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REVIEW ARTICLE

Catalase and ascorbate peroxidase—representative H_2O_2-detoxifying heme enzymes in plants

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Abstract Plants have to counteract unavoidable stress-caused anomalies such as oxidative stress to sustain their lives and serve heterotrophic organisms including humans. Among major enzymatic antioxidants, catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) are representative heme enzymes meant for metabolizing stress-provoked reactive oxygen species (ROS; such as H_2O_2) and controlling their potential impacts on cellular metabolism and functions. CAT mainly occurs in peroxisomes and catalyzes the dismutation reaction without requiring any reductant; whereas, APX has a higher affinity for H_2O_2 and utilizes ascorbate (AsA) as specific electron donor for the reduction of H_2O_2 into H_2O in organelles including chloroplasts, cytosol, mitochondria, and peroxisomes. Literature is extensive on the glutathione-associated H_2O_2-metabolizing systems in plants. However, discussion is meager or scattered in the literature available on the biochemical and genomic characterization as well as techniques for the assays of CAT and APX and their modulation in plants under abiotic stresses. This paper aims (a) to introduce oxidative stress-causative factors and highlights their relationship with abiotic stresses in plants; (b) to overview structure, occurrence, and significance of CAT and APX in plants; (c) to summarize the principles of current technologies used to assay CAT and APX in plants; (d) to appraise available literature on the modulation of CAT and APX in plants under major abiotic stresses; and finally, (e) to consider

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a brief cross-talk on the CAT and APX, and this also highlights the aspects unexplored so far.

Keywords Abiotic stress · Reactive oxygen species · Oxidative stress · Catalase · Ascorbate peroxidase · Plant stress tolerance

Abiotic stresses and their consequences and control measures in plants

Impacts of abiotic stresses at both whole plant and cellular levels are inevitable because of plants' sessile life style. At cellular level, oxidative stress is caused when varied abiotic stress-provoked generations of reactive oxygen species (ROS; such as singlet oxygen, $^1O_2$; superoxide, $O_2^{\cdot-}$; hydrogen peroxide, H_2O_2; hydroxyl radical, $OH^{-}\cdot$) exceeds the pace of their metabolism (Gill and Tuteja 2010). ROS such as H_2O_2 is a water-soluble, longer half-life exhibiting, and non-radical w-electron reduction product of oxygen and acts at subtle levels as a second messenger molecule in biological processes such as development and stress perception (Orozco-Ca′rdenas et al. 2001; Corpas 2015; Del Río 2015). H_2O_2 priming-mediated enhanced plant tolerance to major abiotic stresses has been reported, where H_2O_2 priming was advocated to modulate ROS detoxification and also regulate multiple stress-responsive pathways and gene expression (reviewed by Hossain et al. 2015). However, severe damages to biomolecules (such as cellular lipids and proteins) and subsequent inactivation of key cellular functions can be possible due to elevated and non-metabolized cellular H_2O_2 (Gill and Tuteja 2010; Del Río 2015).

The list of H_2O_2-scavenging enzymatic antioxidants includes catalases (CAT; EC 1.11.1.6), ascorbate peroxidases (APX; EC 1.11.1.11), various types of peroxiredoxins (PRX), glutathione/thioredoxin peroxidases (GPX), and glutathione S-transferases (GST) (reviewed by Mhamdi et al. 2010) (Fig. 1). Glutathione (GSH)-associated H_2O_2-metabolizing systems in stressed plants have been discussed extensively (Mhamdi et al. 2010; Noctor et al. 2012; Gill et al. 2013; Anjum et al. 2014a). However, literature discussing or evaluating the biochemical and genomic characterization as well as techniques for the assays of CAT and APX and their modulation in plants under abiotic stresses are scanty or disorganized. In particular, CAT and APX are distinguished enzymes meant for metabolizing stress-provoked H_2O_2 and for controlling their potential impacts in order to maintain cellular concentration of H_2O_2 to a level necessary for all aspects of normal plant growth and development (Gill and Tuteja 2010; Ray et al. 2012; Anjum et al. 2014b). CAT mainly occurs in peroxisomes while APX occurs in chloroplasts, cytosol, mitochondria, and peroxisomes. In association with other cellular antioxidants, CAT and APX help plants combat H_2O_2-accrued impairments in cellular organelles and redox homeostasis (Gill and Tuteja 2010; Mhamdi et al. 2010; Sofo et al. 2015). Though APX and CAT possess heme, these enzymes differ in terms of their affinity for H_2O_2 and in their requirement for reducing power during H_2O_2 metabolism (Gill and Tuteja 2010; Anjum et al. 2014b). Since APX efficiently eliminates even very low levels of H_2O_2 using ascorbate (AsA) as its substrate and CAT degrades H_2O_2 without any reducing power and is mainly active at relatively high H_2O_2 concentrations (Gechev et al. 2006), differences can be obvious in the role and modulation of these enzymes in abiotic-stressed plants. Hence, it is imperative and timely to discuss and provide readers a comprehensive overview on the biochemical and genomic characterization of CAT and APX, techniques and underlying principles for their assays, and the modulation and significance of these enzymes in plants of economic importance under abiotic stresses. With the major aim of contributing with novelties on the distinguished heme enzymes and plant abiotic stress tolerance, this paper (a) overviews structure, occurrence, and significance of CAT and APX in plants; (b) summarizes principles of current technologies used to assay CAT and APX in plants; (c) appraises available literature on the modulation of CAT and APX in plants under major abiotic stresses; and finally, (d) considers a brief cross-talk on the CAT and APX, and it also highlights aspects unexplored so far.

Catalase

Localization and biochemical characterization

Catalase (H_2O_2/H_2O_2 oxidoreductase, EC 1.11.1.6; CAT) is a tetrameric, heme-containing enzyme that catalyzes the dismutation of H_2O_2 into H_2O and O_2 and plays important role not only in plant metabolism and defense but also in signal perception (Loew 1901; Redinbaugh et al. 1990; Scandalios et al. 1997; Mhamdi et al. 2010; Hu et al. 2010; Nie et al. 2015; Liu et al. 2015). Historically, the degradation of H_2O_2 in plant and animal tissues was first observed by Thenard in 1881; whereas, Loew (1901) proved that a new enzyme, which he named "catalase," was responsible for the degradation of H_2O_2 in tissues (Aebi and Sutter 1971). CAT is mainly localized in the peroxisomes. In higher plants, it is present in all differentiated peroxisomes including the peroxisomes of leaves, cotyledons, roots, and glyoxysomes and unspecialized peroxisomes (Su et al. 2014). There are evidences for the presence of CAT in mitochondria too (Scandalios 1990; Heazlewood et al. 2004; Shugaev et al. 2011). In *Zea mays*, CAT3 was found to be located in mitochondria (Roupakias et al. 1980). Higher peroxidase activity was reported for

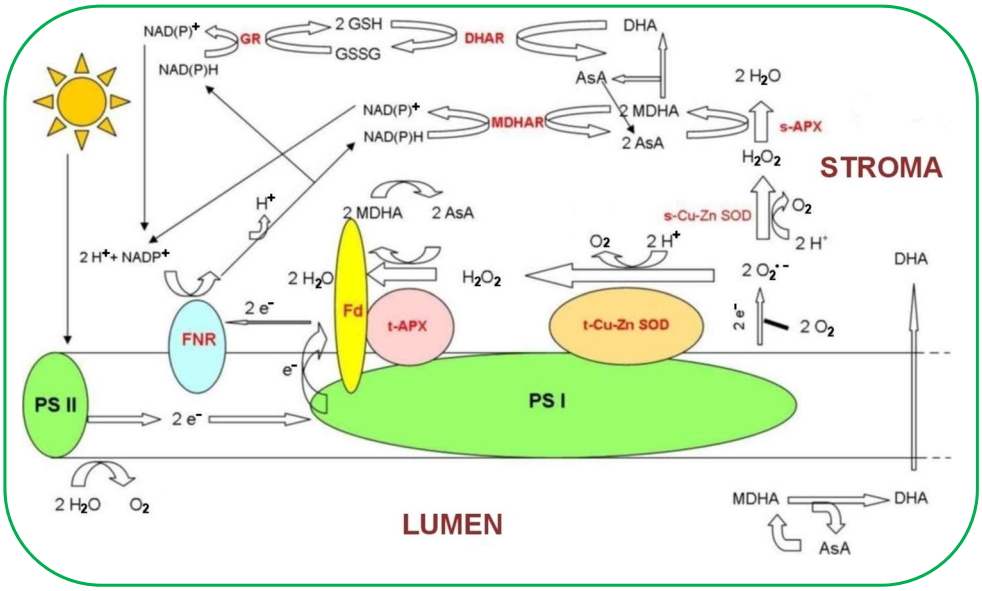

Fig. 1 Enzymatic and non-enzymatic antioxidant system in plant chloroplasts. *AsA* ascorbate, *DHA* dehydroascorbate, *DHAR* dehydroascorbate reductase, *Fd* ferredoxin, *FNR* ferredoxin-NADP+ reductase, *GR* glutathione reductase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *MDHA* monodehydroascorbate, *MDHAR* monodehydroascorbate reductase, *s-APX* stromatic ascorbate peroxidase, *s-Cu/Zn-SOD* stromatic Cu-Zn-superoxide dismutase, *t-APX* thylakoidal ascorbate peroxidase, t-*Cu/Zn-SOD* thylakoidal Cu-Zn-superoxide dismutase

putative mitochondrial *Zea mays* CAT isoform compared to other *Zea mays* isoforms and was relatively sensitive to 3-AT (Havir and McHale 1989). Identification of *CAT2* and *CAT3* peptide sequences was done in Arabidopsis through proteomic analysis of highly purified mitochondria (Heazlewood et al. 2004). The submitochondrial localization of CAT analyzed using proteinase K has also established that CAT is localized in the mitochondrial matrix (Shugaev et al. 2011).

CAT, first purified and crystallized from beef liver in its high purity, has been purified and characterized from various sources including plants (Sumner and Dounce 1937). CAT is an iron-containing enzyme and can be inhibited by cyanide (Warburg 1923). CATs from various sources display similarities in number of subunits, molecular weight, and types of prosthetic groups. The *enzyme* is a tetramer consisting four *subunits* of 54–59 kDa and a *total molecular mass* of approximately 240 kDa. Bovine and human CATs also contain four tightly bound molecules of NADPH (Kirkman and Gaetani 1984). Although not essential for the activity of CAT, NADPH can decrease CAT susceptibility to inactivation when the enzyme is exposed to low concentrations of H_2O_2, its toxic substrate. By playing its role as a regulatory protein, CAT releases $NADP^+$ in peroxidative stressed cells (Kirkman and Gaetani 1984). The reaction catalyzed by CAT is very fast and their reaction rate constant is $K \approx 10^7\ M^{-1}se^{-1}$ (Deisseroth and Dounce 1970). When the concentration of H_2O_2 is low ($<10^{-6}$ M), CAT works in peroxidatic mode, where it can oxidize most of hydrogen donors (e.g., ethanol, ascorbic acid, phenols, formaldehyde) in the following manner.

$$RH_2 + H_2O_2 \rightarrow R + 2H_2O$$

At high concentrations of H_2O_2 ($>10^{-6}$), it works "catalytically," where H_2O_2 acts as both acceptor and donor of hydrogen molecules.

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

The peroxidatic as well as catalytic reactions of CAT takes place in two steps (Deisseroth and Dounce 1970; Dounce 1983). First, the H_2O_2 molecule oxidizes the heme iron of CAT to an oxyferryl species leading to the formation of oxygen-rich iron peroxide. In this step, one oxidation equivalent is removed from the iron and one from the porphyrin ring to generate a porphyrin cation radical.

$$CAT{-}Fe{-}OH + H_2O_2 \rightarrow CAT{-}Fe{-}OOH + H_2O$$

Intermediate iron peroxide (CAT–Fe–OOH), referred to as compound I, can be detected in vitro and in vivo (Oshino et al. 1975) as it alters the spectrophotometric properties of the CAT heme. Further, in step two, at low concentrations of H_2O_2, compound I is reduced by hydrogen donors such as ethanol, peroxidatically.

$$CAT{-}Fe{-}OOH + C_2H_5OH \rightarrow CAT{-}Fe{-}OH + H_2O + CH_3CHO$$

At high H_2O_2 concentrations, the resting-state enzyme is then regenerated from compound I using the second H_2O_2 molecule as redundant, catalytically. Additionally, in the catalytic mode, CAT is not easily saturated with substrate due to

very high apparent Michaelis constant, and hence, enzyme activity increases linearly over a wide range of H_2O_2 concentrations. Unlike other antioxidant enzymes, CATs degrade H_2O_2 in an energy-efficient manner as they do not require cellular reducing equivalent.

$$CAT{-}Fe{-}OOH + H_2O_2 \rightarrow CAT{-}Fe{-}OH + H_2O + O_2$$

The presence of a heme prosthetic group in CATs is confirmed by the inhibition of this enzyme with hemeprotein inhibitors such as cyanide, azide, and hydroxylamine. Additionally, the inhibition of CAT by aminotriazole and mercaptoethanol indicates the participation of a thiol group (present at the active center of CAT) in the CAT-mediated reactions. Between the two main groups of CAT, a monofunctional form is present in prokaryotes, fungi, animals, and plants and can also catalyze some H_2O_2-dependent peroxidation of organic substrates (Mutsada et al. 1996; Regelsberger et al. 2002). However, structurally distinct bifunctional forms of CATs are found in some fungi and prokaryotes and are more similar to the heme-containing peroxidases such as APX and fungal cytochrome c peroxidase (Mutsada et al. 1996; Regelsberger et al. 2001, 2002; reviewed by Mhamdi et al. 2010). A wide range of biochemical and kinetic properties have been reported among the purified and characterized CATs. CATs have a very high turnover rate. CAT protein has a degradation constant of 0.263 day^{-1} (Eising and Süselbeck 1991); whereas, the degradation constant for heme can be 0.205 day^{-1} (Eising and Gerhardt 1987). Purification and the characterization of CATs from 16 different organisms (including representatives from all three phylogenetic clades) reported the maximal turnover rates in the range of 54,000 to 833,000/s and the specific activities ranged between 20,700 to 273,800 units/mg of protein (Switala and Loewen 2002). Compared to a high affinity of APX and peroxidase for H_2O_2 (K_M values below 100 μM; Mittler and Zilinskas 1991a; König et al. 2002), CATs exhibit much lower affinity for H_2O_2 where their apparent K_M for H_2O_2 ranges between 40 and 600 mM (Del Rio et al. 1977; Arabaci 2011). Notably, the kinetic constants such as K_m and V_{max} for CATs were advocated to be labeled as "apparent" (Switala and Loewen 2002). CATs can exhibit activity at a broad pH range (5.0–10.5) (Stansell and Deutsch 1965; Hochman and Goldberg 1991), where pH for their optimal activity may be in the range of 6.8–7.5 (Aebi 1984). A broad range of sensitivities to heat inactivation has also been observed. Optimum temperature for CAT activity can also vary in different plants. In *Oryza sativa*, the optimum temperature for CAT activity was found to be 35 °C, whereas 15 °C was reported as an optimal temperature for the spinach leaf CAT (Mitsuda and Yasumatsu 1955). It was argued that these optimum temperatures for CAT activity can closely resemble the temperature in which plants grow (Mitsuda and Yasumatsu 1955). Maximum CAT activity was found at 30 °C in *Anethum graveolens* and chard (Arabaci 2011) and 50 °C in Van apple (Yoruk et al. 2005).

Catalase genomic characterization, genes and isoforms

Based on the comparison of the exon-intron structure of 12 genomic sequences from 6 plants, it was assumed that the putative single primordial CAT gene had 7 introns (Iwamoto et al. 1998). After the evolutionary divergence of monocots from dicots, the occurrence of consecutive duplications of the primordial gene followed by the differential loss of introns resulted in three (or possibly four in dicots) diverse isozyme genes (Iwamoto et al. 1998). *CAT1*, *CAT2*, and *CAT3* genes of *Zea mays* inbred line W64A have already been isolated and fully characterized, where their sequencing revealed almost identical coding region with variable introns (Abler and Scandalios 1993; Guan and Scandalios 1993; 1995). The *CAT1*, *CAT2*, and *CAT3* genes are interrupted by 6, 5, and 2 introns, respectively. The identical positioning of these introns suggests an evolutionary link between all three *Zea mays* CAT genes. The *CAT2* gene lacks the third intron of the *CAT1* gene, whereas the *CAT3* gene contains only the first and the last introns in the same position as of the *CAT1* gene (Guan and Scandalios 1995; reviewed by Scandalios et al. 1997). Three *Zea mays* CAT promoters differed in their sequence homology. In particular, promoter sequences of approximately 2.6 kb and CAAT consensus sequences were evidenced in the *CAT1* and *CAT3* genes, whereas the promoter region of the *Zea mays CAT2* gene, which possesses the TATA-like sequence, exhibited only about 1.6 kb and was lacking CAAT consensus sequences (Abler and Scandalios 1993; Guan and Scandalios 1993, 1995; reviewed by Scandalios et al. 1997). Notably, each CAT promotor gene possesses a unique set of putative cis-acting elements that play role in eukaryotic gene regulation. Two GC-rich sequences (CCGCCG, GGGCTG) were identified in the promoter region of the *CAT1* gene (Dynan and Tjian 1985). Several other protein-binding motifs were also identified within 800 bp upstream from the transcriptional start site. Similar to ABA response element (ABRE, CACGTGGC), two 8-bp sequences (CACGTACG, CACGTGGA) were located at −110 and −220 relative to the start of transcription of the *CAT1* gene (Guiltinan et al. 1990). In addition, several transcription factor binding sites and a transposable element were also identified in the 3′–5′ upstream region of the *CAT3* gene (Polidoros and Scandalios 1997). An entirely new short intron was found in one of the *Oryza sativa CAT A* gene which was not found in any other plant CAT gene examined (Iwamoto et al. 1998). In silico modeling and H_2O_2 binding study of *Oryza sativa* CAT has also been done (Sekhar et al. 2006). The coexpression of chaperone GroEL/ES was reported to enhance the expression of plant CAT in bacterial cytosol (Mondal et al. 2008a).

Molecular identification and properties were performed in a light-insensitive *Oryza sativa* CAT B that was expressed in *Escherichia coli* (Mondal et al. 2008b). The purification and expression of a soluble bioactive *Oryza sativa* CAT A from recombinant *Escherichia coli* have also been done (Ray et al. 2012). Efforts have also been made to perform expression analysis and biochemical characterization of intracellular CAT/peroxidase from the phytopathogenic *Oryza sativa* blast fungus *Magnaporthe grisea* (Zamocky et al. 2009).

Regarding CAT genes and isoforms, CATs can exist in multiple molecular forms or isozymes encoded by multiple genes (Scandalios 1968). Notably, unlike animals which contain a single CAT gene, *all angiosperm/flowering plants* including *Oryza* sp., *Arabidopsis*, *Zea mays*, *Nicitiana tabacum*, and pumpkin are composed of a multigene family (Scandalios 1990; Frugoli et al. 1996; Guan and Scandalios 1996; Iwamoto et al. 2000; reviewed by Mhamdi et al. 2010; Liu et al. 2015). Multiple forms of CATs in plants are usually expressed in different tissues and at their developmental stages (reviewed by Scandalios et al. 1997). The classification of CAT genes is based on based on the naming of the *Nicotiana tabacum* genes. The division of CATs into three classes was also supported by the outcomes of the comparisons of gene structure done between *Zea mays* and *Oryza sativa* CAT genes (Iwamoto et al. 2000). Class I, II, and III CATs are strongly expressed in photosynthetic tissues, vascular tissues, and seeds and reproductive tissues, respectively. In particular, *Class I CATs* are most abundant in photosynthetic tissues and remove excess H_2O_2 produced during photorespiration; no exact role is known for *Class II CATs*, the most prominent in vascular tissues, and is assumed to express during lignification. The *Class III* are expressed in seeds and young seedlings at high levels and their activity is related to the removal of H_2O_2 produced during the degradation of fatty acids in glyoxylate cycle in glyoxysomes (reviewed by Mhamdi et al. 2010; Sharma et al. 2012). Arabidopsis genome has three catalase genes namely *At1g20630*, *At4g35090*, and *At1g20620* (Frugoli et al. 1996). Additionally, these genes possess highly conserved nucleotide and corresponding amino acid sequences, encode three individual subunits (that associate to form at least six catalase isoforms), and are highly expressed in inflorescences (Frugoli et al. 1996). However, only CAT2 and CAT3 are highly expressed in leaves. In plants, day–night rhythms in transcript abundance of catalase have been observed. To this end, *CAT2* and *CAT3* were reported to be expressed in different areas of leaf tissues (Zimmermann et al. 2006). On the other hand, *CAT2* expression was observed in photosynthetically active tissues and was downregulated during leaf senescence, whereas changes in age modulated the expression of *CAT3* (Zimmermann et al. 2006). Because the expression and activity of the *CAT1* gene are activated by abiotic stresses, this gene was advocated to act as a feedback regulating ROS signaling (Xing et al. 2007; Du et al. 2008). Regarding the insights into the functions of the three CAT genes, significant growth retardations and distinct accumulation of H_2O_2 were reported in leaves of CAT2 Arabidopsis mutants (Hu et al. 2010).

The expression of CAT genes is regulated both temporally and spatially and depends on developmental (Kwon and An 2001) and environmental oxidative stimuli (Su et al. 2014). The expression of Cat genes in some plants is under circadian control (Kabir and Wang 2011). Studies have demonstrated that Cat genes/transcripts are differentially expressed in different organs (Purev et al. 2010; Kabir and Wang 2011; Su et al. 2014). *PgCAT1* gene was expressed relatively high in the leaves and stems of *Panax ginseng*, whereas it was moderately expressed in the roots (Purev et al. 2010). The expression of *SlCAT1* gene was high in the stems and flowers of tomato, whereas *SlCAT2* was expressed more in the leaves (Kabir and Wang 2011). Su et al. (2014) found that the expression of *ScCAT1 (sugarcane catalase gene)* was the highest in buds, followed by stem epidermis and stem pith, and was the least in leaves. Dark and light conditions and their intensity can modulate CAT activity at molecular level (Redinbaugh et al. 1990). In tomato, *SlCAT1* had high expression during the late light phase, whereas the expression of *SlCAT1* was high during the early dark phase (Kabir and Wang 2011). The induction of Cat under metal stress is also dependent upon the presence or absence of light (Azpilicueta et al. 2007). In addition, CAT activity can also be modulated by calmodulin, a calcium-binding protein that binds to and activate plant CAT in the presence of calcium (Yang and Poovaiah 2002).

Assay methods in plants

CAT activity may be determined employing ultraviolet spectroscopy, permanganate titration, iodometric assay, nanometric method, and visual approaches. A few of these methods are highlighted hereunder. In plant homogenates, the spectrophotometric method which measures the decomposition of H_2O_2 at 240 nm has been widely used. A relatively low H_2O_2 concentration (≈10 mM) is used to avoid inactivation of CAT during the assay or formation of bubbles in the cuvette due to the liberation of O_2. Measurements are generally made at room temperature and pH 7.0 (Aebi 1984). There are several disadvantages associated with this method which includes low sensitivity and limitations with turbid tissue preparations. Additionally, interference by peroxidase can use H_2O_2 to oxidize the phenolic and thereby produce an apparent CAT activity. The assay of CAT activity by titration although good for high UV absorption samples or where samples have high pigmentation or precipitate has generally not been used in plant homogenate. The principle behind titration method that uses starch as an indicator is that the decomposition of H_2O_2 is followed by the measurement of peroxide which remains undecomposed in the mixture after a certain time by back-

titration with $KMnO_4$ (Artenie and Tanase 1981). CAT activity can also be assayed calorimetrically (Sinha 1972). The principle behind the calorimetric assay of CAT activity is that herein, dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically at 570–610 nm. Dichromate has no absorbency in this region. To determine CAT activity, one can adopt a method to measure the volume of oxygen which is generated under the chemical action of enzyme upon H_2O_2. The measurement of oxygen concentration in the solution by means of the Clark oxygen electrode had been applied for the CAT assay (Rorth and Jensen 1967; Schepartz 1974). However, only oxygen concentration greater than the air-saturated solution (i.e., oversaturated oxygen) can be measured by this method which limits the sensitivity and time of linear oxygen production. Further, oxygen generated from CAT reaction can form bubbles, and the Clark oxygen electrode fails to measure the oxygen generated/released in the two phases. Later, oxygen-free buffer solutions for the determination of CAT activity were reported. Herein, oxygen is removed by boiling the buffer or oxygen is displaced by flushing with nitrogen (Breidenbach et al. 1968; Del Rio et al. 1977). An assay method combining the ease and simplicity of the qualitative approach for measuring CAT activity has also been developed (Iwase et al. 2013). Herein, the assay reagents comprise only H_2O_2 and Triton X-100, and the height of the Triton X-100-trapped enzyme-generated oxygen bubbles is estimated that is visualized as foam.

Modulation in abiotic stressed plants

Metals/metalloids

The effect of metal(oids) on the activities of antioxidant enzymes, including CAT, involved in ROS detoxification and counteracting metal-induced damage is not consistent and varies with plant species, tissue type, plant age/developmental stage, and metal type and with its concentration and duration of exposure. Previously, several studies have reported modulation (decline/increase) in the activities of CAT under metal/metalloid exposure (Table 1). Under metal stress, plant-CAT activity showed differential responses. An improved antioxidant system via higher CAT activity was argued in cadmium (Cd)-exposed *Brassica napus* (Hasanuzzaman et al. 2012b), *Oryza sativa* (Hsu and Kao 2004), *Brassica juncea* (Mobin and Khan 2007), *Triticum aestivum* (Khan et al. 2007), and arsenic-exposed *Triticum aestivum* seedlings (Hasanuzzaman and Fujita 2013a, b). In contrast, Cd exposure can also cause decline in CAT activity (Balestrasse et al. 2001; Agami and Mohamed 2013). Elevated levels of plant-beneficial elements such as Mn (100 μM $MnCl_2$) decreased CAT activity by 45 % in *Helianthus annuus*, whereas a 34 % increase in the activity of CAT was recorded when supplementation of Mn-exposed plants was done with 5.0 μM Se (Saidi et al. 2014). Unchanged CAT activity was noted in *Zea mays* treated with Cd (25 mM), whereas Z. mays pretreatment with salicylic acid (SA) (500 μM, 6 h) reduced CAT activity by 50 % (Krantev et al. 2008). Elevated CAT activity was also reported in Cd-exposed plants but exogenously supplemented with IAA and SA (Agami and Mohamed 2013) and Se (both 50 and 100 μM) (Hasanuzzaman et al. 2012b). Sodium nitroprusside (SNP, NO donor)-mediated enhancement in CAT activity detoxified H_2O_2 in As-stressed *Triticum aestivum* (Hasanuzzaman and Fujita 2013a, b). Cd (25 and 50 μM)-accrued significant increase in CAT activity in *Pisum sativum* seedlings was not retrieved even with supplementation of $CaCl_2$ (1.0 and 5.0 mM) (El-Beltagi and Mohamed 2013). This clearly suggests an adaptive mechanism in plants to compensate the higher level of H_2O_2 under metal toxicity. In a similar report, lower concentrations of Cd enhanced CAT activity in *Coffea arabica* (Gomes-Júnior et al. 2006). The activity of CAT can be modulated by the development stages of plants. In Cu- and Zn-exposed *Vigna mungo*, a significantly higher activity of CAT was noted at germination stage, whereas its activity was inhibited later (Solanki and Poonam 2011). This may be due to the fact that CAT are photosensitive antioxidant enzymes and need constant fresh synthesis (Feierabend et al. 1992). Additionally, at the germination stage, metals are usually sequestered properly by these CATs; however, later, either their synthesis is inhibited or there occurred some change in their confirmation (Sreedevi et al. 2008). It is not always obvious that CAT takes part in the detoxification of H_2O_2 in metal/metalloid-exposed plants, and some peroxidases respond well in such cases. To this end, the activity of CAT was not affected by Cd treatment in *Zea mays* , rather its activity dropped to approximately 50 % in SA-pretreated plants (Krantev et al. 2008). This indicates a different role of CAT in the oxidative stress induced by metals/metalloids. In another report, the incubation of *Helianthus annuus* leaf disks with 300 and 500 mM $CdCl_2$ under light conditions increased *CATA3* transcript level; however, this transcript was not induced by Cd in etiolated plants (Azpilicueta et al. 2007). Moreover, in the roots of the transgenic CAT-deficient tobacco lines (CAT 1AS), the DNA damage induced by Cd was higher than in wild-type tobacco roots (Gichner et al. 2004). A CAT gene from *Brassica juncea* (*BjCAT3*) was cloned and upregulated in tobacco under Cd. CAT activity of transgenic *Brassica juncea* was approximately 2-fold higher when compared to wild plant type under similar stress (Guan et al. 2009). Exposure of plants to different metals/metalloids enhances or declines CAT activity and has been summarized in Table 1.

The literature is full on the molecular insights into the metal/metalloid-accrued modulation of CAT activity, expression, isozyme, and genes. $Cd2^+$ exposure (50 μg L^{-1})

Table 1 Changes in catalase (CAT) activity in plants and its role in abiotic stress tolerance

Stress type	Plant species	Stress level	Stress-induced changes in activity	Protectant	Protectant-mediated changes in activity	Tolerance attributes	References
Salt stress	*Brassica napus* cv. BINA sharisha 3	100 mM NaCl, 48 h	Increased by 35 %	100 μM SA, 48 h	Increased compared to salt alone	MDA decreased by 29 %; inhibited the H_2O_2 generation	Hasanuzzaman et al. (2014a)
	Oryza sativa L. cv. BRRI dhan49	150 mM NaCl, 48 h	Decreased by 31 %	5.0 mM Pro, 48 h	Increased compared to salt alone	RWC increased by 16 %; decreased chl *a*, *b*, MDA, H_2O_2 and LOX	Hasanuzzaman et al. (2014b)
	Brassica napus cv. BINA sharisha 3	200 mM NaCl, 48 h	Increased by 45 %	Selenium (25 μM Na_2SeO_4), pre-treatment, 48 h	Increased compared to salt alone	MDA decreased 26 %; reduced H_2O_2	Hasanuzzaman et al. (2011a)
	Suspension cell culture of *Nicotiana tabacum*, cv. BY-2	200 mM NaCl, 7 d	Decreased	20 mM Pro and Bet, 7 d	No further change	Exogenous Pro or Bet increased the activities of all enzymes except MDHAR involved in NaCl-induced ASC–GSH cycle	Hoque et al. (2006)
	Zea mays L. cv. BR-5011	100 mM NaCl, 4d	Unchanged	Pretreatment with 1.0 mM H_2O_2, 2 d	Increased by 42 %	Increased SDM, RDM and LA(Leaf area) of 35 %, 26 % and 23 % respectively; decreased MDA	Neto et al. (2005)
	Triticum aestivum L. cv.Wenmai No.6	150 mM NaCl, 4 d	Decreased	Pretreatment with 2 mM JA, 7 d	Increased and same as control	Decreased MDA, H_2O_2 and the production rate of $O_2^{\cdot-}$	Qiu et al. (2014)
	Oryza sativa L. var. Pusa Basmati-1 cv. indica	75 mM NaCl, 12 d	Increased by 37 %	10^{-7} M 24-epibrassinolide, 8 h	Further increase by 26 %	Increased shoot, root length, chl *a*, total chl and decreased MDA 36 %	Sharma et al. (2013)
	Cucumis sativus L. cv. Jinlu4	50 mM NaCl, 10 d	Decreased	1.0 mM Si, 10 d	Not increased	Increased dry weight of shoots and roots, leaf soluble protein, and decreased ELP, LPO level and H_2O_2 content	Zhu et al. (2004)
Drought stress	*Brassica juncea* cv. BARI Sharisha 11	15 % PEG, 48 h	Increased by 20 %	5.0 mM Tre, 48 h	Unaltered	Increased FW, DW, RWC, chl *a*, chl *b*, total chl and decreased MDA, H_2O_2	Alam et al. (2014c)
	Brassica juncea cv. BARI Sharisha 11	15 % PEG-6000, 48 h	Unchanged	0.25, 0.5, 0.75 and 1.0 mM JA, 48 h	Unable to enhance CAT	Increased FW, DW, RWC, chl a, total chl and decreased MDA, H_2O_2, LOX	Alam et al. (2014b)
	Brassica juncea cv. BARI Sharisha 11	20 % PEG, 48 h	Unchanged	50 μM SA, 48 h	Enhanced CAT activity	Increased 50 % RWC, 31 % chl *a*, 79 % chl *b*, decreased H_2O_2 and 32 % MDA	Alam et al. (2013)
	Fragaria × *ananassa* Duch. cv. Kurdistan	-1 MPa water potential	Slightly increased	0.1 mM SA at flowering stage	Slightly increased	Increased leaf number, leaf area, leaf dry matter, root dry matter, shoot dry matter, carbohydrate content	Ghaderi et al. (2015)
	Triticum aestivum L. cv. SN215953	Drought stress was induced through withholding water	Declined	100 mM GB, 3 d	No change	Photosynthetic rate increased because GB-treated plants maintain higher maximal photochemistry efficiency of PSII	Ma et al. (2006)
	Olea europaea L.	Substrate water content about 15 % on dry weight basis	Increased by 89 %	50 mg Se_L^{-1}	Further increase by 38 %	Increased RWC and MDA decreased 33 %	Proietti et al. (2013)

Table 1 (continued)

Stress type	Plant species	Stress level	Stress-induced changes in activity	Protectant	Protectant-mediated changes in activity	Tolerance attributes	References
	Triticum aestivum L. cv. Longchun 8139	50 % FC, 12 d	Decreased	2.11 mM Na_2SO_4, 12 d	Increased	Increased water status of leaf, photosynthetic pigments and decreased H_2O_2	Gong et al. (2005)
Heavy metal stress	*Brassica napus* cv. BINA sharisha 3	1.0 mM $CdCl_2$, 48 h	Decreased by 28 %	Pretreatment with 100 μM Na_2SeO_4, 24 h	Significantly higher CAT activities	Significant decrease of MDA and H_2O_2	Hasanuzzaman et al. (2012b)
	Triticum aestivum L. cv. Pradip	0.5 mM Na_2HAsO_4 .$7H_2O$, 72 h	No change	0.5 mM SNP, 72 h	Increased by 65 %	Increased chl *a*, chl *b*, total chl, and adequately lower level of MDA content , decreased H_2O_2 26 %	Hasanuzzaman and Fujita (2013a, b)
	Vigna radiata cv. Binamoog-1	1 mM $CdCl_2$, 48 h	Decreased by 55 %	5.0 mM Pro or 5 mM Bet, 48 h	47 and 38 % decrease by Pro and Bet, respectively	Decreased MDA 29 % and 30 % by Pro and Bet, respectively. Decreased H_2O_2	Hossain et al. (2010)
	Helianthus annuus	100 μM $MnCl_2$, 4 d	Decreased by 45 %	5.0 μM Se, 24 h	Increased by 34 %	Decreased MDA 43 % and decreased H_2O_2 37 %	Saidi et al. (2014)
	Helianthus annuus	72 mM flurochloridone, ,15 d	Decreased by 57 %	Pretreatment with 0.5 mM SA, 6 h	Decreased further by 96 %	Increased RWC; slight decrease of MDA	Kaya and Yigi (2014)
	Zea mays L. cv. Norma	25 mM $CdCl_2$, 3 d	Unaffected	Pretreatment with 500 mM SA, 6 h	Decreased by 50 %	Increased chl and decreased MDA	Krantev et al. (2008)
	Brassica juncea L. cv. Varuna	$_{100\ mg\ L}{}^{-1}$, $CdSO_4{\cdot}8H_2O$), from 11 to 45 d	Decreased	1.0 mM SA, 11 to 45 d	Further decreased	Decreased 42.8 % H_2O_2 and slight decreased of MDA	Ahmad et al. (2011)
	Brassica juncea Czern & Coss cv. T-59	150 μM $CdCl_2$	Significantly increased	0.01 μM of 28-homobrassinolide	Significantly increased	Increase leaf RWC and Chlorophyll pigments	Hayat et al. (2007)
	Triticum aestivum L. cv.Giza168	500 μM Cd	Decrease by 20 %	500 μM IAA	Increased by 7 %	Increased plant fresh mass , dry mass , chl, carotene and RWC	Agami and Mohamed (2013)
	Kentucky bluegrass	50 μM $CdCl_2$, 7 d	Small change	Pretreatment (seed) with 500 μM SA, 12 h	Decreased by 11.2 %	Enhanced the length, DW of shoots , roots, 46 % chl *a*, and decreased 23 % MDA , 29 % H_2O_2	Guo et al. (2013)
High temperature	*Triticum aestivum* L. cv. Pradip	38 °C, 24 h	Decreased by 20 %	0.25 mM SNP, 24 h	Significantly enhanced	Increased 14 % chl a, 10 % chl b, 13 % total chl and decreased 27 % MDA , 29 % H_2O_2	Hasanuzzaman et al. (2011a)
	Vigna radiata cv. Binamoog-1	42 °C , 24 h	Decreased by 28 %	0.5 mM GSH, 24 h	Increased by 39 %	Increased leaf RWC 15 %, chl *a* 12 %, chl *b* 40 %, total chl 25 % and decreased MDA and H_2O_2 levels, O_2 generation rate, LOX	Nahar et al. (2015c)

significantly modulates the *m*RNA expression of the *CAT2* gene and not *CAT1* gene in *Suaeda salsa*, a pioneer halophyte (Cong et al. 2013). In *Suaeda salsa*, the *CAT2* gene was reported to play a major role in increased CAT activity under Cd stress and has been considered as a gene marker to indicate Cd pollution (Cong et al. 2013). Studies have also been done to elucidate the alteration in the expression of CAT genes under metal(oid) stress. Cd caused significant DNA damage in the roots of the transgenic CAT-deficient tobacco lines (*CAT 1AS*) compared to wild-type tobacco roots (Gichner et al. 2004). *CAT3* (mitochondrial) transcript level was enhanced in the leaf disks of *Helianthus annuus* incubated with 300 and 500 μM $CdCl_2$ (Azpilicueta et al. 2007). *BjCAT3*, a CAT gene cloned from *Brassica juncea* was upregulated (by 2-fold) and protected transgenic tobacco plants against Cd stress (Guan et al. 2009). Cd stress linearly increased CAT activity in a number of macroalga including *Nannochloropsis oculata* (Lee and Shin 2003), *Gracilaria tenuistipitata* (Collén et al. 2003), and *Ulva fasciata* (Wu et al. 2009). A study on *Ulva fasciata* (*UfCAT*) confirmed that CAT transcripts may not be affected by Cd stress; hence, the induction of CAT may not always be under transcript control (Wu et al. 2009). Under Al stress, *m*RNA level declined in *Arabidopsis thaliana* clones encoding for enhanced CAT (Richards et al. 1998). The total CAT activity and induction of the *CAT* gene expression was enhanced in in vitro grown plants of *Prunus cerasifera* under Cu stress (Lombardi and Sebastiani 2005). Al exposure can enhance the expression of CAT cDNA from *Capsicum annuum*, and the transcript induction can be greater in the stem and during early stages of fruit development (Kwon and An 2001). Hg (20–40 μM $HgCl_2$)-accrued induction in the *CAT3* gene was reported in *Arabidoposis thaliana* Columbia wild type (Heidenreich et al. 2001). Mn treatment in plants can also increase the specific activity of CAT and upregulate the expression of gene encoding CAT (CAT, TDF no. 103-2) (Zhou et al. 2013). A high transcript level of *SlCAT1* and *SlCAT2* was reported in Pb-exposed tomato (*Solanum lycopersicum*) (Kabir and Wang 2011). *PgCAT1*, a novel gene isolated from leaves of 4-year-old *Panax ginseng*, showed a high homology to *CAT1* from alpine snowbell, upland cotton, eastern cottonwood, and peach (Purev et al. 2010). Heavy metals such as Cu brought a 2-fold increase in the transcript level of *PgCAT1* until 24 h of exposure to 50 μM Cu, whereas a declined expression was observed at 8 h posttreatment of 500 μM Cu (Purev et al. 2010). It was in sharp contrast to the increased expression of *CAT1* observed in *Prunus cerasifera* for 10 days when grown in media containing 100 μM Cu (Lombardi and Sebastiani 2005). Transcript levels of the cat3 (mitochondrial) protein were increased in sunflower by 2-fold after 8 h of incubation in 300 μM Cd^{2+}, under light and not in dark (Azpilicueta et al. 2007) and also in *Brassica juncea* by 2- to 5-fold after 48 h of exposure to 22.4 ppm of Cd (Minglin et al. 2005). A higher accumulation of CAT protein and enhanced expression of molecular chaperones were noted in soybean under Cd (100 μM $CdCl_2$) stress (Hossain et al. 2012).

The induction of new isoenzymes and alterations in isoenzyme profile can also play significant roles in cellular defense against heavy metal-induced oxidative stress. In leaf peroxisomes from Cd-stressed *Pisum sativum*, the total activity of CAT was increased and isoelectric focusing (IEF) analysis showed the presence of five different CAT isoforms (*CATs 1–5*) with small differences in their isoelectric points (Romero-Puertas et al. 1999). Notably, the most acidic isoforms, i.e., *CATs 3–5*, were enhanced with increase in Cd concentration and protected *Pisum sativum* against Cd impacts (Romero-Puertas et al. 1999). In the same plant, native PAGE analysis of *CAT* showed one widespread band of activity that decreased with increase in Cd concentration (Sandalio et al. 2001). Additionally, most acidic isoforms (*CAT2* and *CAT3*) were enhanced by low Cd concentration (10–30 μM), whereas higher concentrations decreased the activity of these CAT isoforms (Sandalio et al. 2001). *CAT* isoenzyme varied in its density in both the roots and leaves of *Raphanus sativus* under Cd exposure (El-Beltagi et al. 2010). The exhibition of similar mobility was noted in control as well as Cd-stressed leaf tissues, whereas the density increased with increase in Cd concentration and was the maximum at highest concentration (50 ppm) (El-Beltagi et al. 2010). In *Solanum nigrum*, Cu was unable to bring changes in the pattern of CAT isozymes (Fidalgo et al. 2013). In *Solanum nigrum* plants, *CAT1* isozyme exhibited a higher activity than *CAT2* did in shoots, whereas *CAT2* had greater activity in roots (Fidalgo et al. 2013). However, these authors noted a decline in CAT1 and CAT2 isozymes at 200 mpl/L Cu that was in parallel with decreases in CAT2 *m*RNA accumulation in shoots and increase in roots (Fidalgo et al. 2013). *ScCAT1*, a novel peroxisomal catalase gene from sugarcane, localized in the plasma membrane and cytoplasm, is induced in response and provided tolerance to Cu and Cd exposures (Su et al. 2014). A transient overexpression of *ScCAT1* in leaves of *Nicotiana benthamina* induced hypersensitive reaction response and cell death (Su et al. 2014). Two isoforms of CAT alleles (CAT1 and CAT2) detected in leaves of poplar (*Populus deltoides* × *Populus nigra*) cuttings increased sharply followed by a gradual decrease and then increased under Cd exposure (Zhang et al. 2014). Exposure to Pb (400 ppm) caused a significant increase in CAT expression, while no detectable isoform bands appeared at 600 ppm Pb, suggesting denaturation of the protein (Ibrahim and Bafeel 2009). Pb treatment (50–500 ppm) increased CAT isozyme profile in the roots and leaves of *Raphanus sativus* (El-Baltagi and Mohamed 2010). Pb (20 μg/L) and Zn (100 μg/L) alone or in combination significantly upregulate the expression of CAT genes in *Suaeda salsa* (Wu et al. 2012). In *Oryza sativa*, CAT activity declined in the roots but was enhanced in the shoots in response to 500 μM Pb; however, a decline was noticed at

1000 μM (Verma and Dubey 2003). Two CAT isoforms (with R_f 0.15 and 0.29) were detected in the roots, whereas three isoforms (with R_f 0.15, 0.29, and 0.42) were found in the shoots at 500 μM Pb (Verma and Dubey 2003). However, at 1000 μM Pb, these authors evidenced an increase in intensity of band with R_f 0.29 in shoots, whereas the band with R_f 0.15 disappeared (Verma and Dubey 2003).

Salinity, drought, and other abiotic stresses

Drought stress and soil salinity are the main causes of reduced plant growth and productivity in semiarid regions and causes a complex of responses at molecular, cellular, physiological, and developmental level, including antioxidant enzymatic and non-enzymatic defenses, mostly due to a photon intensity that exceeds the capacity of stressed plants of absorbing it (Fig. 2a, b). Response of CAT to salinity and drought in different plant species follows. Increasing soil salinity can significantly impair the H_2O_2-scaveging system in plants. To this end, varying NaCl concentration-mediated decrease in the activity of CAT, a major H_2O_2-scavenging enzyme was observed in a number of plant species including *Anabaena doliolum* (Srivastava et al. 2005), *Oryza sativa* (Sharma et al. 2013), *Cicer arietinum* (Eyidogan and Oz 2005), and *Brassica napus* (Hasanuzzaman et al. 2011a). In two *Oryza sativa* varieties differing in their salt tolerance, Hasanuzzaman et al. (2014b) reported decreases in CAT activity by 31 and 55 % at 150 and 300 mM NaCl, respectively, in salt-sensitive BRRI dhan49. However, the salt-tolerant BRRI dhan54 exhibited significant increase in CAT activity under mild stress (150 mM NaCl) and a slightly decreased CAT activity (11 %) at severe salt stress (300 mM NaCl). Recently, Nahar et al. (2015a) reported decreases of 28 and 44 % in the CAT activity of *Vigna radiata* seedlings exposed to salt stress for 24 and 48 h, respectively. In contrast to previous reports, Hasanuzzaman et al. (2011a) observed no change in the CAT activity in *Brassica napus* exposed to 150 mM NaCl. Drought stress-mediated impairment in H_2O_2-metabolizing system has also been reported. Proietti et al. (2013) reported that water deficit stress reduced CAT

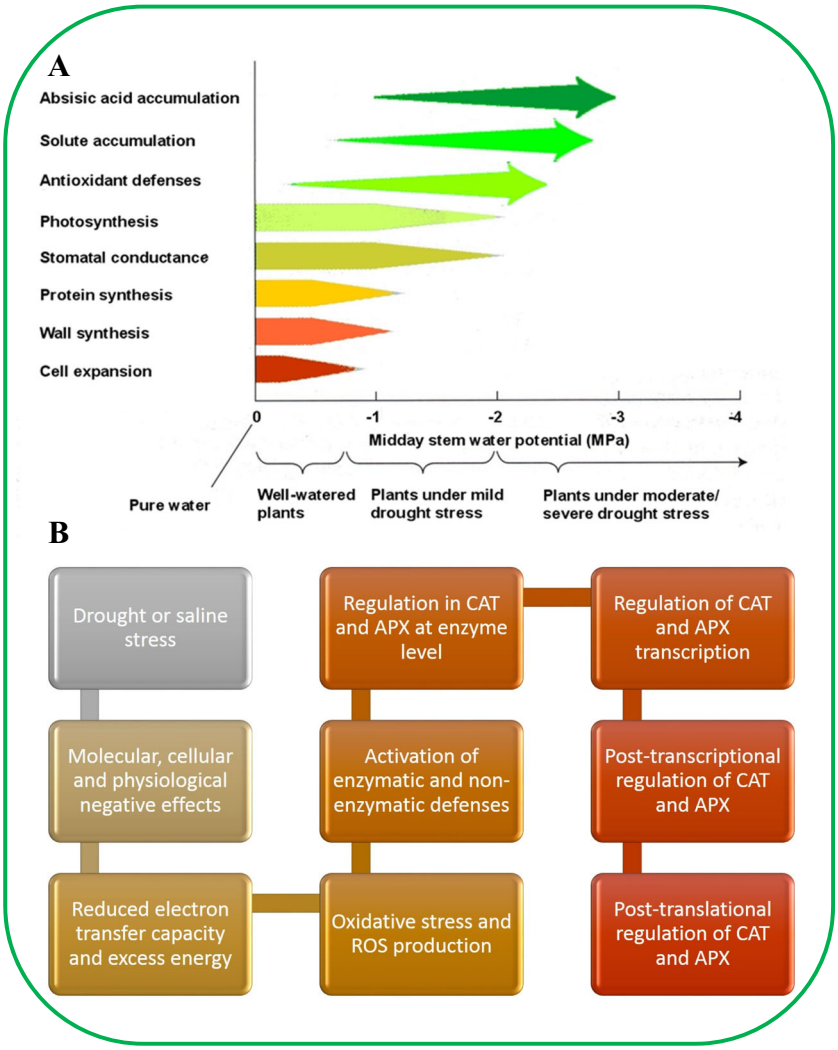

Fig. 2 Schematic representation of the main physiological and biochemical changes affected during the progression of drought, evaluated by means of stem water potentials measured at midday (**a**) and major biochemical and genetic effects of drought and saline stress in plants (**b**)

activity in *Olea europaea* by 89 %. A decreased CAT activity was observed in rice plant under drought stress (Sharma and Dubey 2005). In Strawberry (*Fragaria* × *ananassa* Duch.) cv. Kurdistan, CAT activity slightly increased under reduced water condition (Ghaderi et al. 2015). In *Brassica napus* seedling, a sharp decrease in CAT activity was observed under drought stress, which was measured as 25 and 36 % lower with 10 and 20 % polyethylene glycol (PEG), respectively, compared with control seedlings (Hasanuzzaman and Fujita 2011). Alam et al. (2014a) observed a 35 and 27 % decrease in the CAT activity of *Brassica napus* and *Brassica campestris*, respectively, when exposed to osmotic stress (15 % PEG), but the activity remained similar in *BRASSICA juncea*. In *Vigna radiata* seedlings, Nahar et al. (2015b) observed a marked decline in CAT activity under osmotic stress (−0.7 MPa), compared to the non-stressed control seedlings, with a reduction of 25 and 28 % in CAT activity after 24 and 48 h of stress, respectively. Transgenic rice plants overexpressing *OsMT1a* showed increase in CAT activity and thus enhanced tolerance to drought (Yang et al. 2009). Shikanai et al. (1998) observed that the expression of *Escherichia coli* CAT (KatE) in the chloroplast improved drought tolerance in tobacco and protected thiol-regulated chloroplast enzymes from oxidative inactivation. Drought-mediated increase in CAT activity was reported in wheat (*Triticum aestivum*) (Simova-Stoilova et al. 2010). In *Triticum aestivum*, heat treatment for 24 and 48 h resulted in a decrease of 20 and 27 % in CAT, respectively, over control (Hasanuzzaman et al. 2012a). In another report, the activities of CAT markedly decreased, due to heat stress, which were 45 or 43 % lower at 24 or 48 h of stress, respectively (Hasanuzzaman et al. 2014c). *Vigna radiata* seedlings exposed to HT stress of 42 °C (24 h) showed a 28 % decrease in CAT activity (Nahar et al. 2015c). Earlier, there was found a close relationship between the relative growth rate of tobacco seedlings and CAT activity under chilling stress condition (Xu et al. 2010). The authors argued that regression equations containing CAT could be used in predicting the seedling growth rate of tobacco under chilling stress conditions. In another study, elevated activity of CAT was correlated with tolerance to dark chilling stress (Wang et al. 2009).

Ascorbate peroxidase

Biochemical characterization

Ascorbate peroxidase (APX; EC 1.11.1.11) belongs to the class I heme-peroxidases and is found in most eukaryotes including higher plants (Groden and Beck 1979; Kelly and Latzko 1979; Nakano and Asada 1981; De Leonardis et al. 2000; Battistuzzi et al. 2001; Sharma and Dubey 2004; Yadav et al. 2014). Historically, soluble ascorbate (AsA)-dependent peroxidase was reported in pea leaves (Kelly and Latzko 1979), where it was observed later in lamellae isolated from spinach chloroplasts (Groden and Beck 1979). In plants, cytosolic isoforms (cAPX), mitochondria isoforms (mit APX), and microbody, including peroxisomal and glyoxysomal isoforms (mAPX), and chloroplastic isoforms (chAPX) of APX have been identified and all these function as scavengers of the H_2O_2, generated continuously in cells (Miyake and Asada 1996). Hence, H_2O_2 produced in the organelles are efficiently scavenged by the organelle themselves. Cytosolic APX, first isolated from pea shoots and later purified, exhibited stability in the absence of AsA (Mittler and Zilinskas 1991a). Isoforms of APX have also been purified and characterized from several other plant species including tea (Chen and Asada 1989), cotton (Bunkelmann and Trelease 1996), cucumber (Battistuzzi et al. 2001), tobacco (Madhusudhan et al. 2003), rice (Sharma and Dubey 2004), olive (Lopez-Huertas and del Río 2014), and ber (Yadav et al. 2014). Plant APX isoforms differ in molecular weight, optimal pH, stability, and substrate specificity. Two isoforms of APX with 57,000 and 34,000 Da molecular weights and distinct molecular properties occur in tea leaves (Chen and Asada 1989). cAPX is a dimer consisting of identical subunits with a molecular mass of 28 kDa, but chAPX isoenzymes exist in a monomeric form (Mittler and Zilinskas 1991a; Miyake et al. 1993). Plastidic APX with a molecular weight of 34,000 has been purified from plastids of tobacco (Madhusudhan et al. 2003), whereas non-plastidic isozymes of APX with molecular masses of 28–31 kDa were reported in pumpkin (Yamaguchi et al. 1995a) and cotton (Bunkelmann and Trelease 1996). Chloroplasts exhibit two APX isoenzymes namely thylakoid-bound (tAPX; molecular mass 37.5 kDa) and stromal (sAPX; molecular mass 33.2 kDa) (Chen and Asada 1989; Ishikawa et al. 1996). The molecular masses of mAPX and mitAPX were found to be approximately 31 kDa (Yamaguchi et al. 1995a; De Leonardis et al. 2000). Optimum pH for the optimum activity of APX isoforms ranges between 7 and 8 (Mittler and Zilinskas 1991a; Sharma and Dubey 2004; Yadav et al. 2014). Notably, APX isoenzymes are unstable in the absence of AsA. In particular, chAPX is more AsA-specific than cAPX is; hence, the former is more labile. In fact, the presence of Trp-175 in chAPX instead of Phe-175 in cAPX controls its greater AsA specificity (Jespersen et al. 1997). The concentration of AsA lower than 20 μM results into the quick loss in the activity of chAPX (sAPX and tAPX). AsA is oxidatively decomposed by very low (nanomolar) levels of H_2O_2 when AsA is unable to reduce compound I of APX to compound II (Miyake and Asada 1996). Both cAPX and mAPX exhibit around 1 hour or more as half-

inactivation time; whereas, for mitAPX and chAPX, it is less than 30 s (Chen and Asada 1989; Ishikawa et al. 1998; Yoshimura et al. 1998; De Leonardis et al. 2000).

Cyanide and azide-mediated inhibition in the activities of all APX isoforms indicates the heme peroxidase nature of APX (Mittler and Zilinskas 1991a). Iron plays an important role in the catalytic site of APX. To this end, the deficiency of iron resulted into bringing the activity of cytosolic APX to half, whereas the AsA concentration was found to be doubled (Zaharieva and Abadía 2003). Inhibition of APX by thiol reagent such as Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid; DTNB) indicates that the thiol group participates at the enzyme's active center. However, the thiol reagents do not inhibit guaiacol peroxidases. The blocking of the ability of APX to oxidize AsA but not the other small aromatic phenolic substrates was found as a result of the chemical modification of the single Cys32 residue near Arg172 and the heme propionates in APX with DTNB (Mandelman et al. 1998a). Mutation and crystallographic studies also supported the above findings. The basic properties of APX differ significantly from those of the guaiacol peroxidases. Unexpectedly, sequencing of first available APX cDNA showed 33 % amino acid identity with yeast cytochrome c peroxidase (CCP) (Mittler and Zilinskas 1991b).

In APX-catalyzed reactions, APX first reacts with H_2O_2 to produce compound I, where the heme (iron V) is oxidized to the oxyferryl (Fe^{4+}=O) species. It is the fully oxidized form of APX. Resting ferric APX state is then regenerated from compound I by two successive one-electron reactions with the substrate.

$$\mathrm{APX} + \mathrm{H_2O_2} \rightarrow \mathrm{Compound\ 1} + \mathrm{H2O}$$
$$\mathrm{Compound\ 1} + \mathrm{HS} \rightarrow \mathrm{Compound\ II} + \mathrm{S}$$
$$\mathrm{Compound\ II} + \mathrm{HS} \rightarrow \mathrm{APX} + \mathrm{S'} + \mathrm{H_2O}$$

Although both CCP and APX contain tryptophan in the proximal heme pocket at the same location, only Trp191 in compound I of CCP forms stable free-radical cations (Houseman et al. 1993). The electron paramagnetic resonance (EPR) spectrum of APX is totally different and indicates formation of porphyrin π-cation radical in compound I (Benecky et al. 1993; Patterson et al. 1995). Roles of various amino acid residues participating in the interaction between subunits of APX and binding of heme and AsA are described in Table 2. Presence of two kinetically competent binding sites for AsA in APX has been confirmed (Lad et al. 2002). APX participates in AsA–GSH cycle or the Foyer–Halliwell–Asada pathway (Noctor and Foyer 1998; Asada 1999) (Fig. 3). The reaction catalyzed by APX produces two molecules of monodehydroascorbate (MDHA) which is reduced to AsA by enzyme monodehydroascorbate reductase (MDHAR). Some dehydroascorbate (DHA) is also always produced when AsA is oxidized in leaves and other tissues. Eventually, DHA is reduced back to AsA by the enzyme dehydroascorbate reductase (DHAR) which uses GSH as the reducing substrate and the oxidized glutathione (GSSG) thus produced is reduced back to GSH by glutathione reductase (GR).

Major molecular mechanisms underlying APX regulation in plants are largely known. *S*-nitrosylation is a well-known redox-based posttranslational protein modification. It has been shown that APX is one of the potential targets of posttranslational modification mediated by nitrous oxide (NO)-derived molecules. Peroxynitrites ($ONOO^-$) which mediate protein nitration can inhibit APX activity, whereas S-nitrosoglutathione (GSNO) can enhance APX activity. Tyr5 and Tyr235 were exclusively nitrated to 3-nitrotyrosine by peroxynitrite, whereas Cys32 was S-nitrosylated. Tyr235 is found at the bottom of the pocket, where the heme group is enclosed. However, Cys32 is located at the AsA-binding site. Salinity increases APX activity and S-nitrosylated APX and the contents of NO and S-nitrosothiol (Begara-Morales et al. 2014). Rapid decreases in the activity of APX have been observed in vivo and in vitro in tobacco Bright Yellow-2 cells (De Pinto et al. 2013). Auxin was reported to cause APX1 denitrosylation and also partial inhibition of APX1 activity in *Arabidopsis* roots (Correa-Aragunde et al. 2013). In *Arabidopsis*, the requirement of the zinc finger protein Zat12 for cAPX1 expression under stress (hydrogen peroxide, heat, paraquat, and wounding)-accrued oxidative stress (Rizhsky et al. 2004). Several phytohormones can also be involved in APX activation. To this end, in sweet potato, abscisic acid treatment caused a strong increase in the transcription of APX1 (Park et al. 2004; Hu et al. 2005). In another study, ethylene treatment showed no effect on AsA content and on the expression of genes involved in AsA metabolism (Nishikawa et al. 2003). Using phylogenetic, genomic, and functional analyses, Lazzarotto et al. (2011) identified and characterized a new class of putative heme peroxidases, called APX-related (APx-R), which are targeted to the chloroplast and can physically interact with chloroplastic APx proteins.

Localization

Plant APXs are found in several cellular compartments including chloroplasts, the cytosol, mitochondria, peroxisomes, and microbodies. *Arabidopsis thaliana* has been reported to have eight isozymes (soluble cytosolic APXs: APX1, APX2, APX6; microsome membrane-bound APXs: APX3, APX4, APX5; and chloroplastic APXs: sAPX and tAPX) (Jespersen et al. 1997; Panchuk et al. 2002). APX has also been found in mitochondria in pea and potato (Jimenez et al. 1997; De Leonardis et al. 2000). In rice genome, in silico analysis revealed eight APX genes: two cytosolic (OsAPx1 and OsAPx2), two putative peroxisomal isoforms (OsAPx3 and OsAPx4), and four putative chloroplastic ones (OsAPx5 to

Table 2 Amino acid residues participating in the interaction between subunits of ascorbate peroxidase (APX) and binding of heme and ascorbate (AsA)

Sl. no.	Amino acid residue	Role	References
1.	Lys-18, Arg-21, Lys-22, Arg-24, Asp-112, Glu-228, Asp-229 Participate in electrostatic interactions between subunits	Jespersen et al. (1997)	
2.	Arg-172	AsA utilization to form compound II	Bursey and Poulos (2000) Kovacsa et al. (2013)
3.	Cys-32	Binding of ascorbate	Mandelman et al. (1998a)
4.	Glu-112	Alteration in solvent structure	Mandelman et al. (1998b)
5.	Arg-38	Control of substrate binding and orientation	Celik et al. (2001)
6.	Arg-172, Lys-30	Hydrogen bonds of these amino acids to heme 6-propionate play a role in stabilization of the bound ascorbate	Sharp et al. (2003), MacDonald et al. (2006)

OsAPx8) (Teixeira et al. 2004). In particular, OSAPX8 was confirmed as a putative thylakoid-bound isoform (Teixeira et al. 2004). Although sequence analysis predicted localization of OSAPX6 in chloroplast, OSAPX6-GFP fusion proteins was found in mitochondria of the BY-2 tobacco cells indicating complementation and coordination of the antioxidant defenses in different cellular compartments (Teixeria et al. 2006).

In fact, APX subcellular location is usually determined by the presence of targeting peptides and transmembrane domains in the NH2- and COOH-terminal regions of the protein (Shigeoka et al. 2002; Teixeira et al. 2004). In chloroplast, superoxide and H_2O_2 are produced in high amounts as a consequence of the highly energetic reactions taking place during photosynthetic activity. Since CATs are not present in chloroplast, APX has a critical role in this organelle. A chloroplastic

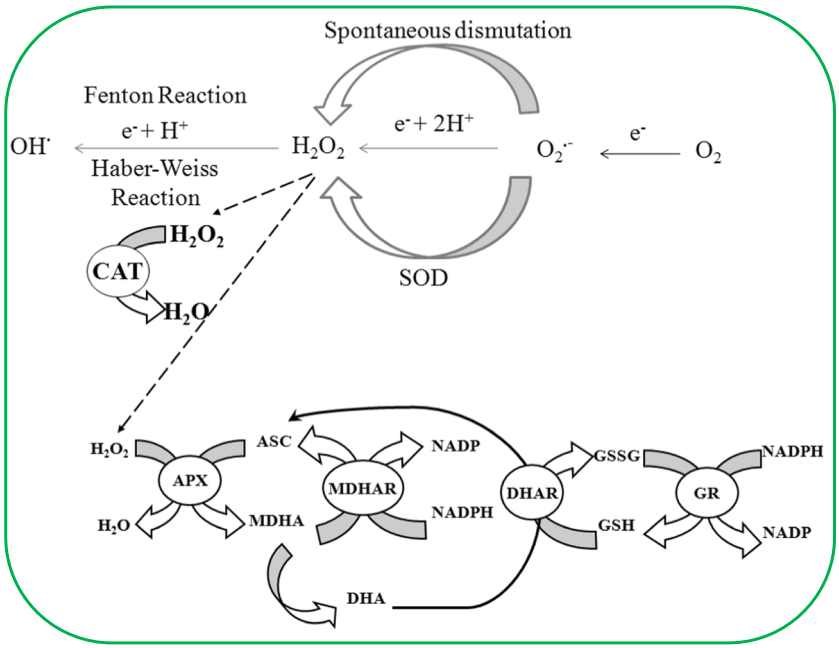

Fig. 3 Stepwise monovalent reduction of O_2 leads to formation of $O_2^{\cdot-}$, H_2O_2, and ·OH. $O_2^{\cdot-}$ is easily dismutated to H_2O_2 either non-enzymatically or by superoxide dismutase (*SOD*) catalyzed reaction to H_2O_2. H_2O_2 is converted to H_2O by catalase (*CAT*) and ascorbate peroxidase (*APX*). APX participates in the ascorbate–glutathione cycle or Foyer–Halliwell–Asada pathway. Ascorbate (*ASC*) is converted to monodehydroascorbate (*MDHA*) in a reaction catalyzed by APX. Monodehydroascorbate is reduced to ascorbate by enzyme monodehydroascorbate reductase (*MDHAR*). Some dehydroascorbate (*DHA*) is also always produced when ascorbate is oxidized. DHA is reduced back to ASC by enzyme dehydroascorbate reductase (*DHAR*) which uses glutathione (*GSH*) as the reducing substrate and the oxidized glutathione (*GSSG*) thus produced is reduced back to GSH by glutathione reductase (*GR*

transit peptide consisting of 19 residues are identified in the chAPX. All chloroplastic isoforms possess a hydroxylated peptide at the N-terminus that is processed in the mature proteins (Madhusudhan et al. 2003). Two signatures clearly identified the chloroplastic isoforms in higher plants. The first signature consisted of 7 residues (KNIEEWP) and the second had 16 (ETKYTKDGPGAPGGQS). Chloroplastic APXs (sAPX and tAPX) scavenge the H_2O_2 within chloroplasts. Two genes encoding APx isoforms, a thylakoid-bound isoform (At1g77490) and a stromal/mitochondrial isoform, (At4g08390) were reported in *Arabidopsis* (Chew et al. 2003; Teixeira et al. 2004; Davletova et al. 2005). In contrast to *Arabidopsis*, plant species including spinach, tobacco, pumpkin, and iceplant were reported to exhibit stromal and thylakoid-bound isoforms generated by alternative splicing of a single gene (Mano et al. 1997). cDNAs isolated from spinach were reported to encode sAPX and tAPX containing common chloroplast transit peptides of 70 amino acid residues (Ishikawa et al. 1996). Notably, the amino acid sequences of tAPX and sAPX were found identical except for C-terminal of tAPX, which is the putative transmembrane segment and was 50 amino acids longer than that of sAPX (Dong et al. 2011). Additionally, no thylakoid membrane domain was observed in the COOH terminus and confirmed the localization of APX in the stroma of the chloroplast (Dong et al. 2011).

ROS (and its reaction products) are regularly produced in the mitochondrial respiratory chain reactions. The presence of APX in mitochondria has been reported by several groups (Jimenez et al. 1997; De Leonardis et al. 2000; Mittova et al. 2004a). The presence of the AsA–GSH pathway in the mitochondria of soybean root nodules was reported; however, immunocytochemical techniques failed to confirm the presence of APX inside the mitochondria (Dalton et al. 1993). The presence of APX in mitochondria purified from pea was evidenced, where APX seemed to be associated with the external side of the outer membrane (Jiménez et al. 1997). However, no latent APX activity was observed for intact mitochondria but remained in the membrane fraction after solubilization assays with 0. 2 M KCl. Later, APX isolated from potato tuber mitochondria was purified, where the treatment with 0.2 M KCl did not solubilize APX (De Leonardis et al. 2000). However, the sonication-mediated disruption of the mitochondria revealed a higher APX activity in the supernatant than in the pellet and also confirmed the localization of APX inside mitochondria. In *Arabidopsis*, dual targets of stromal APX to plastids and mitochondria were reported as a result of the ambiguity of the targeting peptide at the N-terminal of the APX (Chew et al. 2003). However, distinct genes for mitochondrial and stromal isoform generation were also confirmed in tomato plants (Mittova et al. 2004b). No corresponding gene, cDNA, or protein sequences for the specific mitochondrial isoform have been described so far.

In peroxisomes/glyoxysomes, ROS are produced as byproducts of several processes such as photorespiration, fatty acid β-oxidation, and ureide metabolism. Although CAT is capable of scavenging large concentrations of H_2O_2, its localization in the peroxisomal/glyoxysomal matrix along with its low affinity for H_2O_2 limits its ability to keep H_2O_2 concentrations low enough to prevent it from diffusing into other CAT-lacking subcellular compartments (Karyotou and Donaldson 2005). APX prevents H_2O_2 from leaking out of peroxisomes. Peroxisomal APX preferentially accumulates in spongy parenchyma rather than palisade parenchyma and can also be found in large amounts near the central vascular bundles (Pereira et al. 2005). Peroxisomal APX has been reported in several plants including pumpkin (Yamaguchi et al. 1995a, b; Nito et al. 2001), cotton (Bunkelmann and Trelease 1996; Mullen et al. 2001), spinach (Ishikawa et al. 1998), pea (Jimenez et al. 1997; Lopez-Huertas et al. 1999), cucumber cotyledons (Corpas et al. 1994; Corpas and Trelease 1998), *Arabidopsis* (Zhang et al. 1997; Wang et al. 1999), and olive (Lopez-Huertas and del Río 2014). No latent APX activity in the intact microbodies indicates that the APX active sites exposed to the cytosol and the peroxidase can scavenge H_2O_2 leaked from microbodies (Yamaguchi et al. 1995a; Ishikawa et al. 1997). Additionally, the N-terminal active domain of the peroxisomal APX enzyme faces the cytosol, and its C-terminal domain is anchored and eventually facilitates the protein's functioning (Lisenbee et al. 2003). The peroxisomal targeting signal comprises a COOH-terminal transmembrane domain rich in valine and alanine followed by a positively charged domain containing five amino acid residues (Mullen and Trelease 2000). In wild-type *Arabidopsis* cells, peroxisomal APX was reported within a subdomain of rough endoplasmic reticulum (Lisenbee et al. 2003). It was suggested that portions of rough ER (pER) in wild-type cells can serve as a constitutive sorting compartment likely to be involved in the posttranslational routing of constitutively synthesized peroxisomal APX (Lisenbee et al. 2003).

Genomic characterization

Information regarding the genetic organization of APXs came into light through studies on a number of plants including pea, *Arabidopsis*, spinach, and tobacco. Pea gene encoding cytosolic APX1 has been characterized and is known to encode by a single gene (Mittler and Zilinskas 1992). Moreover, the promoter of APX1 gene possesses a TATA box at −28 bp, a CCAAT box at −176 bp, and several regulatory elements (Mittler and Zilinskas 1992). Pea APX1 contains nine introns with high contents of A and T nucleotides. The first intron was found in the 5′ UTR region in mRNA (Mittler and Zilinskas 1992). Notably, regulatory elements present in APX1 include the antiperoxidative element (ARE) that is responsible for H_2O_2-dependent responses. However, CACGCA sequence is

responsible for the xenobiotic response and that of CACGTG sequence constitutes a part of the G box (Mittler and Zilinskas 1992). Additionally, the TGATTCAG sequence was reported to be a part of the GPE I enhancer (a regulatory element for glutathione transferase P) that regulates gene transcription by interacting with transcription factors and RNA II polymerase (Okuda et al. 1989). The heat shock element, characteristic of heat shock genes that bind the heat shock factor, is also present in the promoters of APX1 (Storozhenko et al. 1998) and APX2 (Panchuk et al. 2002). Promoters of APX3 and APX5 are known to contain sequences similar to the heat shock element; however, it does not bind heat shock factors (Storozhenko et al. 1998; Panchuk et al. 2002).

Chloroplastic isoforms namely sAPX and tAPX are encoded by separate genes in *Arabidopsis* (Yoshimura et al. 1999). In species such as spinach and tobacco, sAPX and tAPX are formed by alternative splicing of one gene, where chAPX was evidenced to contain 12 introns (Yoshimura et al. 1999). A putative splicing regulatory *cis* element (SRE) present in the upstream of the acceptor site in intron 12 of chAPX genes is highly conserved in higher plants and controls alternative splicing (Teixeira et al. 2004). Notably, this element is not present in plants that possess separate genes for chloroplast forms (Teixeira et al. 2004). Both spinach and tobacco are reported to possess four types of mRNA variants, one form (tAPX-I) encodes thylakoid-bound APX and the other three forms (sAPX-I, sAPX-II, and sAPX-III) encode the stromal APX that is formed as a result of the alternative splicing events in the 3′-terminal region of chAPX pre-mRNA (Yoshimura et al. 2002). Moreover, the ratio of the level of sAPX mRNAs to tAPX-I mRNA was close to 1 in leaves, whereas the ratio in root was greatly elevated possibly due to an increase in sAPX-III and a decrease in tAPX-I as a result of the alternative excision of intron 11 and intron 12, respectively. Interestingly, the strong interaction of SRE with a nuclear protein from the leaves, but not those from the roots, of spinach and tobacco indicates the splicing enhancer SRE-mediated regulation of the tissue-specific alternative splicing of chAPX pre-mRNA (Yoshimura et al. 2002).

Assay methods in plants

The extraction buffer used to first report soluble AsA-dependent peroxidase in pea leaves contained 10 mM Na phosphate, pH 7.0, 0.5 mM $MgCl_2$, and 1.0 mM EDTA and around 5 % insoluble polyvinylpyrrolidone (Kelly and Latzko 1979). APX activity was determined spectrophotometrically by following the decrease in absorbance at 265 nm (absorption maximum of ascorbate) due to the oxidation of ascorbate to dehydroascorbate. Reaction mixtures contained, in a final volume of 1.0 ml, 50 μmol Na phosphate buffer (pH 7.0), 2.0 μmol EDTA, partially purified APX preparation, and 30 nmol AsA. The reaction was started by adding H_2O_2. Enzyme activity was calculated considering the change in absorbance at 265 nm and an extinction coefficient for AsA at pH 7.0 of 14.0 mM^{-1} cm^{-1} Enzyme activity was directly proportional to the amount of pea-leaf preparation added to the reaction mixture. Either enzyme or H_2O_2 can be used as final component to initiate APX reactions. Ascorbate oxidase (measured at pH 7.0) and CAT (determined with 0.25 mM H_2O_2) were present with activities less than 3 % of that of APX. Later, chAPX was assayed in spinach leaves (Nakano and Asada 1981). Instead of a 265-nm wavelength, these researchers used 290 nm because the absorbance of the assay mixture used was too high at the absorption maximum of AsA. The reaction mixture contained 50 mM K-phosphate, pH 7.0, 0.5 mM AsA, 0.1 mM H_2O_2, and 0.1 mM EDTA in a total volume of 1.0 ml. The absorbance decrease was recorded after 10 to 30 min of addition of either enzyme or H_2O_2. Due to the lack or very low activity of ascorbate oxidase (EC 1.10.3.3) in spinach leaves, no correction for the oxidation of AsA in the absence of H_2O_2 was necessary. However, non-enzymatic oxidation of AsA by H_2O_2 needs to be done. The bioassay method involving the disappearance of AsA at 290 nm has been widely used for CAT assay in plant homogenates.

Modulation in abiotic-stressed plants

Metals/metalloids

As a key enzyme of the AsA–GSH pathway, APX protects plants against abiotic stress-accrued oxidative stress by reducing H_2O_2 into water utilizing AsA as an electron donor (Anjum et al. 2010). In general, APX activity increases in response to metals/metalloids in plants, where upregulation in its activity up to a certain limit has widely been advocated to control scavenging of H_2O_2 and subsequent control of oxidative stress (Mittler et al. 2004; Anjum et al. 2014b). However, inhibition or activation of an enzyme in defense system depends upon metal/metalloid types, concentrations and exposure magnitude, and plant species (Table 3).

In *Brassica oleracea*, low concentrations of Cd (0.5 mM) exposed for longer duration were not sufficient to inhibit or induce APX activity, whereas 2.5 mM Cu resulted in a linear increase in its activity with time (Posmyk et al. 2009). In Pb-treated *Typha latifolia*, a complete inhibition in APX activity was observed; however, the extent of inhibition was lesser when exposed to higher concentrations of As and Cd (Lyubenova and Schröder 2011). Lomonte et al. (2010) reported higher APX activity in the roots of *Atriplex codonocarpa* in response to 0.05 and 0.1 mg Hg l^{-1}. This was directly correlated with more need of a higher APX activity for scavenging H_2O_2 accumulated under Hg-induced oxidative stress up to 0.1 mg Hg l^{-1} (Lomonte et al. 2010). Plant organs can vary in terms of APX activity under metal/

Table 3 Changes in ascorbate peroxidase (APX) activity in plants and its role in abiotic stress tolerance

Stress type	Plant species	Stress level	Stress-induced changes in the activity	Protectant	Protectants-mediated changes in the activity	Tolerance attributes	References
Salt stress	*Brassica napus* cv. BINA sharisha 3	100 mM NaCl, 48 h	Increased by 22 %	100 μM SA, 48 h	No change	MDA decreased 29 %; inhibited the H_2O_2 generation	Hasanuzzaman et al. (2014a)
	Oryza sativa cv. BRRI dhan49	150 mM NaCl, 48 h	Increased by 40 %	5.0 mM Pro, 48 h	Increased by 29 %	RWC increased by16%; decreased Chl *a*, *b*, MDA, H_2O_2 and LOX	Hasanuzzaman et al. (2014b)
	Brassica napus 3 cv. BINA sharisha	200 mM NaCl, 48 h	Increased by 22 %	Selenium (25 μMNa_2SeO_4), pre-treatment, 48 h	Elevated APX activity	MDA decreased 26 %; reduced H_2O_2	Hasanuzzaman et al. (2011a)
	Suspension cell culture of *Nicotiana tabacum*, cv.BY-2	200 mM NaCl, 7 d	Decreased	20 mM Pro and Bet, 7 d	Increased by Pro but slightly by betaine.	Exogenous Pro and Bet increased the activities of all enzymes except MDHAR involved in NaCl-induced ASC–GSH cycle	Hoque et al. (2006)
	Zea mays L. cv. BRS 3003	80 mM NaCl, 96 h	Decreased by 28 %	Pretreatment with 10 mM H_2O_2	No change	Increased shoot and root dry mass; MDA decreased by 17 %	Gondim et al. (2013)
	Zea mays L. cv. BR-5011	100 mM NaCl, 4 d	Increased by 150 %	Pretreatment with 1.0 mM H_2O_2, 2 d	Unchanged	Increased SDM, RDM and LA(Leaf area) of 35 %, 26 % and 23 % respectively, MDA decreased	Neto et al. (2005)
	Triticum aestivum L. cv.Wenmai No.6	150 mM NaCl, 4 d	Decreased	Pretreatment 2 mM JA, 7 d	Increased and same as control	Decreased MDA, H_2O_2 and the production rate of $O_2^{\cdot-}$	Qiu et al. (2014)
	Oryza sativa L. var. Pusa Basmati-1 cv. indica	75 mM NaCl, 12 d	Increased by 46 %	10^{-7} M 24-epibrassinolide, 8 h	Further increased by 3 %	Increased shoot, root length, chl *a*, total chl and decreased MDA 36 %	Sharma et al. (2013)
	Cucumis sativus L. cv. Jinlu4	50 mM NaCl, 10 d	Increased	1.0 mM Si, 10 d	Increased	Increased dry weight of shoots and roots, leaf soluble protein, and decreased ELP, LPO level and H_2O_2 content	Zhu et al. (2004)
Drought stress	*Brassica juncea* cv. BARI Sharisha 11	15 % PEG, 48 h	Increased by 22 %	5 mM Tre, 48 h	Increased further by 14 %	Increased FW, DW, RWC, chl *a*, chl *b*, total chl and decreased MDA, H_2O_2	Alam et al. (2014c)
	Brassica juncea cv. BARI Sharisha 11	15 % PEG-6000, 48 h	Increased by 25 %	0.25, 0.5, 0.75 and 1.0 mM JA, 48 h	Unaltered	Increased FW, DW, RWC, chl a, total chl and decreased MDA, H_2O_2, LOX	Alam et al. (2014b)
	Brassica juncea cv. BARI Sharisha 11	20 % PEG, 48 h	Increased by 25 %	50 μM SA, 48 h	Unaltered	Increased 50 % RWC, 31 % chl *a*, 79 % chl *b*, decreased H_2O_2 and 32 % MDA	Alam et al. (2013)
	Fragaria × *ananassa* Duch. cv. Kurdistan	-1 MPa water potential	Slightly increased	0.1 mM SA at flowering stage	Increased	Increased leaf number, leaf area, leaf dry matter, root dry matter, shoot dry matter, carbohydrate content	Ghaderi et al. (2015)
	Triticum aestivum L. cv. SN215953	Drought stress was induced through withholding water	Increased	100 mM GB, 3 d	Increased	Photosynthetic rate increased because GB-treated plants maintain higher maximal photochemistry efficiency of PSII	Ma et al. (2006)

Table 3 (continued)

Stress type	Plant species	Stress level	Stress-induced changes in the activity	Protectant	Protectants-mediated changes in the activity	Tolerance attributes	References
	Olea europaea L.	Substrate water content about 15 % on dry weight basis	Decreased by 43 %	50 mg Se $_{L}^{-1}$	Increased by 131 %	Increased RWC and MDA decreased 33 %	Proietti et al. (2013)
	Triticum aestivum L. cv. Longchun 8139	50 % FC, 12 d	Unchanged	2.11 mM Na_2SO_4, 12 d	Unchanged	Increased water status of leaf, photosynthetic pigments and decreased H_2O_2	Gong et al. (2005)
Heavy metal stress	*Brassica napus* cv. BINA sharisha 3	1.0 mM $CdCl_2$, 48 h	Increased by 39 %	Pretreatment with 100 μM Na_2SeO_4, 24 h	Unchanged	Significant decrease of MDA and H_2O_2	Hasanuzzaman et al. (2012b)
	Triticum aestivum L. cv. Pradip	0.5 mM $Na_2HAsO_4.7H_2O$, 72 h	Increased by 34 %	0.5 mM SNP, 72 h	Unchanged	Increased chl *a*, chl *b*, total chl, and adequately lower level of MDA content , decreased H_2O_2 26 %	Hasanuzzaman and Fujita (2013a, b)
	Vigna radiata cv. Binamoog-1	1 mM $CdCl_2$, 48 h	Increased	5.0 mM Pro or 5 mM Bet, 48 h	35 and 57 % increase by Pro and Bet, respectively	Decreased MDA 29 and 30 % by Pro and Bet respectively; Decreased H_2O_2	Hossain et al. (2010)
	Helianthus annuus	100 μM $MnCl_2$, 4 d	Decreased by 47 %	5.0 μM Se, 24 h	Increased by 30 %	Decreased MDA by 43 % and decreased H_2O_2 by 37 %	Saidi et al. (2014)
	Helianthus annuus	72 mM flurochloridone, , 15 d	Decreased by 92 %	Pretreatment with 0.5 mM SA, 6 h	Decreased further by 50 %	Increased RWC; slight decrease of MDA	Kaya and Yigi (2014)
	Zea mays L. cv. Norma	25 mM $CdCl_2$, 3 d	Dropped more than 2-fold	Pretreatment with 500 mM SA, 6 h	Alleviated the inhibitory effect of Cd	Increased chl and decreased MDA	Krantev et al. (2008)
	Brassica juncea L. cv. Varuna	$_{100\ mg\ L}^{-1}$, $CdSO_4{\cdot}8H_2O$), from 11 to 45 d	Slightly increased	1.0 mM SA, 11 to 45 d	Decreased	Decreased 42.8 % H_2O_2 and slight decreased of MDA	Ahmad et al. (2011)
	Kentucky bluegrass	50 μM $CdCl_2$, 7 d	Reduced	Pretreatment (seed) with 500 μM SA, 12 h	Increased by 30 %	Enhanced the length, DW of shoots, roots, 46 % chl *a*, and decreased 23 % MDA , 29 % H_2O_2	Guo et al. (2013)
High temperature	*Triticum aestivum* L. cv. Pradip	38 °C, 24 h	Increased by 59 %	0.25 mM SNP, 24 h	Further enhancement	Increased 14 % chl a, 10 % chl b, 13 % total chl and decreased 27 % MDA , 29 % H_2O_2	Hasanuzzaman et al. (2012a)
	Vigna radiata cv. Binamoog-1	42 °C, 24 h	Increased by 30 %	0.5 mM GSH, 24 h	Further improvement	Increased leaf RWC 15 %, chl *a* 12 %, chl *b* 40 %, total chl 25 % and decreased MDA and H_2O_2 levels, O_2 generation rate, LOX	Nahar et al. (2015c)

metalloid exposure. In *Arabidopsis thaliana* under Cu (2 and 5 μM) exposure, APX activity was found to decline in roots, whereas its activity increased in leaves (Cuypers et al. 2011). There was change in APX activity in shoots of *Medicago sativa* under Hg stress (3.0, 10, 30 μM), whereas enhancements of 1.7- to 2.8-fold in APX activity was recorded in the roots (Sobrino-Plata et al. 2009). Irrigation with a combination of Pb^{2+} (5–15 mg l^{-1}), Hg^{2+} (0.5–1.5 mg l^{-1}), and Cd^{2+} (05–1.5 mg l^{-1}) enhanced the activity of APX in the leaves, stem, and roots of *Kandelia candel* and leaves of *Bruguiera gymnorrhiza* (Huang et al. 2010). A genotype-dependent modulation of APX activity has also been reported. In this context, exposure to 5.0 μM Cd for 10 and 15 days resulted in a greater increase or lesser decrease in Cd-tolerant genotype *Weisuobuzhi* than sensitive genotype *Dong 17* (Chen et al. 2010). A reduced activity of APX was reported in roots of less Cd-sensitive pea genotypes namely 3429 and 1658, whereas more Cd-sensitive genotype 188 exhibited an increased APX (Metwally et al. 2005). However, an increased APX activity was observed in Mn-tolerant cultivar *Kingston* of perennial ryegrass (*Lolium perenne*) when compared to sensitive cultivar *Nui* (Mora et al. 2009). In *Brassica napus* seedlings, APX activity increased by 39 and 43 % in response to 0.5 and 1.0 mM $CdCl_2$ stress, respectively (Hasanuzzaman et al. 2012b). *Brassica juncea* exposed to Cd exhibited an increased APX activity (Ahmad et al. 2011). A higher APX activity was correlated to nickel tolerance in *Triticum aestivum* (Gajewska and Sklodowska 2008). *Helianthus annuus* plants exposed to $MnCl_2$ (100 μM, 4 days) showed 47 % decrease in APX activity compared to control (Saidi et al. 2014). Contrarily, two levels of As (0.25 and 0.5 mM) increased in APX activity by 24 and 34 %, respectively, compared to control seedlings of *Triticum aestivum* (Hasanuzzaman and Fujita 2013a, b). In flurochloridone (72 mM, 15 days)-exposed *Helianthus annuus*, the APX activity decreased by 92 % (Kaya and Yigi 2014). In addition to elevated levels of known toxic elements, deficiency of plant-beneficial element including nitrogen, potassium, and phosphorus can modulate APX activity in plants (Tewari et al. 2009; Anjum et al. 2015). In *Zea mays* , APX activity was suppressed by 2-fold less than 25 mM Cd exposure (Krantev et al. 2008). Enhanced Cd tolerance of the MuSI transgenic tobacco was argued as a result of a higher activity of APX, where Cd exposure caused a 2-fold increase in APX activity compared to wild-type tobacco (Kim et al. 2011). Exposure of plants to single metal or two or more metals simultaneously may also differentially modulate APX activity. Under Cd + Zn exposure, *Ceratophyllum demersum* and *Cucumis sativus* exhibited highly increased APX activity compared to Cd- or Zn-alone treated plants and indicated a differential response of APX activity to Cd or Zn (Aravind and Prasad 2005; Khan et al. 2007). In the seedlings of *Sesbania drummondii*, Pb, Cu, Ni, and Zn alone exposure exhibited lower APX activity compared to treatment with combination of Pb + Cu + Ni + Zn (Israr et al. 2011). In another study, a combination of Cu and Cd (5 μM Cu + 50 μM Cd and 50 μM Cu + 100 μM Cd) caused inhibition in the activity of APX activity in *Arabidopsis thaliana* leaves (Drążkiewicz et al. 2010).

Many molecular and genetic evidences have indicated that transgenic plants are more tolerant to heavy metal stress owing to enhanced APX activity. In a genomic survey with *Arabidopsis c*DNA microarray, APX genes and class III peroxidases were expressed at higher levels in Cd/Zn hyperaccumulator *Arabidopsis halleri* compared to non-accumulator *Arabidopsis thaliana* and was argued to contribute to the heavy metal tolerance in the former (Chiang et al. 2006). Transgenic *Arabidopsis thaliana* plants constitutively overexpressing a peroxisomal APX gene (*HvAPX1*; from barley) were found more tolerant to Cd stress (Xu et al. 2008). Likewise, transgenic *Oryza sativa* plants double silenced for *APX1* and *APX2* (*APX1/APX2* plants) exhibited elevated tolerance to toxic concentrations of Al in addition to exhibiting normal development (Rosa et al. 2010). In another instance, an elevated activity of APX was reported in transgenic tall fescue (*Festuca arundinacea*) plants with chloroplast CuZn-SOD and APX-expressing genes under Cu and Cd exposure; however, As exposure resulted into declined APX activity (Lee et al. 2007).

Expression of APX genes is regulated during normal growth and development of plants. The activity and gene expression of APX (and also its isoenzymes) can be modulated in plants by several biotic and abiotic stress factors including toxic metals (Singh et al. 2008; Hasanuzzaman et al. 2011a, b, 2012a, b; Hossain and Fujita 2012). Nevertheless, several APX isoenzymes can be expressed constitutively mainly to efficiently and immediately detoxify H_2O_2 (Hossain and Fujita 2012). An increased APX activity in Cu (10–200 μM)-exposed *Withania somnifera* correlated it with six APX isoenzymes bands (particularly 3, 4, 5 and 6) detected in native PAGE analysis (Khatun et al. 2008). Inhibition in the activity of APX isoforms 3–6, observed at 25 μM Cu, was attributed either to proteolytic degradation or inactivation of APX isoforms due to oxidative protein modification (Khatun et al. 2008). The expression patterns and modulation of APX isoenzymes can also differ in different organs of a plant. To this end, in *Oryza sativa* shoots, two major bands of APX activity (*APX1* and *APX2*) were observed, whereas enzyme preparation from *chl*APX exhibited only one band in both control and Al-stressed plants (Sharma and Dubey 2007). Transcripts of *APX2* (cytosolic ascorbate peroxidase 2) and *APX1* (cytosolic ascorbate peroxidase 1) can exhibit their different responses to similar exposure concentrations of metals such as Cu and Cd. To this end, in 3-week-old *Arabidopsis thaliana*, μ *APX2* transcripts were elevated by Cu (2 and 5 μM) and Cd (5 and 10 μM) in roots, whereas an enhancement in *APX1* expression was enhanced in leaves but was

declined in roots under Cu stress (Cuypers et al. 2011). Genes encoding isoenzymes of *chl*APX are categorized into two groups. In the first group, through posttranscriptional regulation, an alternative splicing regulation single gene encodes two enzymes and includes genes from *Nicotiana tabacum*, *Mesembryanthemum crystaliium*, *Cucurbita sp.*, and *Spinacia oleracea*. On the other hand, a separate regulation of individual genes coding different isoenzymes takes place in the second group. Genes from *Arabidopsis*, *Oryza sativa* and *Lycopersicon esculentum* fall under the category of the second group (reviewed by Caverzan et al. 2012). Notably fundamental mechanism for controlling stromal (*s*APX) and thylakoid (*t*APX) isoenzyme expressions came into light from studies on alternative splicing in *Spinacia oleracea* (Ishikawa and Shigeoka 2008). Nine APX genes reported in *Arabidopsis thaliana* APX gene family encode isoforms found in mitochondria (APX6, APX7), peroxisomes (APX3, APX5), cytosol (APX1, APX2, and APX6), and chloroplast (APX3, APX4, APX6) (Chew et al. 2003). Out of six APX-encoding loci identified in *Eucalyptus grandis*, three were predicted to be cytosolic, one as peroxisomal and two chloroplast proteins (Teixeira et al. 2005). Four cDNAs of cytosolic, peroxisomal, and chloroplast (thylakoid and stroma) APX isoforms have also been isolated and characterized in *Vigna unguiculata* (D'Arcy-Lameta et al. 2006). A new class of heme peroxidases identified has been reported to synthesize a new protein, APX-R (ascorbate peroxidase-related) that is functionally associated with rice APX (Lazzarotto et al. 2011). Earlier, Fe-regulated expression of a cytosolic APX was reported to be encoded by the APX1 gene in *Arabidopsis* seedlings (Fourcroy et al. 2004). It was demonstrated that Fe-caused oxidative stress-mediated induction of *AtAPX1* gene encoding cytosolic APX can be possible in *Arabidopsis* leaves, whereas in roots, its constitutive expression can remain unaffected. In tomato also, the leader intron of the *APX20* gene was reported to contain an enhancer element for high constitutive expression in leaves and not in roots (Gadea et al. 1999).

Enhanced APX activity in tobacco plants overexpressing *Arabidopsis thaliana* cytosolic DHAR after 24 h of exposure to 400 μM $AlCl_3$ was linked to their greater tolerance against Al toxicity (Yin et al. 2010). An exposure to excess Fe (III) (500 μM) can result into an accumulation of APX transcripts (APX mRNA) also in cotyledons of plants such as 5-day-old *Brassica napus* (Vansuyt et al. 1997). Notably, the Fe-mediated expression of APX gene was reported to involve a signal transduction pathway different from those of oxidative stress (Vansuyt et al. 1997). Pekker et al. (2002) demonstrated that the Fe stress-induced expression of cytosolic APX can be possible both at *m*RNA and protein levels in plant organ such as leaves (Pekker et al. 2002). These authors also reported an exhibition of a higher Fe-stress sensitivity in transgenic tobacco plants with suppressed *c*APX levels compared to wild type. Fe(II) (7.0 mM) treatment was reported to enhance the expression of two cytosolic APX genes in 18-day old rice seedlings (Finatto et al. 2015). The genes encoding L-APX were downregulated by 4.7-fold in the shoot of rice after 3 days of exposure to 125 mg/l of $FeSO_4$ (Quinet et al. 2012). Additionally, Zn (1.0 mM), Cu (0.5 mM), and Cd (0.5 mM) treatments also elevated the expression of *NtAPX* genes in transgenic *Nicotiana tabaccum* lines compared to WT plants (Chaturvedi et al. 2014). In addition to an enhanced APX activity, accumulation of *m*RNA and transcripts encoding for various APXs was also evidenced in Pb-exposed plants (Verma and Dubey 2003). In another study, Pb (0.5 and 3.2 mM) upregulated the expression of APX genes after 4 h of exposure (Li et al. 2012). Al concentrations (such as 20 ppm) can also lead to increased transcript levels of *Os*APX genes (except *Os*APX6) in plants such as *Oryza sativa* (Rosa et al. 2010). In pea shoots, an Al-mediated-enhanced expression of *c*APX was not correlated with APX activity (Panda and Matsumoto 2010). An enhanced expression of APX was reported to protect perennial ryegrass (*Lolium perenne*) against Cd (0.2 and 0.5 mM) exposure (Luo et al. 2011). In a similar study, protection of cuttings of poplar (*Populus deltoides* × *Populus nigra*) against Cd stress was reported with elevated genes encoding APX (Zhang et al. 2014). In contrast to a greater expression of cytosolic APX protein in shoots and roots of Cd (6 and 30 μM)-exposed maize Hg (30 μM) was reported to have diminuted APX cytosolic protein (and also APX ativity) (Rellán-Álvarez et al. 2006). It was argued that this might happen as a result of a high degree of protein oxidation that in turn was translated into extreme toxic damage. It is also conceivable that at higher concentrations of Cd, peroxidase activity decreases due to oxidation of proteins in pea leaf (Cd up to 50 μM; Romero-Puertas et al. 2002) and poplar (Schützendübel et al. 2002). In addition, the failure of the metabolism resulting into the attenuation of APX activity could also be another reason for its declined activity under extreme metal/metalloid exposure (Schützendübel et al. 2001, 2002).

Salinity, drought, and other abiotic stresses

Soil salinity can decrease plant growth and productivity via impacting plant processes at physiological, biochemical, and molecular levels (Shahbaz and Ashraf 2013). It can disturb the homeostasis of Na^+ and Cl^- ions, uptake of major nutrients, and cause oxidative stress via alleviating cellular ROS (Tuteja 2007). Significant role and modulation of APX have been reported in plants exposed to varying levels of soil salinity. Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. Salt-tolerant tomato cultivar exhibited higher activities of both mitochondrial and peroxisomal APXs (Mittova et al. 2003a). No change in APX activity can be possible in plants under 5 days of exposure to salt stress (50 mM NaCl) (Zhu et al. 2004). However, 4 days

of exposure of plants to 150 mM NaCl was reported to bring significant decreases in APX activity in *Triticum aestivum* (Qiu et al. 2014). In general, an elevated activity of APX was argued to contribute to plant salt tolerance (Hernandez et al. 2000; Hasanuzzaman et al. 2011a). In salt-exposed rapeseed seedlings, Hasanuzzaman et al. (2014a) observed increases of 22 and 19 % in APX activity upon exposure to 100 and 200 mM of NaCl, respectively. In another recent study, salt stress (200 mM NaCl) for 24 h increased the activity of APX by 31 %, compared with control, whereas 48 h of stress did not significantly increase its activity (Nahar et al. 2015a). Rice seedlings were reported to exhibit salt treatment levels and salt sensitivity-dependent activity of APX (Hasanuzzaman et al. 2014b). To this end, 150 mM significantly increased APX activity (by 40 %) in salt-sensitive BRRI dhan49, whereas in salt tolerant BRRI dhan54, the similar treatment level caused an increase of 45 % (vs. control). Notably, under severe salt stress (300 mM NaCl), APX activity was decreased by 27 % in salt-sensitive cultivar; however, its activity increased by 27 % in salt-tolerant cultivar (Hasanuzzaman et al. 2014b). Organs of the same salinity-exposed plant can exhibit a differential APX activity. In 120 mM NaCl-exposed *Solanum lycopersicum*, 8 days of exposure was reported to bring no change in leaf APX activity, whereas in roots, similar stress brought increases by 28 % in APX activity (Manai et al. 2014). In halophyte *Avicennia marina*, salt stress elevated the expression of peroxisomal APX (Kavitha et al. 2008). Tissue-specific and tissue-dependent expressions of cAPX, mAPX, and chlAPX in response to salinity (450 mM NaCl) can also be possible in plants such as sweet potato (Lin and Pu 2010). These authors reported a higher APX activity in salt-tolerant genotypes when compared to the salt-sensitive ones (Lin and Pu 2010). Cytosolic APX2 (*OsAPX2*) can significantly contribute in conferring salt tolerance in rice (Zhang et al. 2013). One of salt treatments (50 mM and 100 mM NaCl) caused 4.5-fold increase in the accumulation of *OsAPX2* transcripts. Additionally, the gene expression was also increased by 7-fold after 150 mM NaCl treatment but was reduced to control level when treated with 250 mM NaCl (Zhang et al. 2013). In a recent study, peanut (*Arachis hypogaea*) plants with *SbpAPX* gene from *Salicornia brachiata* were reported to overexpress peroxisomal APX (SbpAPX) gene and were found tolerant to 150 mM NaCl (Singh et al. 2014a).

In context with APX responses to drought, an increased APX activity and subsequent reduction in H_2O_2 levels and plant drought tolerance was reported in several studies. *Zea mays* exhibiting correlation of higher APX with GR activity was drought tolerant (Pastori and Trippi 1992). In a similar recent study, a higher APX activity in tolerant pigeon pea cultivars had higher decreasing levels of H_2O_2 and lipid peroxidation under water logging stress, compared to sensitive cultivar (Kumutha et al. 2009). Improved drought tolerance in "Berangan" cultivar of poplar was argued as a result of an increased APX activity, whereas "Mas" cultivar showed no change in APX activity under the same stress condition (Yang et al. 2009). Water deficit stress-mediated decrease (43 %) in APX activity was also evidenced in *Olea europaea* (Proietti et al. 2013). In *Fragaria* × *ananassa* Duch. cv. Kurdistan, water deficit brought insignificant increases in APX activity (Ghaderi et al. 2015). No change in the activity of APX was observed in rapeseed seedlings under drought stress (10 and 20 % PEG) was argued to cause elevation in H_2O_2 content (Hasanuzzaman and Fujita 2011). Exposure of Brassica seedlings to 15 % PEG caused enhancements in APX activity (vs. control) (Alam et al. 2014a). Drought stress (−0.7 MPa) increased the APX activity by 21 % after 24 and 48 h of exposure in *Vigna radiata* (Nahar et al. 2015b). Drought-tolerant *Vigna unguiculata* cultivar exhibiting about 60 % higher APX activity (vs. and drought-sensitive) also showed a lower increase in the transcript levels of cytosolic and peroxisomal *APX* genes (D'Arcy-Lameta et al. 2006). However, chloroplastic *APX* genes expression was stimulated earlier in the tolerant cultivar when exposed to drought stress. It was argued that an enhanced drought tolerance can be associated with the capacity of APX (activity and genes) to efficiently detoxify ROS at their production sites. In a recent study, transgenic plants overexpressing *SbpAPX* gene showed enhanced salt and drought stress tolerance compared to wild-type plants (Singh et al. 2014b). Transgenic plants also reported to exhibit enhanced vegetative growth and germination rate both under normal and stressed conditions which indicate the role of APX both in conferring drought stress tolerance and growth enhancement as well. Overexpression of a *Populus* peroxisomal APX (PpAPX) gene in transgenic tobacco enhanced cellular protection against drought (Li et al. 2009). A correlation of the enhanced tolerance of *OsMT1a* overexpressing transgenic rice plants to drought stress with the increase in APX activity has also been reported (Yang et al. 2009).

The significance of APX has also been extensively reported in plants exposed to other major abiotic stresses including heat (Hasanuzzaman et al. 2012a, b), chilling (Sato et al. 2001), and flooding (Lin et al. 2007). In wheat seedlings, exposure to 24 and 48 h of heat treatment resulted in 59 and 44 % increase of APX activity (vs. control) (Hasanuzzaman et al. 2012a). Additionally, further enhancement (29 and 23 % higher) in APX activity was observed in seedlings supplemented with SNP, which resulted in the lower accumulation of H_2O_2 (Hasanuzzaman et al. 2012b). In contrast, in another instance, instead of APX, CAT was the main enzyme to detoxify H_2O_2 in heat-stressed *Brassica napus* (Hasanuzzaman et al. 2014c). The effect of prior HT exposure on the susceptibility of rice seedlings to chilling injury and the role therein of APX has also been studied (Sato et al. 2001). The authors found a higher level of APX activity in

heated seedlings that sustained after 7 days of chilling. It was argued that the heat shock-mediated APX gene expression provided the protection against chilling injury in rice seedlings. Tobacco plants overexpressing chloroplastic APX were reported to detoxify H_2O_2 and tolerate photooxidative damage during temperature stress (Sun et al. 2010). The authors noted a higher photochemical efficiency of photosystem II in transgenic plants (vs. wild-type plants) under cold and heat stresses. Involvement of cAPX gene expression in H_2O_2 detoxification and protection of egg plants were revealed under flooding stress (Lin et al. 2007).

Conclusions and future perspectives

Though varied ROS have been evidenced to be involved in plant signal transduction and thereby control the activation of plant stress defense (Neill et al. 2002; Corpas 2015; Del Río 2015), excess and/or non-metabolized ROS can bring severe consequences in plants (Gill and Tuteja 2010). Being major components of plant antioxidant defense system, both CAT and APX efficiently scavenge varied ROS including H_2O_2 and avert their impacts in plants. Through appraising recent literature, this paper presented an orchestrated view of the structure, occurrence, and significance of CAT and APX in plants; principles of current technologies used to assay CAT and APX in plants; and the modulation of CAT and APX in plants under major abiotic stresses. Much has been achieved in context with the roles of CAT and APX in isolated studies on stressed plants. However, molecular insights into points of interaction between CAT and APX and their potential synergistic role in the control and improvement of plant stress tolerance are yet to be enlightened. Reports are available on the recombinant expression of CAT in transgenic plants (Sørensen and Mortensen 2005; Switala and Loewen 2002; Engel et al. 2006) and on the expression of the soluble rice CAT B partially in *Escherichia coli* with coexpression of chaperone groEL-ES (Mondal et al. 2008a), but little report is available on the expression and purification of soluble bioactive rice plant CAT A from recombinant *Escherichia coli* (Ray et al. 2012). In particular, the literature is full on isolation, production, and purification of CATs with different techniques from various microbial sources (Sooch et al. 2014); improved, efficient, and reproducible techniques for the assays of CAT and also that of APX required exploration. If done, these information can have double benefits: in improving plant oxidative stress tolerance as well as their application in various analytical and diagnostic methods (as biosensors and biomarkers). Also, it would be very interesting to unveil potential outcome and underlying molecular genetic mechanisms of interaction of CAT and APX with varied ROS known to be involved in plant signal transduction under stress conditions.

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