 4.2 Factors Affecting the Catalytic Activity of Catalase**

| S. No. | Factors | Details |
|---|---|---|
| 1 | Concentration of substrate | Amount of $H_2O_2$ defines the type of reaction as catalytic and peroxidative activities. |
| 2 | Temperature | Should be below 40–50°C |
| 3 | pH | 6.8 and 7.5 |
| 4 | Presence of inhibitor | Inhibitors strongly binding to the heme of catalase and stopping the enzyme's action.<br>Noncompetitive inhibitor: e.g. copper Cations in copper (II) sulfate.<br>Competitive inhibitor: e.g. cyanide |
| 5 | Osmolytes | Sucrose induces a stimulatory characteristic to CAT |

oxidative stress and induction of the antioxidant defense system in different plant species. Table 4.2 shows physical factors affecting the catalytic activity of catalase.

According to a report by Scandalios et al. (1997) catalase genes demonstrate alterations in response to fungal toxin (Cercosporin), exogenously applied $H_2O_2$ and temperature (−4°C to 40°C).

Similarly, plant hormones like salicylic acid (acts as an endogenous signal molecule in the induction of systemic acquired resistance (SAR) in plants) can bind to catalase and inhibit its activities in dicot plants (Chen et al., 1993). However, the catalytic activities increased in monocot plants exposed to salicylic acid (Scandalios et al., 1997). Shim et al. (2003) have also reported that a fall in catalase activity under oxidative stress is related to accumulation of salicylic acid in treated plants. Other phytohormones, abscisic acid (ABA) and IAA, are responsible for increased catalase activities (Scandalios et al., 1997).

The multiple catalase genes respond differentially to light intensity, color and spectrum. It has been reported that a constant dark period is involved in inhibition of translocation of *Cat2* mRNA, whereas exposure of that leaf to white light or UV light (290–400 nm) leads to induction of CAT-2 isozymes (Fig. 4.2).

The transcription of *Cat3* gene in maize (encoding Class II catalase), *Cat2* (encoding class I catalase) and *Cat 3* (encoding Class II catalase) in *Arabidopsis*, and *Cat1* gene of *Nicotiana plumbaginifolia* (encoding class III catalase) have been regulated by circadian rhythm of corresponding plants (Willekens et al., 1994a). In C3 plants (*Arabidopsis* and *N. plumbaginlfolia*) the phase of accumulation of catalase transcripts early in the light period coincides with that of photosynthetic genes, which indicates a role of catalase in

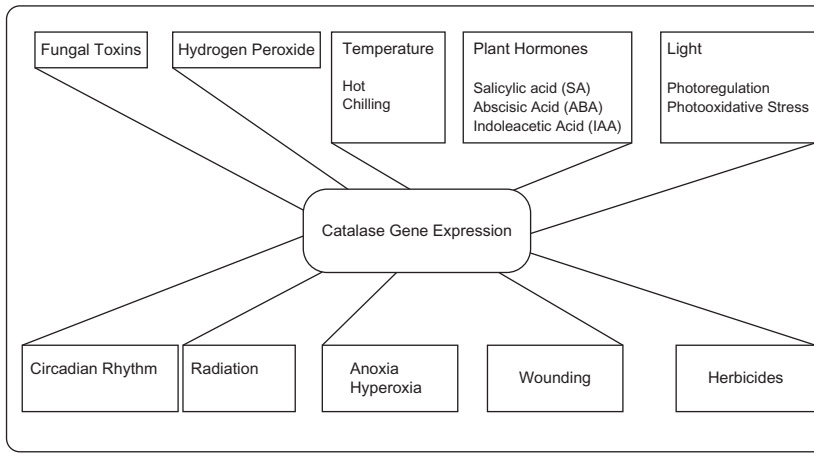


**FIGURE 4.2** Environmental factors affecting catalase gene expression.

decomposing $H_2O_2$ generated during the photorespiratory oxidation of glycolate in the peroxisome (Ogren, 1984). However, phasing of *Cat3* gene with maximum expression in maize during photorespiratory conditions indicates that this gene is not associated with photorespiration in C4 plants.

The inhibitory effect of radiation on CAT activity was also reported by several researchers (Liang et al., 2000; Al-Rumaih and Al-Rumaih, 2008). Moreover, osmolytes such as sorbitol, mannitol and sucrose facilitate maintenance of high activity during salt stress (Shau et al., 2010).

### 4.5.6 Catalase Deficiency in Plants

A complete deficiency of catalase leads to autosomal recessive peroxisomal disorder acatalasemia (also known as Takahara's disease) in humans (Zamocky et al., 2008; Patnaik et al., 2013). Similarly, in plants catalase-mediated tolerance has been reported in *Pennisetum typhoides* exposed to arsenic-induced oxidative stress (Sharma, 2013b). Prominent catalase deficiency symptoms have been characterized in catalase-deficient mutants (Woodson and Kerdnaimongkol, 1999). Kendall et al. (1983) reported that catalase-deficient barley mutant was unable to survive under photorespiratory conditions, but stayed alive in a high $CO_2$ atmosphere. High catalase activities in mutants of *Nicotiana tabacum* balance the photorespiratory $CO_2$ loss more than wild type plants. High catalase activity inhibits the chemical decarboxylation of *a*-keto acids by peroxisomal $H_2O_2$, which may cause the decrease in photorespiration. So, mutants with cloned catalase genes into C3 plants can handle more photorespiratory carbon loss than wild plants. Plants deficient in catalase activity are able to grow to maturity but sustain chlorosis and significant head sterility. The important role of catalase in C3 photosynthetic metabolism has been

well-established by analysis through antisense technology, as spontaneous lesion formation and oxidative stress are observed in catalase deficient mutant (Willekens et al., 1997; Chamnongpol et al., 1998). An undetectable increase in $H_2O_2$ during *Cat2* deficiency generates significant effects on gene expression and cell redox state (Chaouch et al., 2010; Mhamdi et al., 2010). Michelet et al. (2013) proposed that under high light intensity, the redox state of the photosynthetic electron transport chain is sensed and transmitted to the cytosol to regulate the catalase activity. This allows a temporary accumulation of $H_2O_2$, inducing a signalling event that is transmitted to the nucleus to modulate the expression of chloroplast-directed protection enzymes. Catalase-deficient barley plants exhibit disturbed glutathione status due to the enhanced burden on catalase-independent pathways to metabolize intracellular $H_2O_2$ (Smith et al., 1984). Similar alteration in glutathione level has also been observed in catalase-deficient tobacco and *Arabidopsis* plants (Rizhsky et al., 2002; Queval et al., 2007). In *Nicotiana tabacum* and barley, Cat1 deficiency caused white necrotic lesions on the leaves when plants were exposed to higher light intensities (300−1000 mmol/$m^2$/s to increased $H_2O_2$ production rather than to a reduced PPFR) (Kendall et al., 1983; Willekens et al., 1997). The reduced ability of Cat1-deficient plants to remove exogenous $H_2O_2$ manipulates the other components of the defense system. At least three central players, namely APX, CAT, and GPX, are involved in the removal of $H_2O_2$ in plants. Mittler (2002) has reported that CAT deficiency results in the induction of APX mitAOX, MDA reductase and GPX, suggesting that these enzymes may be induced to compensate for CAT suppression. Reduced peroxisomal catalase activity leads to increased sensitivity towards ozone and photorespiratory $H_2O_2$ induced cell death in transgenic *Arabidopsis thaliana*. It has been observed that deleterious effects of salinity may be reduced by $H_2O_2$ pretreatment and elevated activity of catalase in the maize plant (Gondim et al., 2012). Reports suggest that alteration of thiol-disulphide status appears to balance catalase deficiency and establish a more oxidized cellular redox state in mutant plants (Queval et al., 2009). Catalase-deficient tobacco being shifted to high light induces photorespiration and accumulation of GPX proteins along with APX (Willekens et al., 1997). The induction of GPX transcript has also been observed in *Cat2* deficient mutants that act as alternative paths for $H_2O_2$ decomposition in *Arabidopsis* (Queval et al., 2007; Mhamdi et al., 2010). Thus, catalase deficiency is a noteworthy loss to plants, but plants have a well-defined alternative pathway to manage the situation.

## 4.6 CONCLUSIONS

Plants often experience different stress conditions and a basic theme in most of their physiological challenges is the generation of harmful reactive oxygen species. To combat the deleterious effects of ROS, plants are equipped with an antioxidant defense system. Catalase, which is an important antioxidant

enzyme, has excellent enzyme kinetics and plays a key role in removal of $H_2O_2$ from cells. It occurs in peroxisomes, mitochondria, cytosol and chloroplast. Catalase isozymes exhibit developmental stages and tissue specificity in plants. Moreover, differential expression of *cat* genes against different chemical and physical stimulations has been reported in various plant species, indicating the role of catalase as a versatile antioxidant enzyme present in aerobic organisms. On the basis of *cat* gene isolation and characterization, phylogenic relationships may be established among different plant species. Catalase is extensively studied in animal and microbial cells but information is restricted to a narrow range among plant systems. It would be desirable to explore various aspects of enzyme kinetics, enzyme structure and mechanism in plant systems.

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## REFERENCES

Acevedo, A., Scandalios, J.G., 1990. Expression of the catalase and superoxide dismutase genes in mature pollen in maize. Theor. Appl. Genet. 80, 705–711.

Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S., 2010a. Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30, 161–175.

Ahmad, P., Nabi, G., Jeleel, C.A., Umar, S., 2011. Free radical production, oxidative damage and antioxidant defense mechanisms in plants under abiotic stress. In: Ahmad, P., Umar, S. (Eds.), Oxidative Stress: Role of Antioxidants in Plants. Studium Press Pvt. Ltd., New Delhi, India, pp. 19–53.

Ahmad, P., Umar, S., 2011. Antioxidants: Oxidative Stress Management in Plants. Studium Press Pvt. Ltd., New Delhi, India.

Ahmad, P., Umar, S., Sharma, S., 2010b. Mechanism of free radical scavenging and role of phytohormones during abiotic stress in plants. In: Ashraf, M., Ozturk, M., Ahmad, M.S.A. (Eds.), Plant Adaptation and Phytoremediation. Springer, Dordrecht Heidelberg London, NY, pp. 99–108.

Al-Rumaih, M.M., Al-Rumaih, M.M., 2008. Influence of ionizing radiation on antioxidant enzymes in three species of *Trigonella*. American Journal of Environmental Sciences 4, 151–156.

Andre, C., Kim, S.W., Yu, X.-H. Shanklin, J. (2013). Fusing catalase to an alkane-producing enzyme maintains enzymatic activity by converting the inhibitory byproduct $H_2O_2$ to the cosubstrate $O_2$. Proc. Natl Acad. Sci. USA 110, 3191–3196.

Becana, M., Moran, J.F., Iturbe-Ormaetxe, I., 1998. Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. Plant Soil 201, 137–147.

Beulah, K., Ramana, T., 2013. Purification, properties and kinetic studies of catalase from leaves of *phyllanthus reticulatu*. Int. J. Pharm. Chem. Bio. Sci. 3, 940–948.

Bienert, G.P., Moller, A.L.B., Kristiansen, K.A., Schulz, A., Moller, I.M., Schjoerring, J.K., et al., 2007. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membrane. J. Biol. Chem. 282, 1183–1192.

Bočová, B., Huttová, J., Liptáková, L'., Mistrík, I., Ollé, M., Tamás, L., 2012. Impact of short-term cadmium treatment on catalase and ascorbate peroxidase activities in barley root tips. Biol. Plant. 56, 724–728.

Boon, E.M., Downs, A., Marcey, D., 2007. Proposed Mechanism of Catalase *Catalase:* $H_2O_2$: $H_2O_2$ Oxidoreductase: Catalase Structural Tutorial. Available from: http://biology.kenyon.edu/BMB/Chime/catalase/frames/cattx.htm

Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann, J.r.H., Van Montagu, M., et al., 1998. Defense activation and enhanced pathogen tolerance induced by $H_2O_2$ in transgenic plants. Proc. Natl. Acad. Sci. USA 95, 5818–5823.

Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59, 527–605.

Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois–Meurinne, M., et al., 2010. Genetic reversion of cell death in the *Arabidopsis* cat2 knockout mutant shows that peroxisomal $H_2O_2$ is coupled to biotic defense responses by isochorismate synthase 1 in a daylength-related manner. Plant Physiol. 153, 1692–1705.

Charles, S.A., Halliwell, B., 1981. Light activation of fructose bisphosphate in isolated spinach chloroplasts and deactivation by $H_2O_2$. Planta 151, 242–246.

Cheeseman, J.M., 2007. Hydrogen peroxide and plant stress: A challenging relationship. Plant Stress 1, 4–15.

Chen, Z., Silva, H., Klessig, D.F., 1993. Active oxygen species in the indication of plant systemic acquired resistance by salicylic acid. Science 262, 1883–1886.

Corpas, F.J., Palma Sandalio, L.M., Valderrama, R., Barroso, J.B., Del Río, L.A., 2008. Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum L.*) leaves. J. Plant Physiol. 165, 1319–1330.

Deisseroth, A., Dounce, A., 1970. Catalase: Physical and chemical properties, mechanism of catalysis and physiological role. Physiol. Rev. 50, 319–375.

Dounce, A.L., 1983. A proposed mechanism for the catalatic action of catalase. J. Theor. Biol. 105, 553–567.

Drews, G., 2011. The evolution of cyanobacteria and photosynthesis. In: Peschek, G.A., Obinger, C., Renger, G (Eds.), Bioenergetic Processes of Cyanobacteria. Springer, Dordrecht, pp. 265–284.

Eising, R., Trelease, R., Ni, W., 1989. Biogenesis of catalase in glyoxysomes and leaf-type peroxisomes of sunflower cotyledons. Arch. Biochem. Biophys. 278, 258–264.

Foyer, C.H., Noctor, G., 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxidants Redox Signal 11, 861–905.

Fridovich, I., 1975. Superoxide dismutases. Annu. Rev. Biochem. 44, 146–159.

Frugoli, J.A., Zhong, H.H., Nuccio, M.L., McCourt, P., McPeek, M.A., Thomas, T.L., et al., 2012. Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol. 112, 327–336.

Gianinetti, A., Cantoni, M., Lorenzoni, C., Salamini, F., Marocco, A., 1993. Altered levels of antioxidant enzymes associated with two mutations in tomato. Physiol. Plant. 89, 157–164.

Gondim, F.A., Gomes-Filho, E., Costa, J.H., Mendes, Alencar, N.L., Prisco, J.T., 2012. Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. Plant Physiol. Biochem. 56, 62–71.

Gu, Y., Sattayasamitsathit, S., Kaufmann, K., Vazquez-Duhalt, R., Gao, W., Wangb, C., et al., 2013. Self-propelled chemically-powered plant-tissue biomotors. Chemical Communication 49, 7307–7309.

Guan, L., Polidoros, A.N., Scandalios, J.G., 1996. Isolation, characterization and expression of the maize *Cat2* catalase gene. Plant Mol. Biol. 30, 913–924.

Halliwell, B., Gutteridge, J.M.C., 1992. Free radicals, antioxidants and human diseases: where are we now? J. Lab. Clin. Med. 119, 598–620.

Havir, A.E., McHale, N.A., 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84, 450–455.

Isah, T., Mujib, A., 2012. Studies on antioxidant enzymes activity during in vitro morphogenesis of *Caladium bicolor Linn*. Int. J. Modern Cell. Mol. Biol. 1, 1–9.

Kato, J., Yamahara, T., Tanaka, K., Takio, S., Satoh, T., 1997. Characterization of catalase from green algae *Chlamydomonas reinhardtii*. J. Plant Physiol. 151, 262–268.

Kendall, A.C., Keys, A.J., Turner, J.C., Lea, P.J., Miflin, B.J., 1983. The isolation and characterization of a catalase-deficient mutant of barley. Planta 159, 505–511.

Koyro, H.W., Ahmad, P., Geissler, N., 2012. Abiotic stress responses in plants: an overview. In: Ahmad, P., Prasad, M.N.V. (Eds.), Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. Springer, New York, USA, pp. 1–28.

Lenton, T.M., 2003. The coupled evolution of life and atmospheric oxygen. In: Rothschild, L., Lister, A. (Eds.), Evolution of Planet Earth. Elsevier, London, UK, pp. 35–53.

Liang, Y.E., Hui, Y.G., Qi, Z., 2000. Response of the antioxidant systems and xanthophylls cycle in *Phaseolus vulgaris* to the combined stress of high irradiation and high temperature. Photosynthetica 38, 205–210.

Loew, O., 1901. Catalase, new enzyme of general occurrence, with special reference to the tobacco plant. US Dep Agric Rep. 68, 47.

Loewen, P.C., Klotz, M.G., Hassett, D.J., 2002. Catalase – an "old" enzyme that continues to surprise us. ASM News 66, 76–82.

Mhamdi, A., Hager, J., Chaouch, S., Queval, G., Han, Y., Taconnat, L., et al., 2010. *Arabidopsis* Glutathione Reductase 1 plays a crucial role in leaf responses to intracellular $H_2O_2$ and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. Plant Physiol. 153, 1144–1160.

Michelet, L., Roach, T., Fischer, B.B., Bedhomme, M., Lemaire, S.D., Krieger-Liszkay, A., 2013. Down-regulation of catalase activity allows transient accumulation of a hydrogen peroxide signal in *Chlamydomonas reinhardtii*. Plant Cell Environ. 36, 1204–1213.

Mittler, R., 2002. Oxidative stress, antioxidants, and stress tolerance. Trends in Plant Sci. 7, 405–410.

Mullen, R.T., Gifford, D.J., 1993. Purification and characterization of catalase from Loblolly pine *(Pinus taeda* L.) mega gametophytes. Plant Physiol. 103, 477–483.

Mullen, R.T., Lee, M.S., Trelease, R.N., 1997. Identification of the peroxisomal targeting signal for cottonseed catalase. Plant J. 12, 313–322.

Ni, W., Turley, R., Trelease, R., 1990. Characterization of a cDNA encoding cottonseed catalase. Biochim. Biophys. Acta. 1049, 219–222.

Ogren, W.L., 1984. Photorespiration: Pathways, regulation and modification. Annu. Rev. Plant Physiol. 35, 415–442.

Patnaik, S.C., Sahoo, D.K., Chainy, G.B., 2013. A comparative study of catalase activities in different vertebrates. Webmed Central Zoology 4, WMC004270.

Perl, A., Perl-Treves, R., Galili, S., Aviv, D., Shalgi, E., Malkin, S., et al., 1992. Enhanced oxidative-stress defense in transgenic potato expressing tomato Cu, Zn superoxide dismutases. Theor. Appl. Genet. 85, 568–576.

Queval, G., Issakidis-Bourguet, E., Hoeberichts, F.A., Vandorpe, M., Gakiere, B., Vanacker, H., et al., 2007. Conditional oxidative stress responses in the *Arabidopsis* photorespiratory

mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of $H_2O_2$-induced cell death. Plant J. 52, 640–657.

Queval, G., Thominet, D., Vanacker, H., Miginiac-Maslow, M., Gakiére, B., Noctor, G., 2009. $H_2O_2$-activated up-regulation of glutathione in *Arabidopsis* involves induction of genes encoding enzymes involved in cysteine synthesis in the chloroplast. Mol. Plant 2, 344–356.

Ray, M., Mishra, P., Das, P., Sabat, S.C., 2012. Expression and purification of soluble bio-active rice plant catalase-A from recombinant *Escherichia coli*. J. Biotechnol. 157, 12–19.

Redinbaugh, M.G., Sabre, M., Scandalios, J.G., 1990. Expression of the maize Cat3 catalase gene is under the influence of a circadian rhythm. Proc. Natl Acad. Sci. USA 87, 6853–6857.

Regelsberger, G., Jakopitsch, C., Plasser, L., Schwaiger, H., Fürtmuller, P.G., Peschek, G.A., et al., 2002. Occurrence and biochemistry of hydroperoxidase in oxygenic phototrophic prokaryotes (cyanobacteria). Plant Physiol. Biochem. 40, 479–490.

Rizhsky, L., Hallak–Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J., Rodermel, S., et al., 2002. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. 32, 329–342.

Sagi, M., Fluhr, R., 2006. Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol. 141, 336–340.

Sahu, S., Das, P., Ray, M., Sabat, S.C., 2010. Osmolyte modulated enhanced rice leaf catalase activity under salt-stress. Adv. Biosci. Biotechnol. 1, 39–46.

Scandalios, J.G., 1974. Subcellular localization of catalase variants coded by two genetic loci during maize development. J. Hered. 65, 28–32.

Scandalios, J.G., 1983. Multiple varieties of isozymes and their role in studies of gene regulation and expression during eukaryote development. Curr. Top. Biol. Med. Res. 9, 1–31.

Scandalios, J.G. (Ed.), 1992. Current Communications in Cell And Molecular Biology: Molecular Biology of Free Radical Scavenging Systems (5). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Scandalios, J.G., 1994. Regulation and properties of plant catalases. In: Foyer, C.H., Mullineaux, P.M. (Eds.), Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. CRC Press, Boca Raton, Florida, pp. 275–315.

Scandalios, J.G., Guan, L., Polidoros, A.N., 1997. Catalases in plants: gene structure, properties, regulation, and expression. Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, pp. 343–406.

Scandalios, J.G., Tsaftaris, A.S., Chandlee, J.M., Skadsen, R.W., 1984. Expression of the developmentally regulated catalase *(Cat)* genes in maize. Dev. Genet. 4, 2–293.

Sharma, I., 2012. Arsenic induced oxidative stress in plants. Biologia. (Bratisl). 67, 447–453.

Sharma, I., 2013a. Arsenic stress in plants: An inside story. In: Khalid, R.H., Ahmad, P., Munir, O. (Eds.), Crop Improvement New Approaches and Modern Techniques. Springer, New York Dordrecht Heidelberg London, pp. 379–400.

Sharma, I., 2013b. Arsenic induced oxidative stress and antioxidant defense system of *Pisum sativum* and *Pennisetum typhoides*: A comparative study. Res. J. Biotechnol. 8, 48–56.

Sharma, I., Singh, R., Tripathi, B.N., 2007. Biochemistry of arsenic toxicity and tolerance in plants. Biochem. Cell. Arch. 7, 165–170.

Sharma, I., Travlos, I.S., 2012. Phosphate supply as a promoter of tolerance to arsenic in pearl millet. Int. J. Plant Production 6, 443–456.

Sheptovitsky, Y.G., Brudig, G.W., 1996. Isolation and characterization of spinach photosystem II membrane-associated catalase and polyphenol oxidase. Biochemistry 35, 16255–16263.

Shim, I.S., Momose, Y., Yamamoto, A., Kim, D.W., Usui, K., 2003. Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. Plant Growth Regul. 39, 285–292.

Shim, I.S., Naruse, Y., Kim, Y.H., Kobayashi, K., Usui, K., 1999. Scavenging activity of NaCl-induced activated oxygen in two rice (*Oryza sativa* L.) cultivars differing in salt tolerance. Japanese J. Tropical Agricul. 43, 32–41.

Singh, S., Braus-Stromeyer, S.A., Timpner, C., Valerius, O., Tiedemann, A., Karlovsky, P., et al., 2012. The plant host *Brassica napus* induces in the pathogen *Verticillium longisporum* the expression of functional catalase peroxidase which is required for the late phase of disease. Mol. Plant Microbe Interact. 25 (4), 569–581.

Smith, I.K., Kendall, A.C., Keys, A.J., Turner, J.C., Lea, P.J., 1984. Increased levels of glutathione in a catalase-deficient mutant of barley (*Hordeum vulgare* L.). Plant Sci. Letters 37, 29–33.

Spanou, C.I., Veskoukis, A.S., Stagos, D., Liadaki, K., Aligiannis, N., Angelis, A., et al., 2012. Effects of Greek legume plant extracts on xanthine oxidase, catalase and superoxide dismutase activities. J. Physiol. Biochem. 68, 37–45.

Sumner, J.B., Dounce, A.L., 1937. Crystalline catalase. J. Biol. Chem. 121, 417–424.

Tsaftaris, A.S., Bosabalidis, A.M., Scandalios, J.G., 1983. Cell-type-specific gene expression and acatalasemic peroxisomes in a null *Cat2* catalase mutant of maize. Proc. Natl. Acad. Sci. 80, 4455–4459.

Wadsworth, G.J., Scandalios, J.G., 1989. Differential expression of the maize catalase genes during kernel development: The role of steady-state mRNA levels. Dev. Genet. 10, 304–310.

Warburg, O., 1923. Ueber die antikatalytische Wirkung der Blaus ure. Biochem. Z. 136, 266–272.

Willekens, H., Langebartels, C., Tire, C., Van Montagu, M., Inze, D., Van Camp, W., 1994a. Differential expression of catalase genes in *Nicotiana plumbaginifolia*. Proc. Natl. Acad. Sci. USA. 91, 10450–10454.

Willekens, H., Villarroel, R., Inze, D., Van Montagu, M., Van Camp, W., 1994b. Molecular identification of catalases from *Nicotiana plumbaginifolia*. FEBS Lett. 352, 79–83.

Willekens, H., Inze, D., Van Montagu, M., Van Camp, W., 1995. Catalases in plants. Mol. Breeding 1, 207–228.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., et al., 1997. Catalase is a sink for $H_2O_2$ and is indispensable for stress defense in C3 plants. EMBO J. 16, 4806–4816.

Woodson, W.R., Kerdnaimongkol, K., 1999. Inhibition of catalase by antisense RNA increases susceptibility to oxidative stress and chilling injury in transgenic tomato plants. J. Amer. Soc. Hort. Sci. 124, 330–336.

Xu, J., Duan, X., Yang, J., Beeching, J.R., Zhang, P., 2013a. Enhanced ROS scavenging by over-production of superoxide dismutase and catalase delays post-harvest physiological deterioration of cassava storage roots. Plant Physiol. 161, 1517–1528.

Xu, J., Duan, X., Yang, J., Beeching, J.R., Zhang, P., 2013b. Coupled expression of Cu/Zn-superoxide dismutase and catalase in cassava improves tolerance against cold and drought stresses. Plant Signaling Behav. 8 (6), 24525.

Yamaguchi, J., Nishimura, M., Akazawa, T., 1986. Purification and characterization of hemecontaining low-activity form of catalase from greening pumpkin cotyledons. Fur. J. Biochem. 159, 315–322.

Youssef, M.M., Azooz, M.M., 2013. Biochemical studies on the effects of zinc and lead on oxidative stress, antioxidant enzymes and lipid peroxidation in Okra (*Hibiscus esculentus* cv. *Hassawi*). Sci. Int. 1, 12–16.

Yu, L., Lamb, C.J., Dixon, R.A., 1992. Purification and biochemical characterization of proteins which bind to the H-box *cis*-element implicated in the transcriptional activation of plant defense genes. Plant J. 3, 805–816.

Zamocky, M., Furtmüller, P.G., Obinger, C., 2008. Evolution of catalases from bacteria to humans. Antioxidants Redox Signal. 10, 1527–1547.

Zamocky, M., Gasselhuber, B., Furtmüller, P.G., Obinger, C., 2012. Molecular evolution of hydrogen peroxide degrading enzymes. Arch. Biochem. Biophys. 525, 131–144.

Zeile, K., Hellstrom, H., 1930. Ueber die aktive Gruppe der Leberkatalase. *Z.* Physiol. Chem. 192, 171–174.

Zhong, H.H., Young, J.C., Pease, E.A., Hangarter, R.P., McClung, R.C., 1994. Interactions between light and the circadian clock in the regulation of the *CAT2* expression in *Arabidopsis*. Plant Physiol. 104, 889–898.