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Review Article

Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection

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Abstract

When plants are exposed to stressful environmental conditions, the production of Reactive Oxygen Species (ROS) increases and can cause significant damage to the cells. Antioxidant defenses, which can detoxify ROS, are present in plants. A major hydrogen peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor. Different APX isoforms are present in distinct subcellular compartments, such as chloroplasts, mitochondria, peroxisome, and cytosol. The expression of *APX* genes is regulated in response to biotic and abiotic stresses as well as during plant development. The APX responses are directly involved in the protection of plant cells against adverse environmental conditions. Furthermore, mutant plants *APX* genes showed alterations in growth, physiology and antioxidant metabolism revealing those enzymes involvement in the normal plant development.

Keywords: ascorbate peroxidase, antioxidant system, reactive oxygen species, abiotic stress, mutant plants.

Plant Responses to Stresses

The exposure of plants to unfavorable environmental conditions increases the production of reactive oxygen species (ROS) such as, singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The ROS detoxification process in plants is essential for the protection of plant cells and their organelles against the toxic effect of these species (Apel and Hirt, 2004; Mittler, 2002). The differences in subcellular localization and biochemical properties of antioxidant enzymes and the distinct responses in gene expression, in addition to the presence of non-enzymatic mechanisms, result in a versatile and flexible antioxidant system able to control the optimum ROS levels (Vranova *et al.*, 2002). The ROS detoxification systems include enzymatic and non-enzymatic antioxidant components (Scandalios, 2005). Ascorbate (AsA) and glutathione (GSH), non-enzymatic antioxidants are crucial for plant defense against oxidative stress, playing a key role as antioxidant buffers (Foyer and Noctor, 2005; Mittler, 2002). Other non-enzymatic antioxidants involved include flavonoids, phenolic compounds, alkaloids, tocopherol and carotenoids (Gratão *et al.*, 2005).

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Enzymatic antioxidants comprise superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR). These enzymes are present in practically all subcellular compartments. Usually, an organelle has more than one enzyme able to scavenge a single ROS (Mittler, 2002; Mittler *et al.*, 2004; Scandalios, 2005). The main hydrogen peroxide-detoxification system in plant chloroplasts is the ascorbate-glutathione cycle, in which APX is a key enzyme (Asada, 1992). APX utilizes AsA as specific electron donor to reduce H_2O_2 to water. The importance of APX and ascorbate-glutathione cycle is not restricted to chloroplasts; it also plays a role in ROS scavenging in cytosol, mitochondria and peroxisomes (Asada, 1992, 1999; Mittler *et al.*, 2004; Noctor and Foyer, 1998; Shigeoka *et al.*, 2002). The ROS-scavenging enzymes in plants have been widely studied and the results have demonstrated that, in response to environmental stress, APX activity generally increases along with other enzymes activities, such as CAT, SOD, and GSH reductase (Shigeoka *et al.*, 2002). Over the past ten years substantial efforts have been made to understand plant antioxidant system mechanisms (Figure1). The in-

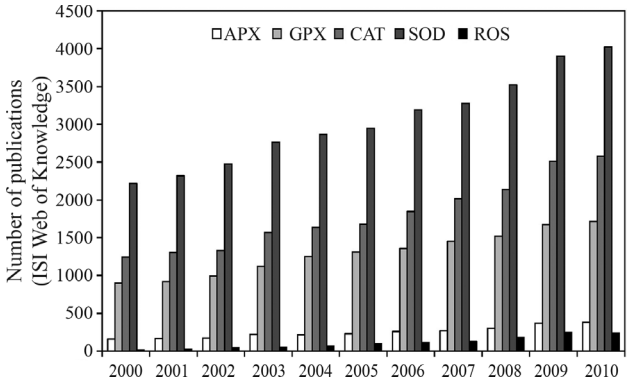

Figure 1 - Number of publication evolution addressing antioxidant enzymes in plants in the last ten years. CAT (catalase), SOD (Superoxide dismutase), GPX (Gluthatione peroxidase) and APX (Ascorbate peroxidase) enzymes, Reactive Oxygen Species (ROS).

creasing number of publications addressing CAT, SOD, GPX and APX enzymes in plants are examples of this tendency, especially APX, which had the number of articles doubled from 158 published in 2000 to 368 in 2010 (ISI Web of Knowledge database). Publications related more specifically to ROS in plants increased 18 times in the same period. The data presented in this study confirm those reported by Azevedo and Azevedo (2006) in which the number of publications addressing antioxidant mechanisms increased after 2000. This shows the relevance of studying these enzymes to further understand the biological processes dealing with oxidative stress responses in plants. The focus of this review is to discuss the main findings related to the APX enzyme at molecular and physiological levels, in different plant species. *APX* gene modulation in response to abiotic stress conditions, especially temperature, high light, drought, salinity and heavy metals will also be reviewed.

Ascorbate Peroxidase in Plants

Ascorbate peroxidase (APX) (EC 1.11.1.11) belongs to the class I heme-peroxidases that is found in higher plants, chlorophytes (Takeda *et al.*, 1998, 2000), red algae (Sano *et al.*, 2001), and members of the protist kingdom (Shigeoka *et al.*, 1980; Wilkinson *et al.*, 2002). APX and other peroxidase sequences from all kingdoms of life are stored in the database Peroxibase (Oliva *et al.*, 2009), which also provides a series of bioinformatics tools useful for analyzing the peroxidases stored sequences.

Genomic and cDNA *APX* sequences were obtained from a great variety of plant species, showing that APX are widely distributed in the vegetal kingdom. These enzymes are encoded by small gene families in these organisms (Passardi *et al.*, 2007). The different isoforms are classified according to their subcellular localization. Soluble isoforms are found in cytosol (cAPX), mitochondria (mitAPX) and chloroplast stroma (sAPX), while membrane-bound isoforms are found in microbody (including peroxisome and glyoxisome) (mAPX) and chloroplast thylakoids (tAPX). The presence of organelle-specific targeting peptides and transmembrane domains found in the N- and C- terminal protein regions determine the final subcellular localization of the isoenzyme (Shigeoka *et al.*, 2002; Teixeira *et al.*, 2004, 2006).

Plant chloroplastic APX (chlAPX) isoenzymes encoding genes are divided into two groups. The first group comprises single genes encoding two isoenzymes through a post-transcriptional alternative splicing regulation. This group includes genes from spinach (*S. oleracea*), tobacco (*N. tabacum*), pumpkin (*Cucurbita sp*) and ice plant (*M. crystallium*). In the second group, individual genes codify different isoenzymes which are individually regulated. This group includes genes from *Arabidopsis,* rice, and tomato. The mechanism of alternative splicing in chlAPX has been studied in spinach (Ishikawa and Shigeoka, 2008) and the results showed that alternative splicing is fundamental for controlling the expression of stromal (sAPX) and thylakoid (tAPX) isoenzymes. This regulation occurs in a tissue-dependent manner.

Ascorbate peroxidases have been partially characterized in some plant species. In spinach, the APX family is formed by genes encoding one cytosolic and two chloroplastic (sAPX and tAPX membrane) isoenzymes, one targeted to microbody membrane and an unknown putative cytosol-soluble isoenzyme (Ishikawa *et al.*, 1995, 1996, 1998). In cowpea, four cDNAs were isolated and characterized, corresponding to putative cytosolic, peroxissomal and chloroplastic (thylakoid and stromal) APX isoforms (D'Arcy-Lameta *et al.*, 2006). Six loci encoding *APX* were identified in *Eucalyptus grandis* and their subcellular localizations were indicated by prediction programs. Among the six isoforms, three were putatively identified as cytosolic, one as a putative peroxisomal protein and two predicted to be associated with chloroplasts (Teixeira *et al.*, 2005). In tomato, seven members were identified, three cytosolic, two peroxisomal, and two chloroplastic (Najami *et al.*, 2008). In the model plant *Arabidopsis thaliana*, the presence of nine *APX* genes was described, two choroplastic, one thylakoid-bound and one member whose product is targeted to both chloroplast stroma and mitochondria (Chew *et al.*, 2003); the intracellular localization of an additional member is yet unknown. In addition, three cytosolic and three microsomal proteins were also described (Mittler *et al.*, 2004; Narendra *et al.*, 2006; Panchuk *et al.*, 2002). In another important model plant, rice, the *APX* gene family comprises eight members, *viz.* two cytosolic, two peroxisomal, two chloroplastic (stromal and thylakoid-bound) and two mitochondrial ones (Teixeira *et al.*, 2004, 2006). Recently, a new protein has also been identified as functionally associated with APX in rice, the APX-R (Ascorbate peroxidase-related) (Lazzarotto *et al.*, 2011). Detailed analyses of evolution and structure of *APX-R* genes indi-

cate that these genes correspond to a new class of heme-peroxidases (Lazzarotto *et al.*, 2011).

APX isoenzymes are labile in the absence of AsA. Thus, high level of endogenous AsA is essential to effectively maintain the antioxidant system that protects plants from oxidative damage (Asada, 1992; Shigeoka *et al.*, 2002). Under special conditions in which the concentration of AsA is lower than 20 μM, the APX activity is quickly lost, this making the chlAPX the least stable isoform. Both cAPX and mAPX have half-inactivation times of around one hour or more, while that for mitAPX and chlAPX is less than 30 seconds (Chen and Asada, 1989; Miyake *et al.*, 1993; Ishikawa *et al.*, 1998; Yoshimura *et al.*, 1998; Leonardis *et al.*, 2000).

APX enzyme Responses Under Abiotic Stress

The expression of *APX* encoding genes is modulated by various environmental stimuli, such as drought and salt stress, high light, high and low temperatures, pathogen attacks, H_2O_2 and abscisic acid (Zhang *et al.*, 1997; Yoshimura *et al.*, 2000; Agrawal *et al.*, 2003; Fryer *et al.*, 2003; Menezes-Benavente *et al.*, 2004; Teixeira *et al.*, 2006; Rosa *et al.*, 2010; Bonifacio *et al.*, 2011). Furthermore, the transcriptional expression of *APX* genes is tissue and developmental stage dependent (Agrawal *et al.*, 2003; Teixeira *et al.*, 2006).

Salt stress

Plants are greatly affected by salinity, which causes alteration in nutrient uptake, accumulation of toxic ions, osmotic stress, and oxidative stress (Verslues *et al.*, 2006). Consequently, salinity results in molecular damage, growth arrest, and even cell death (Wang *et al.*, 2008). Salt stress induces the production of ROS, and the response of *APX* genes to this condition is tissue and developmental stage regulated. When the response of major antioxidant enzymes transcripts was analyzed for different developmental stages in salt stressed rice, *cAPX* was up-regulated in 11-day-old seedlings, while in 6-week-old plants salt had no significant effect on this gene (Menezes-Benavente *et al.*, 2004). In addition to *APX* expression alteration, discrimination on *CAT* transcript accumulation was also noticed in the basal region of rice leaves under salinity (Yamane *et al.*, 2010). Concerning the APX rice isoforms, induction was observed for the *OsAPX1*, *OsAPX4*, *OsAPX6* and *OsAPX7* genes, whereas cytosolic *OsAPX2* gene expression was not altered by salinity (Yamane *et al.*, 2010).

Teixeira *et al.* (2006) reported that three rice *APX* genes, *OsAPX2*, *OsAPX7*, and *OsAPX8*, showed altered transcript levels in response to NaCl treatment. The expression of *OsAPX2* and *OsAPX7* was increased, whereas *OsAPX8* transcript accumulation was strongly suppressed in plants undergoing salt stress (Teixeira *et al.*, 2006). The transcript level of *OsAPX8* was slightly decreased by salinity in the basal region of rice leaves (Yamane *et al*, 2010). On the other hand, *OsAPX8* expression in rice roots was enhanced by all NaCl concentrations tested (150, 200 and 300 mM), and *OsAPX7* expression was down-regulated by 300 mM NaCl. This discrepancy in regulation for the *OsAPX* genes might be due to differences in cultivars, organs, plant age and growth conditions (Hong *et al.*, 2007). An increase in rice cytosolic *APX2* gene transcript levels after treatment with salt has previously been shown by our group (Menezes-Benavente *et al.*, 2004; Teixeira *et al.*, 2006). In accordance, transgenic *Arabidopsis* plants over-expressing cytosolic *OsAPXb* (*OsAPX2*) showed higher tolerance to NaCl than those over-expressing cytosolic *OsAPXa* (*OsAPX1*) (Lu *et al.*, 2007). A similar increment in salt stress tolerance was also observed in transgenic tobacco over-expressing the *Arabidopsis cAPX* gene (Badawi *et al.*, 2004) and also in tobacco plants over-expressing a *Solanum lycopersicum* thylakoid-bound ascorbate peroxidase gene (*StAPX*) (Sun *et al.*, 2010b). Transgenic tobacco plants that simultaneously expressed CuZnSOD, APX, and DHAR in chloroplasts presented increased protection against salt induced injury (Lee *et al.*, 2007b). Transgenic tobacco BY-2 cells with 50 and 75% lower cAPX activity showed higher intracellular content of ROS. On the other hand, the tobacco cells showed a potential enhancement in tolerance to heat and salt stress, perhaps by induction of stress-related gene expression. However, no substantial differences were observed in the activity levels of the other antioxidant enzymes (Ishikawa *et al.*, 2005).

In barley, the transcript level of peroxisomal *APX* gene (*HvAPX1*) increased significantly under salt stress (Shi *et al.*, 2001). However, *Arabidopsis apx3* knockout mutants exposed to normal or stressful conditions did not present disturbed growth or development. In these plants, other antioxidant enzymes possibly compensate the lack of the peroxisomal isoform (Narendra *et al.*, 2006). In contrast, the overexpression of a *Populus* peroxisomal *APX* (*PpAPX*) gene in transgenic tobacco improved salt tolerance at the vegetative stage and plants were more resistant to oxidative damage induced by methyl viologen (MV) and, in addition, the plants had longer roots (Li *et al.*, 2009). Lin and Pu (2010) studied changes in enzymes involved in ROS scavenging in sweet potato plants tolerant and sensitive to salinity. After exposure to salinity (450 mM NaCl), APX activity increased in plants at 24 and 48 h, and this response was higher in a salt-stress tolerant genotype than in the salt sensitive ones. The expression of *cAPX*, *mAPX* and *chlAPX* in response to salinity was tissue specific and dependent on stress duration (Lin and Pu, 2010). Taken together, these studies put in evidence that salt stress causes disturbances in antioxidant gene expression by producing alterations in the transcriptional pattern in several plant species, and that the expression of distinct APX isoform may result in redox homeostasis regulation in each cellular compartment.

Temperature stress

Extreme temperatures affect the growth, yield and quality of plant production. ROS levels tend to increase if plants are exposed to stressful conditions such as low or high temperatures (Mittler *et al.*, 2004; Scandalios, 2005). In potato tubers, the transient accumulation of *cAPX* mRNA after storage at low-temperature was greater than after high-temperature storage, showing that *APX* expression was induced in response to low temperature (Kawakami *et al.*, 2002). Likewise, the two rice *cAPX* (*OsAPX1* and *OsAPX2*) genes were induced after rice plants were exposed to low temperatures. Furthermore, *OsAPX3*, *OsAPX4, OsAPX6* and *OsAPX7* were also significantly induced, while *OsAPX8* were repressed after 24 h under low temperature (unpublished data). The sweet potato *cAPX* gene was highly induced in leaves after exposure to high temperature (Park *et al.*, 2004). In cucumber plants submitted to heat treatment, the activities of cAPX, sAPX and mAPX increased after an initial slight decline during the course of the experiment. The expression of *sAPX* followed a similar pattern (Song *et al.*, 2005). In response to cold, the expression of a peroxisomal *APX* gene increased slightly in *Arabidopsis* (Zhang *et al.*, 1997).

These results were corroborated in *Arabidopsis* by overexpressing a putative peroxisomal membrane-bound *APX* from barley, resulting in an increased tolerance to higher temperature treatment (Shi *et al.*, 2001). Furthermore, the overexpression of chloroplastic *tAPX* in tobacco plants improved the tolerance to chilling stress combined with high light intensity (Yabuta *et al.*, 2002). On the other hand, *Arabidopsis* plants lacking *tAPX* had enhanced tolerance to heat stress (Miller *et al.*, 2007). Recently, Sato *et al.* (2011) showed that transgenic rice plants overexpressing a cytosolic *APX1* gene (*OsAPXa*) which exhibited higher APX activity in spikelets than in wild type (WT) plants, sustained higher levels of APX activity under cold stress, resulting in enhanced cold tolerance at the booting stage.

Plants with enhanced tolerance to multiple environmental stresses were obtained through induced expression of *CuZnSod* and *APX* genes. *Sod* and *APX* genes were expressed in chloroplasts of potato plants under the control of an oxidative stress inducible promoter - *SWPA2.* These plants showed enhanced tolerance to MV and when exposed to 42 ºC for 20 h, the photosynthetic activity of these transgenic plants decreased by only 6%, whereas in non-transformed (NT) plants it decreased by 29% (Tang *et al.*, 2006). Sweet potato plants expressing both *CuZnSod* and *APX* in chloroplasts through the inducible promoter also showed higher tolerance to MV-mediated oxidative stress and chilling stress (Lim *et al.*, 2007). The tolerance to high and low temperature stresses was studied in tobacco plants overexpressing a tomato *tAPX* gene. The overexpression of chloroplastic *APX* played a significant role in H_2O_2 detoxification and in minimizing photooxidative damage during temperature stress. The transgenic plants showed a higher photochemical efficiency of photosystem II when compared to WT plants under cold and heat stresses (Sun *et al.*, 2010a). These results put in evidence that the manipulation of the antioxidative mechanism in chloroplasts may be applied in the development of plants with increased tolerance to multiple environmental stresses.

High light stress

Plants exposed to excessive light can suffer photoinhibition, serious damage to the photosynthetic apparatus, and degradation of photosynthetic proteins (Demmig-Adams and Adams III, 1992). Light stress can also lead to ROS accumulation and antioxidant enzymes activation (Mittler, 2002). The responses of APX isoenzymes to photooxidative stress were studied in spinach leaves during high light stress. cAPX activity and transcripts increased during high light stress, however protein levels were not altered. The activities of chlAPX isoforms showed a gradual decrease, while the other isoenzymes showed no significant variation in transcript and protein levels, as well as activities (Yoshimura *et al.*, 2000). In wheat, a mutant line showing decreased tAPX activity presented reduced photosynthetic activity and biomass accumulation when growing under high-light intensity, suggesting that tAPX is essential for photosynthesis (Danna *et al.*, 2003). Single mutants of *Arabidopsis* lacking tAPX or sAPX presented higher levels of H_2O_2 and oxidized proteins than WT plants when exposed to high light and MV stresses. The strongest effect of photooxidative stress was observed in plants lacking tAPX, these showing increased H_2O_2 accumulation and oxidized proteins (Maruta *et al.*, 2010).

Double mutants deficient in two *APX* genes, thylakoid-bound and a cytosolic one (*tylapx/apx1*), resulted in different signals in *Arabidopsis* plants, such as late flowering, low protein oxidation during light stress and enhanced accumulation of anthocyanins (Miller *et al.*, 2007). Mutants lacking a functional copy of tAPX, sAPX or both, were characterized in *Arabidopsis* under photooxidative stress during germination. The stress led to chloroplast bleaching in *sapx* single-mutant and *tapx/sapx* double-mutant plants, while the greening process of WT and *tapx* plants was partially impaired (Kangasjarvi *et al.*, 2008). When mature leaves of *tapx/sapx* double mutants were submitted to short-term photooxidative stress induced by high light or MV treatment, the plants showed susceptibility (Kangasjarvi *et al.*, 2008). These results indicate that the APXs isoenzymes are indispensable under environmental stresses in different species, especially under light stress conditions.

In *Arabidopsis* leaves, high light treatment induced the expression of cytosolic *APX2*, which has its expression restricted to bundle sheath cells of the vascular tissue (Fryer *et al.*, 2003). In *Arabidopsis,* APX1 knockout plants showed suppressed growth and development, altered stomatal responses and induction of heat shock proteins

during light stress. The inactivation of cytosolic APX resulted in the alteration of several transcripts involved in different functions. In transgenic APX1 plants kept under optimal conditions, the transcripts encoding APX enzymes were not elevated. However, during light stress, certain enzymes were induced in knockout-APX1 plants (Pnueli *et al.*, 2003). In another study (Davletova *et al.*, 2005) with APX1-deficient *Arabidopsis* plants the observation was that the entire chloroplastic H_2O_2-scavenging system collapsed, H_2O_2 levels increased and protein oxidation occurred in leaves subjected to a moderate light stress, suggesting that the absence of cytosolic APX1 resulted not only in the accumulation of H_2O_2 but also in damage to specific proteins in leaf cells. On the other hand, rice plants double silenced for cytosolic APXs up-regulated other peroxidases, making these transgenic plants able to survive under stress, such as salt, heat, high light and MV, similar to NT plants. The antioxidative compensatory mechanism exhibited by the silenced plants was associated with increased expression of *Gpx* genes. The transcript levels of *OsCatA* and *OsCatB* and the activities of CAT and guaiacol peroxidase (GPOD; type III peroxidases) were also up-regulated. In contrast, none of the other isoforms of *OsAPX* were up-regulated under normal growth conditions. These results suggested that signaling mechanisms triggered in rice could be distinct from those proposed for *Arabidopsis* (Bonifacio *et al.*, 2011).

Drought stress

Drought stress in plants leads to severe effects such as reduction in vegetative growth and cell division. As a consequence of drought stress several changes occur inside the cell, including changes in gene expression levels, synthesis of molecular chaperones, and activation of enzymes involved in the production and removal of ROS (Mahajan and Tuteja, 2005). In two cowpea (*Vigna unguiculata*) cultivars, one drought-tolerant and the other drought-sensitive, APX activity was 60% higher in tolerant plants cultivated under control conditions. In response to drought stress, a higher increase in transcript levels of cytosolic and peroxisomal *APX* genes was observed in the sensitive cultivar (D'Arcy-Lameta *et al.*, 2006). Chloroplastic *APX* genes expression was stimulated earlier in the tolerant cultivar when submitted to drought stress. These data suggest the capacity of these enzymes to efficiently detoxify ROS at their production site (D'Arcy-Lameta *et al.*, 2006). Relative *APX* transcript levels showed distinct changes in two genotypes of wheat exposed to mild water deficit. Cytosolic *APX1* expression levels increased in both genotypes, while cytosolic *APX2* was up-regulated only in the drought-tolerant genotype. The transcript level of thylakoid *APX* increased in the drought-tolerant genotype, while stromal *APX2* showed higher expression levels in the drought-sensitive cultivar (Secenji *et al.*, 2010).

APX gene expression patterns in rice were studied after 15 days of drought stress. In marked contrast with the experiments with wheat, thylakoid *APX* (*OsAPX8*) expression was down-regulated in this condition, while the *OsAPX1*, *OsAPX2*, *OsAPX5*, *OsAPX6* and *OsAPX7* genes were up-regulated. The peroxisomal *OsAPX3* gene was not affected, while *OsAPX4* was slightly but significantly down-regulated by this treatment (Rosa *et al.*, 2010). This discrepancy could be due to distinct responses of *APX* genes in different species and to different magnitudes of stress. In *Arabidopsis*, APX1 protein and mRNA accumulated during combination of heat and drought stress. A cytosolic *APX1*-deficient mutant accumulated more H_2O_2 and was more sensitive to stress combination than WT plants when exposed to heat and drought stress combined. In contrast, plants deficient in thylakoid APX were not more sensitive to this stress combination than *APX1*-deficient mutant or WT plants. The cytosolic *APX1* gene may thus play a key role in the acclimation of plants to combined stress such drought and heat (Koussevitzky *et al.*, 2008). Indeed, when the overexpression of cytosolic *APX* was studied in tobacco chloroplasts, its overexpression protected the plant from several oxidative stresses, including drought and polyethylene glycol-induced stress (Badawi *et al.*, 2004). Plants overexpressing other antioxidant enzymes in different species showed increased tolerance to various stresses, including drought resistance. The overexpression of a *Populus* peroxisomal ascorbate peroxidase (*PpAPX*) gene in transgenic tobacco improved drought resistance in these plants (Li *et al.*, 2009). The overexpression of tomato (*Solanum lycopersicum*) thylakoid-bound *APX* (*StAPX*) gene in tobacco plants enhanced the tolerance of these plants to salt and osmotic stress (Sun *et al.*, 2010b).

Heavy metals

The contamination of soils with heavy metals is a serious environmental problem that limits crop production. Exposure at higher concentrations of heavy metals can increase the production of ROS and change antioxidant response (Gratão *et al.*, 2005). Exposure of pea (*Pisum sativum* L.) plants to cadmium changed enzymatic and non-enzymatic antioxidant defenses, however, APX activity or accumulation of its transcripts were not significantly different (Romero-Puertas *et al.*, 2007). However, it was observed that in coffee cells, the activity of APX was increased at the lower cadmium concentration. On the other hand, APX activity was not detectable in cells submitted to the higher cadmium concentration after 24 h of treatment (Gomes-Junior *et al.*, 2006b).

An increase in APX activity was also observed in response to other heavy metals such as aluminum (Sharma and Dubey, 2007). In rice, the transcript levels of all *OsAPX* genes, except *OsAPX6*, were significantly increased after eight hours of 20 ppm aluminum exposure (Rosa *et al.*, 2010). In pea plants, *cAPX* expression increased in the

shoots under aluminum treatment, but APX activity presented a significant decline at 10 μM aluminum in roots and shoots after 24 and 48 h of stress; at 50 μM aluminum treatment, however, APX activity did not show any significant changes (Panda and Matsumoto, 2010). Transgenic rice plants double silenced for *APX1* and *APX2* (APX1/2s plants) exhibited normal development and enhanced tolerance to a toxic concentration of aluminum (Rosa *et al.*, 2010). In bean plants, the expression of cytosolic APX was induced both at mRNA and protein levels in leaves of de-rooted plants in response to iron overload. Likewise, transgenic tobacco plants with suppressed cytosolic APX levels were more sensitive to iron application than WT plants (Pekker *et al.*, 2002). In coffee cells treated with nickel showed a rapid increase in APX activity, although the activity trends were slightly different between the two nickel concentrations tested (0.05 mM and 0.5 mM) (Gomes-Junior *et al.*, 2006a). Transgenic tall fescue plants expressing the CuZnSOD and APX genes in chloroplasts were submitted to copper, cadmium or arsenic treatment. Of the metals tested, copper and cadmium increased SOD and APX activities in control and transgenic plants, with a higher increase observed in transgenic plants. In contrast, in leaves exposed to arsenic, both enzymes exhibited less activity when compared to other treatments and no significant differences were observed between control and transgenic plants (Lee *et al.*, 2007a). These results emphasize the important role of APX and other antioxidant enzymes in H_2O_2 scavenging under toxic metals levels in the soil.

Conclusions

Ascorbate peroxidase is a key enzyme regulating ROS levels acting in different subcellular compartments (Figure 2). The expression of *APX* encoding genes is differentially modulated by several abiotic stresses in different plant species. All the data collected so far firmly indicate that APX isoforms play important and direct roles as protective elements against adverse environmental conditions. The diverse effects of knockdown or knockout of different *APX* genes on the plant growth, physiology and antioxidant metabolism indicate that APX may also regulate redox signaling pathways involved in plant development. These re-

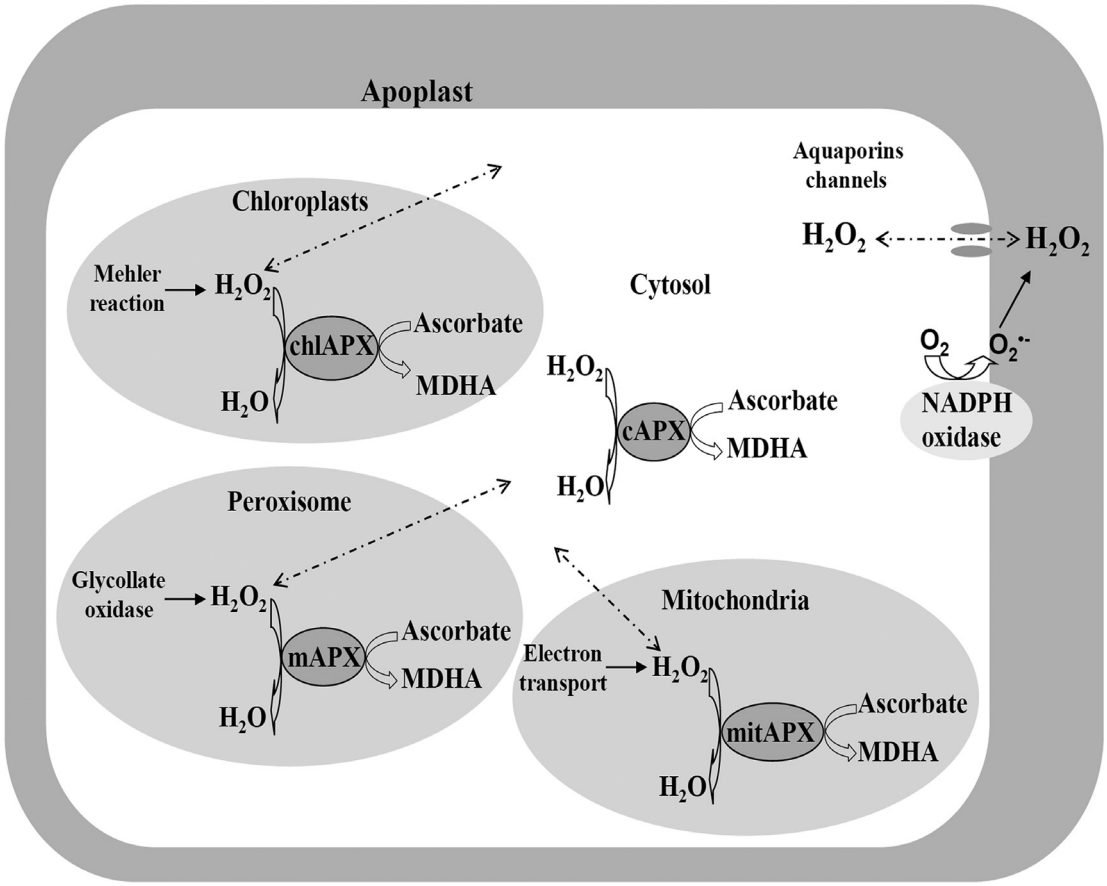

Figure 2 - APX enzymes and the elimination of ROS excess in different subcellular compartments. H_2O_2 is generated in normal metabolism via the Mehler reaction in chloroplasts, electron transport in mitochondria and photorespiration in peroxisomes. Abiotic and biotic stresses enhance H_2O_2 and chlAPX, mAPX, cAPX and mitAPX enzymes which can eliminate ROS excess in different subcellular compartments. The plasma membrane-NADPH oxidases also generate H_2O_2, which can cross membranes through aquaporin channels. Superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), monodehydroascorbate (MDHA).

sults emphasize the importance and complexity of the interactions of APX with other antioxidants in fine tuning the vegetal antioxidant metabolism.

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Internet Resources

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