Chapter 5
Plant Glutathione Peroxidases: Antioxidant Enzymes in Plant Stress Responses and Tolerance

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Abstract In contrast to other eukaryotic organisms, plants are unable to run away from unfavourable conditions; they must cope with different abiotic and biotic stress factors. Under abiotic and biotic stresses, the production of reactive oxygen and reactive nitrogen species (ROS and RNS) can damage the biological membranes, proteins and nucleic acids. However, plants have developed complex defence systems including different non-enzymatic and enzymatic antioxidants as shields to prevent the toxic effects of an increased amount of ROS and RNS. Glutathione peroxidases (GPXs) are important antioxidant enzymes in animals, but plants contain GPX-like (GPXLs) enzymes. In contrast to animal GPXs, plant GPXLs contain cysteine in their active site instead of selenocysteine, and most of them prefer thioredoxin as the electron donor rather than glutathione. In the last 25 years, many researches proved that plant GPXLs also are essential elements of plant stress responses and are important ROS scavengers. Overexpression of GPXLs in different plant species led to increased tolerance against drought, salt, osmotic, heavy metal and particularly oxidative stresses; however, in some cases, it caused decreased tolerance against biotic stresses. In this chapter, we focus on the importance of plant GPXLs in stress responses, highlighting the significance of distinct genes as possible candidates for genetic engineering to improve the yield of agricultural plants under unfavourable environment.

Keywords Abiotic stress • Antioxidant enzymes • Biotic stress • Glutathione peroxidases

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Contents

1 Introduction

Eukaryotic organisms live under constantly changing environmental conditions that could negatively affect their development and reproduction. In contrast to other eukaryotes, plants are sessile organisms, unable to run away from unfavourable conditions; thus they must cope with different abiotic and biotic stress factors. Environmental stresses represent the most limiting factors to agricultural productivity worldwide. Their influence is not only restricted on currently cultivated fields, but they also hamper the introduction of crop plants in non-cultivated areas. A global problem in the improvement of crop productivity is the large variation of annual crop yields due to unpredictable environmental stresses.

2 Environmental Stresses Impair the Development and Yield of Plants

There are two main categories of environmental factors: abiotic and biotic. Biotic stress occurs as a result of damage by other living organisms, for example, by bacteria, viruses, fungi, parasites, insects or weeds. These factors destroy more than 40% of all potential food production each year, despite the huge amount of pesticide or other non-chemical controls used (Pimentel and Greiner 1997).

On the other hand, abiotic stress occurs as the negative impacts of non-living factors on the organisms, for example, water deficiency or flooding, extreme temperature, salinity, insufficient nutrition, radiation and light intensity, mechanical effects, metals, chemicals and pollutants. Among the abiotic stress factors, one of the most limiting for crop production is water. The two ends of water supply are too much (flooding) or too little water (drought). The Food and Agriculture Organization (FAO) analysis in 2015 revealed that 37% of the damage and loss to crops and livestock is because of flood. Flood causes on plants the death of leaves, wilting or epinasty and finally the loss of production. From another side, according to the analysis of the World Resources Institute in 2013, 28% of the cultivated areas are exposed to high or extremely high drought stress, but in the case of some cultivated plants, this number is more extreme: 35% of maize fields, 43% of wheat fields and

57% of cotton fields suffer from high water deficit (Reig et al. 2013). The effects of drought stress on plants lead to reduced germination, development, photosynthesis and production. For example, in the case of sunflower in 2015, the production fell down by 5.5% compared with the 5-year average, mainly driven by strong drought-related yield decreases in the main producing European Member States – Bulgaria, Romania, France and Spain (FAO 2016). In order to maintain the agricultural production in these areas, farmers decide on irrigation. However, the extreme groundwater extraction is leading to the salinization of soils. Most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. About 33% of the cultivated areas are affected by high salt stress, and this number is increasing by 10% of the rate annually (Jamil et al. 2011). Salt as an osmotic stress causes the same symptoms like drought stress, hence reducing the growth and development; moreover, salt imposes ion toxicity, too (Ashraf and Harris 2004). And besides water and salt stresses, 23% of the overall production losses are caused by extreme temperature, radiation, pollution and other factors (FAO 2017).

Every environmental stress may end up in oxidative stress, at least to some degree. The elevated level of reactive oxygen and nitrogen species (ROS and RNS), such as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$), nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$), can cause damage to lipids, proteins and DNA (Mittler 2002; Luis et al. 2006).

3 Glutathione Peroxidases Are Versatile ROS Scavengers

Plants have developed complex antioxidant defence systems to counteract the deleterious effect of increased amount of ROS and RNS. This defence system comprises non-enzymatic and enzymatic components in different cellular compartments. Non-enzymatic components include the major redox buffers glutathione and ascorbate, as well as carotenoids, tocopherols and phenolic compounds. They are important cofactors of the enzymatic antioxidants and elements of redox homeostasis (Sharma et al. 2012). The enzymatic components of the defence system include several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione transferase (GST) and glutathione peroxidase-like enzyme (GPXL). These enzymes and their isoenzymes are located in different subcellular compartments (Noctor and Foyer 1998). The activities of these enzymes are generally increased under stress conditions, and in several cases, their activities correlate well with enhanced tolerance (Foyer et al. 1997). Their role and mechanism in stress responses have been investigated intensively for several decades; however, relatively little is known about plant GPXLs.

The glutathione peroxidase (GPX) enzymes are non-heme thiol peroxidases that catalyse the reduction of H_2O_2 or organic hydroperoxides to water or the corresponding alcohols using reduced glutathione. Numerous GPXs characterized from

various organisms revealed their role in ROS scavenging (Noctor et al. 2012; Yang et al. 2015). The mammalian GPXs are central components of the antioxidant defence system and contribute in the repair of biomembranes (Imai and Nakagawa 2003; Margis et al. 2008; Brigelius-Flohe and Maiorino 2013). The plant GPXLs are closely related to animal phospholipid hydroperoxide glutathione peroxidases; however, they contain cysteine instead of selenocysteine in their active site and prefer the thioredoxin (TRX) regenerating system rather than the glutathione system (Iqbal et al. 2006; Navrot et al. 2006; Herbette et al. 2007; Margis et al. 2008). Nevertheless, GPXLs have also an important role in the elimination of organic hydroperoxides and lipid peroxides (Milla et al. 2003; Bela et al. 2015). For example, *Arabidopsis* plants lacking GPXL8 contain an elevated level of the lipid peroxidation marker malondialdehyde (MDA) compared to wild-type plants after exposure to salt or osmotic stresses (Gaber 2011). They also participate in the H_2O_2 homeostasis. As another example, *Atgpx1* mutant *Arabidopsis* plants had higher basal foliar H_2O_2 levels than the wild-type plants under low-light condition, which were elevated even further under high-light stress (Chang et al. 2009).

4 Role of Plant Glutathione Peroxidases in Plant Stress Tolerance

Several reports provided direct or indirect evidence for the importance of GPXLs in different stress responses. Their very important feature is that they may protect proteins and DNA against oxidative stress. Gaber et al. (2012) proved that *Arabidopsis* plants overexpressing *AtGPXL8* accumulated less oxidized proteins and 8-oxo-2′-deoxyguanosine under oxidative stress. GPXLs also help to protect biological membranes by the reduction of lipid peroxides (Herbette et al. 2002; Jung et al. 2002; Chen et al. 2004; Iqbal et al. 2006; Navrot et al. 2006); thus, the plant GPXLs are thought to be part of the enzymatic antioxidant systems. Although GPXLs mainly take part in the elimination of organic hydroperoxides, in some cases, they also react with H_2O_2.

4.1 GPXLs in Oxidative Stress Responses

H_2O_2 is an important compound of the oxidative stress and is a component of signalling processes. About the role of GPXLs in signalling, see more details in Chap. 4 in this book ("Plant Glutathione Peroxidases: Structural and Functional Characterization and Their Roles in Plant Development").

It was reported that external H_2O_2 treatment resulted in elevated transcript levels of many *GPXL* genes in *Panax ginseng* (Kim et al. 2014) and in *Oryza sativa* (Li et al. 2000; Passaia et al. 2013). The dramatic increase of *OsGPXL* mRNA levels

after the H_2O_2 treatment was reported originally by Li et al. (2000). Passaia et al. (2013) showed that all the five *OsGPXLs* were induced 2–8 h after the 10 μM H_2O_2 treatment. Islam et al. (2015) analysed separately the shoot and the root of rice during oxidative stress, and their results also proved the induction of *GPXL* genes by H_2O_2 treatment; however, *OsGPXL5* was induced only in root tissues. *Osgpxl3* knockdown mutant plants displayed short root and shoot phenotypes and increased H_2O_2 production in root tissues compared to wild-type plants (Passaia et al. 2013). These results indicate that in rice the OsGPXLs are important in H_2O_2 elimination. *AtGPXL3* was also induced by H_2O_2; moreover, *Atgpxl3* knockout mutants are more sensitive to H_2O_2 treatment, and the H_2O_2 level was elevated in these plants compared to wild type. These mutants showed delayed leaf development compared to wild-type plants on H_2O_2-containing media (Miao et al. 2006). However, *Panax ginseng PgGPXL1* expression increased only in the first 24 h of H_2O_2 exposure, and the *PgGPXL2* was parallely downregulated (Kim et al. 2014). In this sense, other authors have suggested that GPXLs may have a role not only in elimination but also in H_2O_2 perception and signalling (see more details in Chap. 4).

One important environmental factor that generates oxidative stress is the ground-level ozone, which enters the leaves through stomata during normal gas exchange. It is a strong oxidant which may cause several types of symptoms including chlorosis and necrosis. Ozone has significant effect on crop yield, and dicot species, like soybean, cotton and peanut, are more sensitive to yield loss caused by ozone than monocot species, such as sorghum, corn and wheat (Heagle 1989). Furthermore, *Nicotiana plumbaginifolia NpGPXL*, together with *CAT2* and *CAT3* genes, showed induction to ozone treatment, indicating the significance of GPXLs also in ozone stress (Willekens et al. 1994).

A series of experiments using paraquat also connect the GPXLs to oxidative stress responses, because it is one of the most widely used quick-acting and non-selective herbicides which generate ROS due to interaction with the free electrons originated from chloroplast photosystem I (PSI) (Upham and Hatzios 1987) or mitochondrial NADH:ubiquinone oxidoreductase (Complex I) (Tawara et al. 1996). The evolved superoxide then may attack biological membranes. Paraquat treatment also caused early transcriptional activation of *GPXLs* in different plants. Treatment of barley leaves dramatically increased the transcript level for cytosolic *HvGPXL1* and chloroplastidic *HvGPXL2* (Churin et al. 1999). Similar induction of *GPXL* was observed due to paraquat in *Raphanus sativus* in the light; however, the gene was downregulated in the dark (Yang et al. 2005). In a *Conyza bonariensis* paraquat-resistant biotype, an elevated transcript level of genes coding antioxidant enzymes, as well as the increase of SOD, APX, DHAR, MDAR, GR and GPXL enzyme activities, was observed after paraquat treatment (Ye and Gressel 2000). Expression of *Citrus sinensis GPXL* gene in *Escherichia coli* enhanced the tolerance against paraquat, but this tolerance depended on the growth stage (Holland et al. 1994). GPXL8 knockout and overexpressing *Arabidopsis* mutants showed decreased and increased tolerance against paraquat, respectively, that was correlated with enlarged and reduced root growth inhibition.

4.2 Involvement of GPXLs in Biotic Stress Responses

Plant diseases cause major economic losses for farmers worldwide, even though the plants have a complex defence system with constitutive and inducible components against pathogen attacks. The endogenous H_2O_2 accumulation is an important feature of the incompatible plant–pathogen interaction. The oxidative burst is a rapid production of large amount of ROS in response to external stimuli that overwhelms the cellular antioxidative defences (Wojtaszek 1997). However, a reasonable regulation of antioxidant systems is part of the signalling pathways, activating defence responses during pathogen attack (De Gara et al. 2003). Inoculation of *Nicotiana sylvestris* with GTAMV (green tomato atypical mosaic virus) resulted in induction of *GPXL* (Criqui et al. 1992), and similar induction was detectable in *Helianthus annuus* during *Plasmopara halstedii* infection (Roeckel-Drevet et al. 1998). Plant glutathione peroxidases are important even in response to insect attack. Colonization of *Zea mays* seedlings by aphids *Sitobion avenae* and *Rhopalosiphum padi* upregulated *ZmGPXL1* and *ZmGPXL3* genes and increased the GPXL enzyme activity (Sytykiewicz 2016). In *Panax ginseng*, *GPXLs* respond to biotic stress differently: *PgGPXL1* expression increased compared to control; conversely, *PgGPXL2* expression gradually decreased during *Colletotrichum gloeosporioides* pathogen attack (Kim et al. 2014). On the other hand, GPXLs do not always have a supportive role in biotic stress responses. Depletion of AtGPXL1 resulted in expanded lesions by *Pseudomonas syringae* on *Arabidopsis* leaves, and bacterial titres were 10 times lower compared to the wild type, where hypersensitive cell death was restricted to the area around the infection. These results showed that the depletion of AtGPXL1 activity improves resistance against virulent bacteria (Chang et al. 2009). *GPXL5* overexpression in tomato plants increased the size of necrotic areas during *Botrytis cinerea* infection. Thus, *GPXL* overexpression counteracted the plant defence response (Herbette et al. 2011).

4.3 GPXLs in Salt Stress Responses

Salinity is one of the most serious factors limiting the yield of agricultural crops. Salt stress causes water deficiency, ion toxicity, nutritional disorders, metabolism alterations, membrane damage and oxidative stress. Salt stress responses of plants include production of different osmolytes and chaperones, ion channel activation and induction of the antioxidant defence system (Carillo et al. 2011). The first salt stress associated *GPXL* was isolated from *Citrus sinensis*: a fast induction of *CsGPXL* was detectable after salt treatment (Avsian-Kretchmer et al. 1999), followed by an increase in the level of CsGPXL protein in cultured cells originating from different organs (Ben-Hayyim et al. 1993; Holland et al. 1993; Beeor-Tzahar et al. 1995). In *A. thaliana*, salt stress increased the transcript level of *AtGPXL1*, *AtGPXL2*, *AtGPXL4*, *AtGPXL5*, *AtGPXL6*, *AtGPXL7* and *AtGPXL8*, but *AtGPXL3* was not affected (Sugimoto and Sakamoto 1997; Milla et al. 2003; Gao et al. 2014).

Atgpxl8 knockout mutants were more sensitive to the salt treatment (Gaber 2011). In *Thellungiella salsuginea*, exposure to salt stress induced or repressed the *TsGPXLs* in organ-specific and tissue-specific manner (Gao et al. 2014). For example, *TsGPXL5*, *TsGPXL7* and *TsGPXL8* were induced in shoots, whereas in roots almost all glutathione peroxidase genes (*TsGPXL1*, *TsGPXL2*, *TsGPXL3*, *TsGPXL5*, *TsGPXL7* and *TsGPXL8*) showed induction by 300 mM NaCl (Gao et al. 2014). *T. salsuginea* is a close relative of *Arabidopsis* which represents a halophytic model for salt stress tolerance studies, but the role of GPXLs in salt stress tolerance has been also reported in crop plants.

Among three barley *GPXLs*, two were activated after salt treatment, the expression of *HvGPXL1* being much higher than *HvGPXL2* (Churin et al. 1999). Similarly, *OsGPXL1* transcript level increased rapidly (Kang et al. 2004), followed by elevated GPXL enzyme activity due to high salinity (Lima-Melo et al. 2016). Similar induction was observed in the case of *OsGPXL3*, but *OsGPXL2* and *OsGPXL4* were activated only in the roots (Islam et al. 2015). *OsGPXL5* was induced in root tissues, but its expression was reduced in shoot tissues; however, *Osgpx5* knockout lines showed increased sensitivity towards high concentration of salt (Wang et al. 2017).

In *Arabidopsis*, the expression of two different *Triticum aestivum TaGPXL* genes led to increased tolerance against salt stress (Zhai et al. 2013). Transgenic plants remained green, and the root inhibition by salinity was reduced; furthermore, germination rate also increased on salt-containing media compared to the wild-type plants. Evaluation of the background of this process revealed that *TaGPXL* overexpression caused an elevated transcript level of *SOS1* (Na^+–H^+ antiporter) and *RbohD* (NADPH oxidase) genes, but downregulated the *ABI1* and *ABI2* (2C protein phosphatases), suggesting a role for TaGPXLs in salt stress signalling (Zhai et al. 2013). Li et al. (2013) also proved the importance of TaGPXLs during high-salinity treatment, because *TaGPXL* transcript levels greatly increased after treatment, together with *MDHAR*, *DHAR* and *glutathione synthetase 3 (GS3)*, and parallely salt stress markedly raised the contents of both glutathione and ascorbate in the leaves of wheat seedlings. Also in *Panax ginseng*, in *Nelumbo nucifera* and in tea plants, *PgGPXL1*, *PgGPXL2*, *NnGPXL* and *CsGPXL2*, respectively, were upregulated by salt treatment (Diao et al. 2014; Fu 2014; Kim et al. 2014). However, in other plants, a role for GPXLs in salt stress response has not been reported; for example, none of the six *Lotus japonicus GPXL* genes were affected by short-term salt treatment, but only after 7 days (Ramos et al. 2009).

4.4 Involvement of GPXLs in Osmotic and Drought Stress Tolerance

One of the components of salt stress is osmotic stress, caused by the change in solute concentrations. So, it is not a surprise that most of the genes which were induced by salt stress are induced also by osmotic stress. For example, the *CsGPXL* in citrus (Ben-Hayyim et al. 1993), *HvGPXL1* and *HvGPXL2* in barley (Churin et al. 1999),

PgGPXL1 in ginseng (Kim et al. 2014), *CsGPXL2* in tea plants (Fu 2014) and *Arabidopsis GPXLs* have been described as osmotic stress-inducible (Milla et al. 2003; Gaber 2011). However, *AtGPXL2* transcript level interestingly decreased under osmotic stress caused by mannitol (Milla et al. 2003), and in contrast with the results during salt stress, *AtGPXL3* was activated (Miao et al. 2007). In *Thellungiella salsuginea*, *TsGPXL1*, *TsGPXL3*, *TsGPXL4* and *TsGPXL7* were significantly upregulated in shoots due to osmotic stress and in roots almost all *TsGPXL* genes, except for *TsGPXL1* (Gao et al. 2014).

The other major limiting factor in crop productivity is the drought. The physiological responses of plants to drought stress generally included the production of antioxidants, osmotic protective compounds and growth regulators (Farooq et al. 2009). In *Euphorbia esula*, among other antioxidant enzymes, GPXLs, GSTs and GR play important roles in plant defence mechanisms against drought (Anderson and Davis 2004). In rice, drought stress induced all of *OsGPXLs* to some degree. After 12 h from drying the seedlings on Whatman sheet, mRNA level is increased for *OsGPXL1*, *OsGPXL2*, *OsGPXL3* and *OsGPXL4* in shoots; however, for *OsGPXL5*, it was reduced. In the roots of rice seedling all the five *OsGPXLs* were activated after 12 h (Islam et al. 2015). Similarly, *OsGPXL1* was activated after removing the source of water from seedlings for 2 days (Kang et al. 2004). In contrast, expression of *OsGPXL4* and *OsGPXL5* was reduced when rice plants were grown without water for 15 days (Passaia et al. 2013). The role of poplar PtGPXLs during water deficit has been also described as not uniform. After 6 days of water withdrawal, the protein level of some PtGPXLs increased, whereas some decreased (Navrot et al. 2006). In case of *Arabidopsis* plants, the important role of the GPXL3 in drought stress responses was reported, because defects of *AtGPX3* reduced drought stress tolerance, whereas *AtGPX3* overexpression in transgenic plants enhanced drought stress resistance (Miao et al. 2006).

4.5 Role of GPXLs Under Low and High Temperatures

Extreme temperatures also cause serious damages in agricultural production. Temperature stresses in plants are classified into three types depending on the stressor, which may be high, chilling or freezing temperature. The three types induce different stress responses, the activation of antioxidant enzymes being part of all of them (Wang et al. 2017). The involvement of GPXLs in chilling stress (4 –10 °C) response of rice is controversial. After 16 h on 4 °C, the mRNA levels of *OsGPXL1* and *OsGPXL3* increased both in shoots and roots; however, *OsGPXL2*, *OsGPXL4* and *OsGPXL5* were downregulated (Islam et al. 2015). When plants were exposed to 10 °C for 24 h, the transcription of either the *OsGPXL1*, *OsGPXL3* or *OsGPXL5* was induced (Passaia et al. 2013), whereas, in other experimental systems, when plants were subjected to 4 °C for 3 days, *OsGPXL1* was not induced by 24 h, but only after 48 h, and the activation disappeared by 72 h (Kang et al. 2004). A similar response was observed in ginseng plants: after 8 h of chilling stress, both *PgGPXL1* and

PgGPXL2 were induced, but later the expression gradually fell down (Kim et al. 2014). Diao et al. (2014) investigated *Nelumbo nucifera GPXL* expression only in short-term chilling, and the expression of this gene increased within an hour, and this activation was maintained until 6 h. In *Arabidopsis* plants, only *AtGPXL6* was activated among the eight genes on 4 °C (Milla et al. 2003). In eggplant, this treatment activated *SmGPXL1* and *SmGPXL2* together with the expression of other genes coding antioxidant enzymes (*GSTs*, *GR*, *MDAR* and *DHAR*) (Chen et al. 2011). On the other hand, overexpression of tomato *SlGPXL5* protected the photosynthetic machinery from chilling treatment under moderate light (Herbette et al. 2005).

Heat stress caused a somewhat different response compared to chilling. For example, in rice plants, all *OsGPXLs* were activated in the shoots and/or roots by heat treatment, contrary to the downregulation observed by chilling (Islam et al. 2015; Wang et al. 2017), and in *Arabidopsis*, heat stress upregulated *AtGPXL1*, instead of *AtGPXL6*, which was induced by chilling stress (Milla et al. 2003). However, a similar *GPXL* gene expression pattern was found after short-term heat stress and chilling in *Nelumbo nucifera* (Diao et al. 2014) and in tea plants (Fu 2014).

4.6 Other Stresses

Mechanical stimulation can also happen during pathogen attack; however, it is considered to be an abiotic stress factor. *NsGPXL* in *Nicotiana sylvestris* and *HaGPXL* in *Helianthus annuus* were induced after wounding in the same way as in biotic stress (Criqui et al. 1992; Roeckel-Drevet et al. 1998). The mRNAs of tomato *GPXLs* were also accumulated after mechanical stimulation; however, the dynamics of the transcription of the two investigated genes were different: *SlGPXL1* was induced within an hour, whereas *SlGPXL2* activation was a bit slower, about 6 h after the treatment (Depège et al. 2000). Moreover, overexpression of *SlGPXL5* led to increased tolerance against wounding-induced growth inhibition (Herbette et al. 2011). Similar rapid gene activation was detectable after injuries in the case of *Nelumbo nucifera GPXL* (Diao et al. 2014). Interestingly, in rice cut-induced *OsGPXL* expression profile showed light dependency: the gene was induced after 12 h from wounding in dark condition, while under light condition this induction delayed to 24 h (Agrawal et al. 2002).

Unfortunately, we cannot turn a blind eye over anthropogenic factors like metal and other chemical pollutions. These factors are also harmful for plants and are continuously increasing factors affecting crop yield (Dukhovskis et al. 2003). As under other environmental stresses, antioxidants are really important elements in metal or chemical stress responses. As an example, the herbicide norflurazon inhibits the synthesis of carotenoids in plant leaves and in this way destructs pigment–protein complexes by photo-oxidation and blocks chloroplast development. Norflurazon treatment on barley leaves caused a dramatic increase in the mRNA level of *HvGPXL1* and *HvGPXL2* but decreased the level of *HvGPXL3* (Churin et al. 1999).

Different metals also affect differently depending on the plant studied. While iron caused the activation of *AtGPXL2*, *AtGPXL5* and *AtGPXL6* in *Arabidopsis* (Sugimoto and Sakamoto 1997; Milla et al. 2003) and *CsGPXL2* in tea plants (Fu 2014), aluminium induced only *AtGPXL6* (Sugimoto and Sakamoto 1997), but did not cause any changes in rice *OsGPXLs* (Passaia et al. 2013), and even downregulated all the *LjGPXLs* in *Lotus japonicus* (Ramos et al. 2009). Copper treatment also induced *AtGPXL2*, *AtGPXL5* and *AtGPXL6* in *Arabidopsis* (Sugimoto and Sakamoto 1997; Milla et al. 2003) and *CsGPXL2* in tea plants (Fu 2014), but decreased the protein level of some poplar PtGPXLs. Cadmium increased the level of particular poplar PtGPXLs (Navrot et al. 2006) and activated the lotus *LjGPXLs*, but later the degree of the induction decreased (Matamoros et al. 2015).

5 GPXL Mutants and Overexpressing Plants Harbour Altered Stress Tolerance

The function of plant GPXs in stress responses was extensively studied by employing transgenic plants engineered to enhance or reduce GPXL pools. Loss-of-function mutations of GPXL in many cases negatively affect the tolerance against environmental stresses in different plants. For example, in *A. thaliana*, depletion of *AtGPXL1* and *GPXL7* gene expression led to decreased tolerance against photo-oxidative stress; however, it increased the resistance against virulent *Pseudomonas syringae* (Chang et al. 2009). Defects of AtGPX3 reduced the drought stress tolerance. The mutants displayed impaired stomatal closure, faster water loss, and lower temperatures of leaves (Miao et al. 2006). Knockout mutation of *AtGPXL8* led to increased sensitivity to salt and osmotic stresses compared to wild type (Gaber 2011); furthermore, paraquat treatment affected the mutant plants more and caused suppressed root growth and higher level of oxidized proteins (Gaber et al. 2012). According to the results of the experiments performed using knockout mutants, the *Oryza sativa* OsGPXL1 mitochondrial enzyme is important for both phases of photosynthesis, root growth, water use efficiency and photorespiration under salinity (Lima-Melo et al. 2016), and depletion of OsGPXL5 also negatively affected the salt stress tolerance (Wang et al. 2017).

In accordance with the above results, overexpression of wheat *GPXL* genes in *Arabidopsis* enhanced early tolerance to high salt stress, and the transgenic plants showed higher germination rate and decreased growth inhibition by NaCl treatment (Zhai et al. 2013). In *Solanum lycopersicum*, overexpression of *SlGPXL5* seemed to protect the photosynthetic activities from chilling treatment under moderate light (Herbette et al. 2005); however, the transformed plants had significantly larger necrotic areas after *Botrytis cinerea* infection than wild-type plants. Thus, GPXL overexpression alleviated the abiotic stress and counteracted the plant defence response against biotic stress factors (Herbette et al. 2011).

6 Concluding Remarks

As global climate becomes more extreme, the abiotic stresses, the rapidly evolving pathogens and weeds cause more adverse environment for plants which can affect the productivity of crops. In the last two decades, many researches proved that plant GPXLs are essential elements of plant stress responses and are important ROS scavengers. Like animal GPXs, GPXLs are also able to reduce H_2O_2 and organic hydroperoxides to water or the corresponding alcohols. Not surprisingly, the involvement of plant glutathione peroxidases in stress responses has been reported in different plants. Overexpression of GPXLs in different plant species led to increased tolerance against abiotic stresses; however, in some cases, it caused decreased tolerance against biotic stress. It is clear that GPXLs could be promising candidates in the genetic engineering or traditional breeding to develop stress-resistant crop plants; however, further intensive research is needed to explore their connection to other elements of the antioxidant system and signalling.

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