Chapter 14
Glutathione-Mediated Biotic Stress Tolerance in Plants

J.A. Hernández, G. Barba-Espín, and P. Diaz-Vivancos

Abstract Glutathione, along with ascorbate, is the main non-enzymatic antioxidant and redox buffers in plant cells. The reduced form of glutathione (GSH) is involved in the protection of cells from the oxidative damage induced by environmental challenges. GSH plays an important role in the recycling of reduced ascorbate in the reaction catalyzed by the enzyme dehydroascorbate reductase in the so-called ascorbate–glutathione cycle. Several studies reported that glutathione is involved in the induction of plant defense genes, and the increase in GSH and/or GSH-related enzymes is correlated with the resistance to different biotic challenges, including plant virus, bacteria, and fungi. Also, different works evidenced that decreases in GSH can be responsible for pathogen-elicited symptom development in susceptible plants. In that respect, it is important to mention that treatments leading to an increase in GSH and/or the redox state of glutathione can reduce the virus contents and/or the symptoms even during compatible plant–virus interactions. In addition, subcellular glutathione contents, reactive oxygen species production, and the antioxidative metabolism are considered valuable biotic stress indicators within plants during situations of pathogen attack.

Keywords Bacteria • Dehydroascorbate reductase • Fungi • Glutathione • Oxidative stress • Redox state • Virus

J.A. Hernández • P. Diaz-Vivancos (✉)
Department of Plant Breeding, CEBAS-CSIC, 30100 Murcia, Spain
e-mail: pdv@cebas.csic.es

G. Barba-Espín
Department of Plant and Environmental Sciences, University of Copenhagen, 1017 Copenhagen, Denmark

M.A. Hossain et al. (eds.), *Glutathione in Plant Growth, Development, and Stress Tolerance*, https://doi.org/10.1007/978-3-319-66682-2_14

Contents

1 Introduction

In plant cells, the reactive oxygen species (ROS) scavenging is dependent on ascorbate (AsA) and glutathione (GSH), the two main non-enzymatic hydrophilic antioxidants and redox buffers (Noctor and Foyer 1998). Both antioxidant molecules act as signaling molecules in many cellular processes and are involved in basic metabolic reactions as well as in the protection of plant cells under environmental stress situations. Ascorbate and glutathione take part in the important ascorbate–glutathione (AsA–GSH) cycle, which plays an important role in the scavenging of H_2O_2 and in the recycling to the reduced forms of ascorbate and glutathione (AsA and GSH). These functions are catalyzed by APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), and GR (glutathione reductase). DHAR is the enzyme that links both antioxidants in the AsA–GSH cycle (Fig. 14.1). APX activity depends not only on the AsA availability but also on GSH through the DHAR activity, a GSH-dependent enzyme that can regenerate AsA from DHA (dehydroascorbate). It is also true that the reaction catalyzed by MDHAR for AsA recycling is more economical for the plant cell, but

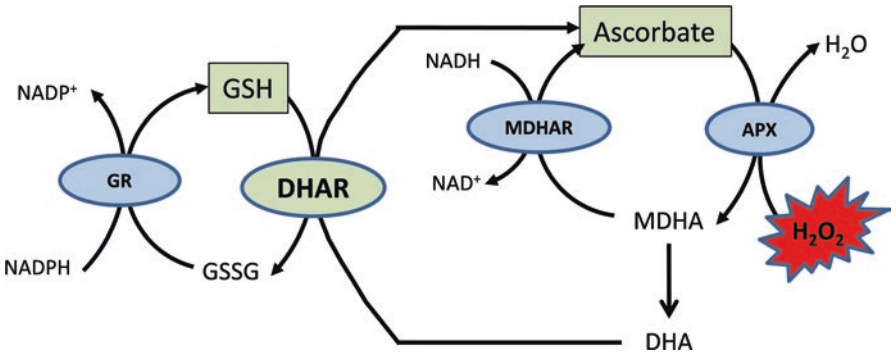

Fig. 14.1 Ascorbate–glutathione (AsA–GSH) cycle in plants. This cycle consists of a series of redox-coupled reactions whose main function is the scavenging of H_2O_2 and the recycling to the reduced forms of ascorbate and glutathione

under certain conditions, DHAR activation can be a good strategy to maintain ascorbate redox state.

Glutathione is the major low-molecular-weight thiol in plants, and its role in plant defense and tolerance against abiotic and biotic stresses has been widely described. In 1998, Wingate et al. reported the importance of GSH for local resistance responses. Treatment of suspension-cultured cells of bean (*Phaseolus vulgaris* L.) with GSH resulted in a massive and selective induction of the transcription of defense genes encoding enzymes related to phytoalexin and lignin biosynthesis, as well as stimulation of genes encoding cell wall hydroxyproline-rich glycoproteins. GSH is considered the most important thiol antioxidant in plant–pathogen interactions (Kuźniak 2010), and a clear relation between GSH increases and pathogen resistance has been reported in different studies (Gullner et al. 1999; Kużniak and Sklodowska 2004; Zechmann et al. 2007a; Clemente-Moreno et al. 2010, 2013).

The redox state of glutathione plays an important role regulating the expression of defense genes. One of the most typical examples corresponds to the activation of NPR1 proteins as well as *NPR1* gene expression. The reduction of NPR1 requires an increase in GSH contents, the NPR1 protein conformation being sensitive to cellular redox changes (Mou et al. 2003). Treatments with L-2-oxo-4-thiazolidine-carboxylic acid (OTC) increased GSH content and glutathione redox state, inducing the expression of the *NPR1* gene in both healthy and *Plum pox virus* (PPV)-infected peach plantlets (Clemente-Moreno et al. 2012). In that regard, GSH seems to be the main antioxidant involved in the activation of plant defense genes (Wingate et al. 1988; Ghanta et al. 2011).

2 Plant–Virus Interaction

Different authors observed the importance of the glutathione content as well as the GSH-related enzymes in the physiological and biochemical responses of plants against virus infection (Hernández et al. 2016). The GSH contents, and therefore the redox state of glutathione, have been often associated with a resistance response to plant viruses (Table 14.1). In addition, the availability of the GSH precursors, cysteine, glycine, and glutamate, which can limit GSH synthesis, is also important in the plant–virus interaction (Zechmann et al. 2007a). In this sense, treatments aimed at increasing GSH levels, or the redox state of glutathione, also induced some kind of resistance in different plant–virus interactions. In 1999, Gullner et al. reported that the exposure of tobacco (*Nicotiana tabacum*) leaf discs to the cysteine precursor L-2-oxo-4-thiazolidine-carboxylic acid (OTC) led to a massive accumulation of GSH, as well as reduced *tobacco mosaic virus* (TMV) coat protein contents and decreased number of necrotic lesions and virus contents in TMV-inoculated tobacco leaf discs (Gullner et al. 1999). Similar results were also recorded by Zechmann et al. (2007a) in pumpkin (*Cucurbita pepo*) seedlings. These authors treated pumpkin seedlings with 1 mM OTC for 48 h previous to the infection with *Zucchini*

Table 14.1 Response of glutathione and GSH-related enzymes in some plant–virus interactions

Interaction	GSH	GSH-related enzymes	Response	References
Tobacco (*Nicotiana tabacum*)–TMV (OTC-treated)	Increase	nd	Reduced necrotic lesions and viral coat proteins	Gullner et al. (1999)
Pumpkin (*Cucurbita pepo*)–ZYMV (OTC-treated)	Increase in roots and cotyledons	nd	Reduced, delayed, or complete suppression of symptoms	Zechmann et al. (2007a, b)
Pea (*Pisum sativum)*–PPV (OTC- or BTH-treated)	Increased redox state of glutathione	Increase in sDHAR, sGR, cDHAR, cGPX	Reduction of percentage of infected leaves	Clemente-Moreno et al. (2010)
In vitro peach (*Prunus persica)*–PPV	Increased redox state of glutathione	Decrease in GST (infected plantlets) Decrease in GR (healthy plantlets)	No protection	Clemente-Moreno et al. (2012)
Peach–PPV (OTC-treated)	Increase	Increase in cGPX	Reduced percentage of infected leaves Chloroplast protection	Clemente-Moreno et al. (2013)
Tobacco–TMV (sulfate-treated)	Increase	Increase in GST	Reduced necrotic lesions	Király et al. (2012)
Apricot (*Prunus armeniaca*) seeds–PNRSV	nd	Decrease in DHAR and GR	Low germination rate	Amari et al. (2007)
N. benthamiana–PMMoV-I	No change	Decrease GR (up to 21 dpi)	Symptom recovery	Hakmaoui et al. (2012)
N. benthamiana–PMMoV-S	Decreased levels Reduced GSH/GSSG ratio	Decrease GR (up to 21 dpi)	Severe symptoms	Hakmaoui et al. (2012)
Cucumber (*Cucumis sativus*)–CMV	nd	Increase in mDHAR, sGR Decreased sGR	Reduced Pn Reduced mitochondrial complex I and II	Song et al. (2009)
Tomato (*Lycopersicon esculentum*)–CMV	nd	Increase in sDHAR, cDHAR, mDHAR, sGR	Reduced Pn (less affected) Reduced mitochondrial complex I	Song et al. (2009)

s soluble fraction, *c* chloroplastic, *m* mitochondrial, *nd* not determined, *dpi* days post-inoculation

yellow mosaic virus (ZYMV) and evaluated the symptoms of ZYMV disease. They observed a general delay, reduction, or complete suppression of symptom development in OTC-treated plants, depending on the time of infection and the severity of symptoms (Zechmann et al. 2007a).

Glutathione content is influenced by sulfate nutrition, and high sulfate supply correlated with increased levels of Cys (cysteine) and GSH (Kopriva and Rennenberg 2004; Király et al. 2012). In a recent work, Király et al. (2012) reported that TMV-resistant tobacco plants grown with sufficient sulfate developed less necrotic lesions response when compared with plants grown with sulfate deficiency. This enhanced virus resistance correlated with elevated levels of Cys and glutathione as well as the induction of glutathione S-transferase (GST) (Király et al. 2012).

In compatible interactions PPV–*Prunus* and PPV–pea, an imbalance in the antioxidant machinery at subcellular level is produced (Hernández et al. 2004, 2006, Diaz-Vivancos et al. 2008), and in all cases an inhibitory effect on some GSH-dependent enzymes was observed. For example, a decrease in soluble and chloroplastic DHAR, GR, or glutathione peroxidase (GPX) was noticed (Hernández et al. 2004, 2006; Diaz-Vivancos et al. 2008).

The treatment of pea and peach plants with 1 mM OTC, previous to the infection with PPV, resulted in a partial resistance response, in terms of PPV symptoms as well as in the percentage of leaves showing PPV symptoms (Clemente-Moreno et al. 2010, 2013). In pea plants, this response was correlated with a higher redox state of glutathione as well as with an increase in APX, POX, and GSH-related enzymes at the subcellular level (Clemente-Moreno et al. 2010). Interestingly, asymptomatic leaves of infected plants displayed a higher redox state of glutathione, a fact that could also play a role in the reduction of symptoms. In peach plants, OTC treatment, in addition to an increased plant growth, provides protection to the photosynthetic machinery and/or the chloroplast metabolism in PPV-infected plants (Clemente-Moreno et al. 2013). However, when OTC was applied to PPV-infected peach plantlets, the OTC treatments did not reduce the virus contents, although GSH levels were increased (Clemente-Moreno et al. 2012). In OTC-treated plantlets, an induction of *NPR1* gene expression took place, mainly in PPV-infected plants, suggesting that GSH could play an important role in the *NPR1* induction under a viral infection (Clemente-Moreno et al. 2012). Accordingly, a similar conclusion was reported by Ghanta et al. (2011) in tobacco plants.

The infection of apricot seeds by *Prunus necrotic ringspot virus* (PNRSV) induced an oxidative stress that was parallel with a general decrease of all AsA–GSH cycle enzymes, including GSH-dependent enzymes such as DHAR and GR. This response was correlated with a more limited ability to eliminate H_2O_2 but also in the recycling of AsA and GSH in infected seeds (Amari et al. 2007).

Hakmaoui et al. (2012) studied the response of *Nicotiana benthamiana* plants against two different strains of *Pepper mild mottle virus*, the Italian (PMMoV-I) and the Spanish (PMMoV-S) strains. The PMMoV-S was the most virulent, inducing dramatic symptoms, whereas plants infected with PMMoV-I were able to recover from their symptoms. This response was linked, among other factors, with the maintenance of GSH levels during the infection phase (up to 21 days). However, the

plants infected with PMMoV-S strain showed a dramatic decrease in both total and reduced glutathione (Hakmaoui et al. 2012).

Cucumber mosaic virus (CMV) infection disrupted electron transport in chloroplast and mitochondria from cucumber and tomato plants. However, the net photosynthesis rate as well as the rate of mitochondrial complex I electron transport in mitochondria was less affected in tomato than in cucumber leaves (Song et al. 2009). This fact could be related with a better response of the antioxidant defenses in CMV-infected tomato plants than in cucumber, including an induction of DHAR in chloroplasts, mitochondria, and soluble fractions and GR in soluble fractions from tomato leaves. Nevertheless, in cucumber leaves, DHAR and GR only increased in mitochondria, whereas in chloroplasts a decrease in GR was recorded (Song et al. 2009).

3 Plant–Bacteria Interaction

A role for GSH and GSH-utilizing enzymes in the resistance against bacteria has been also suggested in different plant–bacteria interactions (Table 14.2). GSH mediates plant–bacteria interaction in both pathogenesis and symbiosis establishment.

The *Arabidopsis* mutant *pad2*–1 showed an increased susceptibility to the bacterial pathogen *Pseudomonas syringae* (Glazebrook et al. 1996) as well as enhanced susceptibility to additional pathogens (Parisy et al. 2007). PAD2 encodes ɣ-glutamylcysteine synthetase (GSH1), the enzyme that catalyzes the first step of de novo GSH biosynthesis, suggesting that in *Arabidopsis* the maintenance of adequate levels of GSH is an important factor during *P. syringae* and other pathogen infections (Parisy et al. 2007). In addition, *rax1–1*, an *Arabidopsis* GSH1 mutant which accumulates less than 50% of wild-type GSH content, was shown to be more susceptible to avirulent strains of *P. syringae* (Ball et al. 2004). However, the *cad2–1* mutant, with approximately about 30% of wild-type amounts of GSH, showed an unaltered disease resistance phenotype to virulent and avirulent strains of *P. syringae* (May et al. 1996).

The response of two different tomato cultivars against *P.syringae* pv. tomato is related with the glutathione levels and its redox state as well as the behavior of GSH-dependent enzymes (Kużniak and Sklodowska 2004). In that regard, the tomato A100 cultivar, susceptible to *P. syringae*, responded to the infection with a decrease in GSH and an accumulation of oxidized glutathione (GSSG) leading to a decrease in the redox state of glutathione. Under the same conditions, increases in GPX and GR were produced that was insufficient to keep the glutathione pool reduced (Kużniak and Sklodowska 2004). In contrast, the resistant tomato cultivar (Ontario), in addition to showing higher constitutive GSH levels than the susceptible cultivar, also maintained the glutathione pool homeostasis in response to *P. syringae*. Moreover, in response to the infection, the Ontario cultivar progressively increased GST activity (Kużniak and Sklodowska 2004). This enzyme not only plays an important role in the detoxification of organic hydroperoxides but also

Table 14.2 Response of GSH and GSH-related enzymes in some plant–bacteria interactions

Interaction	GSH	GSH-related enzymes	Response	References
Arabidopsis pad2–1–P. syringae	Decrease	nd	Increased susceptibility	Glazebrook et al. (1996)
Arabidopsis rax1–1–P. syringae	Decrease	Increased MDHAR	Increased susceptibility to avirulent strains	Ball et al. (2004)
Arabidopsis cad2–1–P. syringae	Decrease	nd	Unaltered disease resistance phenotype	May et al. (1996)
Tomato cv. A100–*P. syringae*	Decrease (GSSG accumulation)	Increased GPX and GR	Susceptible	Kuzniak and Sklodowska (2004)
Tomato cv. Ontario–*P. syringae*	Increase	Increased GST	Resistant	Kuzniak and Sklodowska (2004)
Transgenic tobacco–*P. syringae*	nd	Increased GR	Delayed necrosis	Faize et al. (2012)
Transgenic tobacco–*P. syringae*	Increase	nd	Improved defense response	Matern et al. (2015)
Soybean (*Glycine max*)–*Bradyrhizobium japonicum*	Increase	Increased DHAR and GR	Defense against oxidative stress	Dalton et al. (1986, 1991)
Medicago truncatula–*Sinorhizobium meliloti*	Decrease by BSO or antisense glutathione synthetase genes	nd	Decreased nodulation	Frendo et al. (2005)

nd not determined

displays DHAR activity (Dixon et al. 2002). In that sense, the increase in GST was parallel with a decrease in the concentration of DHA, maintaining the AsA levels and thus increasing the redox state of ascorbate (Kużniak and Sklodowska 2004).

The treatment of apple plants with benzothiadiazole (BTH), a SA analogue, limited the infection by *Erwinia amylovora* (Sklodowska et al. 2011). At short term (2 days post-inoculation, dpi), this response was correlated with an increase in GSH and GSSG, leading to a decrease in the GSH/GSSH ratio. After 7 dpi, BTH-treated plants showed a decline in GSH but a low increase in GSSG, about 20%, in relation to control plants. However, non-treated plants displayed a threefold increase in GSSG. In both cases, a decrease in GSH/GSSG ratio was produced, especially in non-treated plants, where the reduction in GSH/GSSG ratio was near four times. At long term (14 dpi), no significant changes in the redox state of glutathione occurred in any case. However, in this case, the BTH-induced resistance against the bacterial

infection was not correlated with increases in GSH-dependent enzymes, such as GPX or GST (Sklodowska et al. 2011).

Additional evidence of the GSH role in plant–bacteria interaction comes from studies using transgenic plants. Tobacco plants overexpressing cytosolic Cu, Zn-superoxide dismutase (cyt*sod*), and/or ascorbate peroxidase (cyt*apx*) genes displayed a disease tolerance phenotype, with various levels of resistance, against bacterial wildfire caused by *P. syringae* pv. tabaci (Faize et al. 2012). Transgenic lines harboring cyt*apx* and both transgenes showed the best response in terms of resistance. Inoculated transgenic lines displayed increased levels of GR activity when compared with wild-type inoculated plants (Faize et al. 2012), suggesting that the maintenance of adequate glutathione redox state could be an important factor during *P. syringae* infection. More recently, it has been reported that transgenic high-glutathione *Nicotiana tabacum* lines showed also an improved defense response against *P. syringae*, this response being modulated by the GSH redox potential (Matern et al. 2015).

The causal agent of bacterial wilt disease of plants is the bacteria *Ralstonia solanacearum.* This pathogen uses virulence effector proteins leading to the suppression of disease resistance responses to succeed in infection. Mukaihara et al. (2016) have described that the *R. solanacearum* effector protein RipAY is able to degrade GSH. This protein displays ɣ-glutamylcyclotransferase activity and due to its high GSH degradation activity could be considered as an effective mechanism to overcome pathogen plant defenses. Moreover, because GSH is also important for bacterial environmental stress tolerance and growth, RipAY displays a very interesting safety mechanism to avoid unwanted activation, and it is specifically activated by host eukaryotic thioredoxins (Mukaihara et al. 2016).

On the other hand, rhizobial bacteria interact with legume root to establish a symbiotic relationship leading to the formation of a new specialized organ, the nodule, which is capable of fixing atmospheric nitrogen. In root nodules, high level of GSH or homoglutathione (hGSH, GSH homolog present instead of or in addition to GSH in certain legumes) and the presence of an active AsA–GSH cycle have been reported (Becana et al. 2000; Dalton et al. 1986; Frendo et al. 1999). Thus, it has been suggested that GSH and hGSH protect nitrogen-fixing nodules against toxic oxygen species resulting from the active nodule metabolism and from varying physiological conditions, as well as from environmental challenges. In this sense, Dalton et al. (1991) reported an increase in ascorbate peroxidase and ascorbate-recycling enzymes (especially DHAR) and GR activities, as well as an increase in ascorbate and glutathione content in soybean nodules exposed to elevated ambient pO_2, linking N_2 fixation and antioxidative metabolism.

Moreover, during the nodulation process, an active root cell division is triggered in order to establish nodule primordia. Through the characterization of different *A. thaliana* GSH1 mutants, it has been showed that GSH plays a key role during root development (Diaz-Vivancos et al. 2015). The *root meristemless1* (*rml1*) mutant, having only about 5% of the wild-type GSH levels (Schnaubelt et al. 2015), is not able to maintain cell division following germination. The cell cycle in *rml1* is arrested in G1 phase of the cell cycle, being GSH the required factor to reactivate

the cell division in the root apical cells (Vernoux et al. 2000). In addition, buthionine sulfoximine (BSO), a GSH synthesis inhibitor, caused the arrest of root but not shoot development in wild-type seedlings (Schnaubelt et al. 2015). In *Medicago truncatula*, both BSO treatment and antisense glutathione synthetase genes in roots resulted in a decrease of (1) the average number of nodules in inoculated roots and (2) the expression of genes involved in the nodulation process, suggesting an important role for GSH in the symbiotic plant–bacteria interaction during the nodulation process (Frendo et al. 2005).

Taking together, all the reported evidences suggest that the maintenance of adequate levels of GSH is important for both the establishment of pathogen bacteria disease resistance and symbiotic plant–bacteria interactions.

4 Plant–Fungi Interaction

The effects of fungal infection on the glutathione metabolism in different cell compartments have been scarcely studied. Most of the information available has been obtained using crude extracts. In addition, the majority of information corresponds to interactions with a low range of fungi, including *Botrytis cinerea*, *Fusarium oxysporum*, or *Trichoderma harzianum* (Kuźniak and Skłodowska 1999, 2001, 2005; García-Limones et al. 2002; Bernal-Vicente et al. 2015) (Table 14.3).

During a plant–fungi interaction, ROS can be generated by both the pathogen and/or the host plant. In the case of a necrotrophic fungus, ROS overproduction can be a strategy to kill the host tissue in the initial phase of infection (Tiedemann 1997). In such conditions, GSH seems to be the limiting factor for a proper functioning of the AsA–GSH cycle during the progression of the infection. In tomato plants, the infection by the necrotrophic fungus *B. cinerea* caused a progressive decrease in GSH contents, whereas GSSG was barely affected (Kuźniak and Skłodowska 1999). The fungal infection also affected GSH-dependent enzymes. In that regard, an increase in GR activity was produced in tomato leaves in order to try to maintain the redox state of the glutathione. However, a decrease in other GSH-dependent enzymes, such as DHAR, occurred (Kuźniak and Skłodowska 1999). The same authors studied the effect of *B. cinerea* in the antioxidative mechanisms in chloroplasts from tomato plants (Kuźniak and Skłodowska 2001). These authors reported that *B. cinerea* infection promoted senescence symptoms, the chloroplasts being one of the earliest cell compartments affected, as indicated by the loss of chlorophyll observed even after 1 dpi. This effect was correlated with a decrease in chloroplastic GSH and total glutathione pools as well as a decrease in GPX, an enzyme that participates in the reduction of lipid hydroperoxides by using GSH as reducing power (Asada 1999).

Years later, Kuzniak and Sklodowska (2005) studied the changes of the AsA–GSH cycle in different cell compartments (chloroplasts, mitochondria, and peroxisomes) in *B.cinerea*-infected tomato leaves. The oxidative stress caused by the fungal infection affected all cellular compartments, although the authors observed organelle-specific

Table 14.3 Response of glutathione and GSH-related enzymes in some plant–fungi interactions

Interaction	GSH	GSH-related enzymes	Response	References
Tomato–*B. cinerea*	Decrease	Decrease in DHAR Increase in GR	Visible symptoms at 3 dpi Gray mold in oldest leaves	Kuźniak and Skłodowska (1999)
Tomato–*B. cinerea*	Decrease	Decreased cGPX (4–5 dpi). Increased cGR (4–5 dpi) and cGST (3 dpi)	Visible symptoms at 3 dpi Gray mold in oldest leaves	Kuzniak and Sklodowska (2001)
Tomato–*B. cinerea*	Decrease Increased mGSSG and pGSSG	Decreased total DHAR, mDHAR, pDHAR, mGR and pGR. Increased cDHAR, total GR	Dark necrotic lesions (2–3 dpi)	Kuzniak and Sklodowska (2001)
Chickpea (*Cicer arietinum* cv. JG62)–*F. oxysporum*	nd	Increased root GR	Susceptible response Vascular infection (20–22 dpi)	García-Limones et al. (2002)
Chickpea (cv. Ontario)–*F. oxysporum*	nd	Higher constitutive stem GR levels	Resistant response	García-Limones et al. (2002)
Olea europaea–*Glomus claroideum*	nd	Increased DHAR	Increased FW	Alguacil et al. (2003)
Retama sphaerocarpa–*Glomus claroideum*	nd	Increased DHAR Increased GR	Increased FW	Alguacil et al. (2003)
Rhamnus lycioides–*Glomus claroideum*	nd	Increased DHAR Increased GR	Increased FW	Alguacil et al. (2003)
Soybean–*Glomus mosseae*	nd	Increased root GR)	Increased plant biomass	Porcel et al. (2003)
Melon–*T. harzianum*	nd	Increased DHAR and GST	Increased FW	Bernal-Vicente et al. (2015)

S soluble fraction, *c* chloroplastic, *m* mitochondrial, *p* peroxisomal, *nd* not determined, *dpi* days post-inoculation

changes, such variations being masked when data were analyzed in whole-leaf extract. A general decrease in glutathione concentration occurred by the infection in different cell compartments from tomato leaves, mitochondria and peroxisomes being the most affected organelles. In chloroplasts and mitochondria, the total glutathione contents

declined after 2–3 dpi, but in peroxisomes, this decrease started earlier, only 1 dpi (Kuzniak and Sklodowska 2005). The reduction in total glutathione was parallel with a significant increase in GSSG, especially in mitochondria and peroxisomes. As a result, in all cell compartments, the infection produced an important decrease in the GSH/GSSG ratios, appearing earlier in mitochondria and peroxisomes than in chloroplasts, showing lower GSH/GSSG ratio at the initial state of the infection phase (Kuzniak and Sklodowska 2005). GSH-dependent enzymes were also affected by *B. cinerea* infection in the different cell compartments studied. In this sense, the infection induced a decrease in DHAR activity in whole-leaf extracts, mainly from 2–4 dpi. The response of chloroplastic DHAR was somewhat different to that observed in mitochondria or peroxisomes. No important effect was produced in chloroplast, and even an important increase after 3 dpi occurred. However, mitochondrial and peroxisomal DHAR activities decreased after 3 dpi (Kuzniak and Sklodowska 2005). Regarding GR activity, an initial increase at 1 dpi was maintained during the infection period in whole-leaf extracts as well as in chloroplasts. In contrast, mitochondrial GR peaked at 3 dpi and then progressively decreased until the end of the infection period (5 dpi), whereas peroxisomal GR showed a decline only at 2 dpi. The authors suggested that the increases in GR can be an effective protection to avoid an excessive GSSG accumulation in order to maintain the redox state of glutathione (Kuzniak and Sklodowska 2005).

Fusarium oxysporum is another necrotrophic fungus that produces the fusarium wilt (a vascular wilt fungal disease) in many plant species, including tomato, pepper, melon, or legumes, among others. The information about the effect of *F. oxysporum* infection on the glutathione metabolism of higher plants is very scarce. In 2002, García-Limones et al. studied the possible role of ROS production in the development of the fusarium wilt disease in chickpea in compatible and incompatible interactions. However, these authors did not measure glutathione contents but only GR activity (among other antioxidant enzymes). The authors observed that the first symptoms appeared 15–17 dpi in the susceptible cultivar (cv. JG62). During this period, about 50% of plants were systemically infected. At the end of the disease development phase (20–22 dpi), more than 90% of susceptible plants showed vascular infection. In the case of the resistant cultivar (cv. WR315), no evidences of infection were observed (García-Limones et al. 2002). The authors found a constitutive GR activity much higher in stems than in roots in both chickpea cultivars. In infected plants, a transient increase in GR occurred only in roots from susceptible plants, and at the end of the disease development (20–22 dpi), a correlation among GR increase, H_2O_2-scavenging enzymes (APX, CAT), and H_2O_2-producing enzymes (SOD) took place. Although GR activity did not show significant changes in stems during the development of the fungal disease, in the resistant cultivar, GR activity levels were higher than the susceptible cultivar. In stems, APX, CAT, and SOD activities were induced only in susceptible plants during the disease development. All these responses led the authors to suggest that the lack of induction of antioxidant enzymes in the stem of resistant plants can denote a less efficient ROS scavenging defense and thus a higher ROS level accumulation that could be related with the resistance mechanism against *F. oxysporum* infection (García-Limones et al. 2002).

The addition of specific fungus such as *T. harzianum* to plant growth substrates can increase plant yield and also reduce plant diseases produced by other plant pathogens present in soils. However, the mechanisms of action of these biostimulant and biocontrol effects are not fully understood and knowledge about their influence in the antioxidative metabolism is very scarce. The inoculation with *T. harzianum* increased FW of melon plants grown in different organic substrates (Bernal-Vicente et al. 2015). This response was parallel with the increase of some GSH-dependent enzymes, such as DHAR and GST (Bernal-Vicente et al. 2015). More specifically, the combination of *T. harzianum* with either citrus or bentonite compost stimulated DHAR activity in melon leaves, whereas the combination of *T. harzianum* with either peat substrate or bentonite compost increased leaf GST activity (Bernal-Vicente et al. 2015). The increase in DHAR involves a higher AsA-recycling capacity, and according to Gong et al. (2005), ascorbate and GST seem to play key roles in plant growth and development.

Mycorrhizae may help plants to grow in semiarid ecosystems improving their response to the environmental changes that involve the progressive increase in atmospheric CO_2 concentration in a climate change context (Terrer et al. 2016). Mycorrhizal inoculation increased the plant biomass in three Mediterranean shrubs, *Olea europaea* L. ssp. sylvestris, *Retama sphaerocarpa* (L.) Boissier, and *Rhamnus lycioides* L. This stimulant effect in plant growth was related with increased mineral content (N, P, K, Mg, Fe, Ca, etc.) as well as with increased antioxidant capacity, including GSH-dependent enzymes. The inoculation with the allochthonous arbuscular mycorrhizal (AM) fungus *Glomus claroideum* strongly increased DHAR in the three mentioned shrubs. In contrast, the inoculation with a mixture of native AM fungi produced a lower stimulation of DHAR activity (Alguacil et al. 2003). The presence of *G. claroideum* also increased GR activity in *R. sphaerocarpa* and *R. lycioides*, whereas the inoculation with the mixture of native AM fungi only raised GR activity in *R. lycioides* plants, producing even higher increases than *G.claroideum.* However, no effect in GR was observed in the shrub *O. europaea* (Alguacil et al. 2003).

Since the abovementioned experiment was carried out in semiarid conditions, the mycorrhizal-induced increases in antioxidant enzymes could be a strategy used by such shrubs to face the ROS overproduction under the environmental conditions assayed. Specifically, and as far as GSH-enzymes are concerned, the increase in DHAR can involve a better capability to recycle AsA, whereas GR, in addition to GSH recycling, may serve to provide $NADP^+$ availability to accept electrons from the photosynthetic electron chain in order to minimize the reduction of O_2 to O_2^-. In conclusion, these authors suggested that the increase in antioxidant enzymes could be involved, at least partially, in the beneficial effect of mycorrhizal colonization on the performance of shrubs species grown under semiarid conditions (Alguacil et al. 2003).

Oxidative damage to biomolecules is one of the most important mechanisms triggering nodule senescence in stressed nodules (Becana et al. 2000). Mycorrhizal symbiosis can also protect plants against premature nodule senescence induced by drought situations, as observed in soybean plants (Porcel et al. 2003). This response seemed to be linked to a higher GR activity in roots and nodules in mycorrhizal

plants. These authors proposed that the higher GR activity in roots and nodules of mycorrhizal plants has contributed to the protection of nodules from premature senescence (Porcel et al. 2003).

5 Changes in the Subcellular Compartmentalization of Glutathione Under Biotic Stress Conditions

As an antioxidant, glutathione is involved in detoxifying ROS through the ascorbate–glutathione cycle (Foyer and Noctor 2009, 2013). Changes in the subcellular contents of glutathione during biotic stimuli reflect the occurrence of compartment-specific defense mechanisms, which is associated with compartment-specific ROS accumulation and oxidative stress. Since virtually all plant pathogens cause ROS generation and oxidative stress, changes in subcellular glutathione contents are therefore valuable biotic stress indicators within plants during situations of pathogen attack. Whereas the role of glutathione and, by extension, of the antioxidative metabolism in different organelles to abiotic stress has been well reported, the data on the glutathione compartment-specific role during plant–pathogen interaction is poorly understood. The following lines summarize the findings on this subject, discussing the existing connection between subcellular accumulation of glutathione and ROS, and the documented functional differences of the subcellular compartments during biotic stress situations.

5.1 Apoplast

Glutathione concentrations in the apoplast constitute a minor portion of its total pool (Vanacker et al. 1998, 2000; Zechmann et al. 2008; Tolin et al. 2013). In leaf cells, the apoplast contains only 1–2% of the total cell glutathione (Foyer et al. 2001), although higher values has been reported in pea leaves (Hernández et al. 2001). Moreover, the glutathione homeostasis is easily alterable in the apoplast due to the absence of systems to regenerate the reduced glutathione form. Consequently the capacity of redox buffering in the apoplast is weaker than that found inside the cell (Horemans et al. 2000). These facts make the apoplast a sensor of changes in the environmental conditions (Tolin et al. 2013). In response to biotic stimuli, the low buffering capacity of the apoplast favors a rapid accumulation of ROS (Mittler 2002). Herein, ROS overproduction is one of the early events following pathogen attack, occurring mainly in the apoplast via plasma membrane NADPH oxidases (Torres et al. 2002; Suzuki et al. 2011) and cell wall peroxidases (Bindschedler et al. 2006). This has been reported in numerous plant–pathogen interactions as common event of plants' hypersensitive response (HR) leading to programmed cell death (Lamb and Dixon 1997; Wojtaszek 1997; Jones and Dangl 2006).

5.2 Chloroplasts and Peroxisomes

Changes in glutathione contents in chloroplasts and peroxisomes are involved in plant defense against pathogens. *P. syringae* and *B. cinerea* caused enhanced accumulation of glutathione in *Arabidopsis* at early stages of infection, reaching 73% and 450% of control levels in chloroplast and peroxisomes, respectively. At later stages of infection, a pronounced decrease of glutathione in both cell compartments was accompanied by increased ROS accumulation (Großkinsky et al. 2012). This highlights the importance of glutathione during stress signaling. Similar results have been achieved in peroxisomes of tomato plants during *B. cinerea* infection, where the initial increase of glutathione was followed by a pronounced decrease and the disruption of the antioxidative system in peroxisomes (Kuźniak and Skłodowska 2005). Some authors have pointed out the existence of a connection between apoplastic and chloroplastic ROS signaling during the biotic response, in which the chloroplast may act as an amplifier of the signal from the apoplast (Joo et al. 2005; Vahisalu et al. 2010). As antioxidants protect the organelles by counteracting the level of ROS (Green et al. 2006), their contents in chloroplast can be decisive in the tuning of ROS signaling.

5.3 Mitochondria

Mitochondria possess a strong antioxidant system to protect them against the constant generation of ROS, in which GSH is of particular importance (Zechmann et al. 2008). In this sense, the drop of glutathione contents is associated with ROS accumulation and oxidative stress in this compartment, leading to the induction of programmed cell death (Vianello et al. 2007). *Arabidopsis* plants infected with *B. cinerea* displayed a strong drop of total glutathione content in mitochondria 48-h post-inoculation, which correlated with a strong increase of H_2O_2 in this compartment and the development of necrosis symptoms (Simon et al. 2013). In a similar way, the infection of *N. tabacum* with an incompatible strain of *tobacco mosaic virus* (TMV) provoked a depletion of glutathione contents in mitochondria and the development of necrotic spots (Király et al. 2012). In tomato plants, *B. cinerea* produced a decrease of glutathione contents mainly in mitochondria, coupled with the accumulation of oxidized glutathione 48-h post-inoculation (Kuźniak and Skłodowska 2005).

5.4 Nucleus

Glutathione in nucleus plays essential roles in protection of DNA against oxidative damage, cell proliferation, DNA synthesis, and regulation of the nuclear matrix organization and proteins (Green et al. 2006; Díaz-Vivancos et al. 2010b; Go and Jones 2010). Glutathione also regulates the expression of genes involved in the activation of plant defense mechanisms (Han et al. 2013). The roles of glutathione in

nuclei during pathogen attack are not fully understood. However it has been reported that there is a notable accumulation of glutathione in nuclei during early stages of viral, fungal, and bacterial infection. Such is the case of *Arabidopsis* plants infected with *P. syringae* (Großkinsky et al. 2012) and *B. cinerea* (Simon et al. 2013), leaves of *Cucurbita pepo* infected with ZYMV (Zechmann et al. 2005), and TMV-infected *N. tabacum* plants (Király et al. 2012). In *Arabidopsis*, increased GSH accumulation in the nuclei has been reported to be concomitant with decreased levels in the cytosol, followed by enhanced levels in the whole cell (Diaz-Vivancos et al. 2010a). Similarly, the increase of total glutathione in the nuclei of *Arabidopsis* plants infected with *P. syringae* (Großkinsky et al. 2012) and *B. cinerea* (Simon et al. 2013) was followed by a rapid accumulation of glutathione in the chloroplasts and cytosol. It was hypothesized that the initial accumulation of GSH in nuclei is perceived as a signal in order to increase its levels in the whole cell under stress conditions (Diaz-Vivancos et al. 2010a).

5.5 An Outlook of the Methods to Determine Subcellular Glutathione Concentrations

Determination of GSH and GSSG on the subcellular levels is technically challenging as the sample preparation itself can be perceived as a stress, thus altering the GSH levels. There are two major approaches to study the compartment distribution of glutathione in plants, presenting advantages and disadvantages inherent in both types of methods: (1) biochemical determination after subcellular fractionation of plant tissues and (2) microscopical visualization following glutathione labeling. Large amount of starting plant material and cross-contamination among fractions during organelle isolation are the major constraints of the biochemical determination. Moreover, the equivalence of the results obtained in vitro with the actual glutathione levels in vivo is, often, uncertain. Nevertheless, these methods allow the determination of glutathione in millimolar range, and glutathione redox state can be calculated through the measurement of both reduced and oxidized forms (Jiménez et al. 1997; Vanacker et al. 1998; Kuźniak and Skłodowska 2001; Ohkama-Ohtsu et al. 2007; Krueger et al. 2009). Regarding the microscopic approaches, they can be separated into light microscopical methods, in which glutathione is labeled with specific antibodies or dyes (Meyer and Fricker 2000; Müller et al. 2005; Zechmann and Müller 2010), fluorescence microscopy determination following labeling with redox-sensitive green fluorescent protein (GFP)(Meyer et al. 2007; Gutscher et al. 2008), and electron microscopy following immunogold labeling (Gao et al. 2012). Unfortunately, the antibody that is currently used for detecting glutathione cannot differentiate between the reduced and oxidized form (Zechmann et al. 2005).These methods allow determining the localization and concentration of glutathione in vivo in the different cell compartments in a more accurate way, which opens up new prospects in the study of glutathione dynamics in plant defense mechanisms. Main

limitations of the microscopic techniques are intrinsic to the sample preparation and visualization, as mechanical separation of cells and tissues and exposure to light and dehydration under microscope can be perceived as a stress to the sample.

6 Conclusion and Future Perspectives

Biotic environmental stress situations lead to considerable yield drop causing important economic losses worldwide. One common consequence of exposure to stress conditions is the increased production of ROS. The most potentially deleterious effect of ROS is that at high concentrations they trigger genetically programmed cell suicide events. Far from being only damaging agents, ROS are also used by plants as second messengers in signal transduction cascades in a variety of process, their accumulation being crucial for plant development as well as defense (Foyer and Noctor 2013). In plants, ROS production and scavenging are intimately linked, and the balance between them will determine defense responses. Thus, the major low-molecular-weight antioxidants ascorbate and glutathione determine the specificity of this oxidative signaling. The tripeptide glutathione exerts a strong influence on plant responses against pathogens, not only as an antioxidant but also as a defense signaling compound. Due to its relatively high cellular concentration, GSH acts as ROS scavenger or sacrificial nucleophile. Although many other secondary metabolites can function similarly, GSH is distinguished from most of these compounds by three main characteristics: (1) the presence of specific enzymes that couple GSH to the oxidative metabolism, (2) the existence of relatively stable oxidizing form, and (3) the recycling of GSSG to GSH by high-capacity enzyme-based system.

Biological systems adapt to changing environments by reorganizing their cellular and physiological program with metabolites representing one important response level. Glutathione can thus be considered as multifunctional metabolite that is important in redox homeostasis and signaling as well as in developmental and defense reactions (Foyer and Noctor 2011). Changes in redox metabolism will inevitably modify much larger signaling network that integrates information from many pathways regulating plant growth and defense responses. The importance of GSH and GSH-related enzymes in reducing the incidence of plant pathogens and symptom development has been widely reported. In this sense, treatments inducing increases in GSH and/or the redox state of glutathione can be beneficial during plant–pathogen interactions. Nevertheless, how pathogens alter plant metabolism and biochemistry is not fully understood yet. New knowledge on this topic and the discovery of new products stimulating GSH redox homeostasis would lead to develop new strategies to achieve a durable tolerance against pathogens.

Acknowledgments PDV thanks CSIC and the Spanish Ministry of Economy and Competitiveness for their “Ramon &Cajal” research contract, co-financed by FEDER funds.

References

Alguacil MM, Hernandez JA, Caravaca F, Portillo B, Roldán A (2003) Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semiarid soil. Physiol Plant 118:562–570

Amari K, Díaz-Vivancos P, Pallás V, Sánchez-Pina MA, Hernández JA (2007) Oxidative stress induction by *Prunus necrotic ringspot virus* infection in apricot seeds. Physiol Plant 131:302–310

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Ball L, Accotto G, Bechtold U, Creissen G, Funck D, Jimenez A, Kular B, Leyland N, Mejia-Carranza J, Reynolds H, Karpinski S, Mullineaux PM (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell 16:2448–2462

Becana M, Dalton DA, Moran JF, Iturbe-Ormaetxe I, Matamoros MA, Rubio MC (2000) Reactive oxygen species and antioxidants in legume nodules. Physiol Plant 109:372–381

Bernal-Vicente A, Pascual JA, Tittarelli F, Hernández JA, Díaz-Vivancos P (2015) Trichoderma supplementation of compost stimulates the antioxidant defense system in melon plants. J Sci Food Agric 95:2208–2214

Bindschedler LV, Dewdney J, Blee KA, Stone JM, Asai T, Plotnikov J, Denoux C, Hayes T, Gerrish C, Davies DR, Ausubel FM, Bolwell GP (2006) Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. Plant J 47:851–863

Clemente-Moreno MJ, Diaz-Vivancos P, Barba-Espín G, Hernández JA (2010) Benzothiadiazole and L-2-oxothiazolidine-4-carboxylic acid reduced the severity of Sharka symptoms in pea leaves: effect on the antioxidative metabolism at subcellular level. Plant Biol 12:88–97

Clemente-Moreno MJ, Diaz-Vivancos P, Piqueras A, Hernández JA (2012) Plant growth stimulation in *Prunus* species plantlets by BTH or OTC treatments under *in vitro* conditions. J Plant Physiol 169:1074–1083

Clemente-Moreno MJ, Diaz-Vivancos P, Rubio M, Fernandez N, Hernández JA (2013) Chloroplast protection in plum pox virus-infected peach plants by L-2-oxo-4-thiazolidine-carboxylic acid treatments: effect in the proteome. Plant Cell Environ 36:640–654

Dalton DA, Russell SA, Hanus FJ, Pascoe GA, Evans HJ (1986) Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. Proc Natl Acad Sci U S A 83:3811–3815

Dalton DA, Post CJ, Langeberg L (1991) Effects of ambient oxygen and of fixed nitrogen on concentrations of glutathione, ascorbate, and associated enzymes in soybean root nodules. Plant Physiol 96(3):812–818

Diaz-Vivancos P, Clemente-Moreno MJ, Rubio M, Olmos E, Garcia JA, Martinez-Gomez P, Hernandez JA (2008) Alteration in the chloroplastic metabolism leads to ROS accumulation in pea plants in response to *plum pox virus*. J Exp Bot 59:2147–2160

Diaz-Vivancos P, Dong Y, Ziegler K, Markovic J, Pallardo FV, Pellny TK, Verrier PJ, Foyer CH (2010a) Recruitment of glutathione into the nucleus during cell proliferation adjusts whole cell redox homeostasis in *Arabidopsis thaliana* and lowers the oxidative defence shield. Plant J 64:825–838

Diaz-Vivancos P, Wolff T, Markovic J, Pallardo FV, Foyer CH (2010b) A nuclear glutathione cycle within the cell cycle. Biochem J 431:169–178

Diaz-Vivancos P, de Simone A, Kiddle G, Foyer CH (2015) Glutathione – linking cell proliferation to oxidative stress. Free Radic Biol Med 89:1154–1164

Dixon DP, Davis BG, Edwards R (2002) Functional divergence in the glutathione transferase superfamily in plants. J Biol Chem 277:30859–30869

Faize M, Burgos L, Faize L, Petri C, Barba-Espin G, Díaz-Vivancos P, Clemente-Moreno MJ, Alburquerque N, Hernandez JA (2012) Modulation of tobacco bacterial disease resistance using cytosolic ascorbate peroxidase and Cu, Zn-superoxide dismutase. Plant Pathol 61:858–866

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Signal 11(4):861–905

Foyer CH, Noctor G (2013) Redox signaling in plants. Antioxid Redox Signal 18(16):2087–2090

Foyer CH, Theodoulou FL, Delrot S (2001) The functions of inter- and intracellular glutathione transport systems in plants. Trends Plant Sci 6(10):486–492

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155(1): 2–18

Frendo P, Gallesi D, Turnbull R, Van de Sype G, Hérouart D, Puppo A (1999) Localization of glutathione and homoglutathione in *Medicago truncatula* is correlated to a differential expression of genes involved in their synthesis. Plant J 17:215–219

Frendo P, Harrison J, Norman C, Hernandez-Jimenez MJ, Van de Sype G, Gilabert A, Puppo A (2005) Glutathione and homoglutathione play a critical role in the nodulation process of *Medicago truncatula*. Mol Plant-Microbe Interact 18:254–259

Gao R, Ng FK, Liu P, Wong SM (2012) Hibiscus chlorotic ringspot virus coat protein upregulates sulfur metabolism genes for enhanced pathogen defense. Mol Plant-Microbe Interact 25:1574–1583

Garcia-Limones C, Hervás A, Navas-Cortés JA, Jiménez-Diaz RM, Tena M (2002) Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f.sp. ciceris. Physiol Mol Plant Pathol 61:325–337

Ghanta S, Bhattacharyya D, Sinha R, Banerjee A, Chattopadhyay S (2011) *Nicotiana tabacum* overexpressing γ-ECS exhibits biotic stress tolerance likely through NPR1- dependent salicylic acid-mediated pathway. Planta 233:895–910

Glazebrook J, Rogers EE, Ausubel FM (1996) Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. Genetics 143:973–982

Go YM, Jones DP (2010) Redox control system in the nucleus: mechanisms and functions. Antioxid Redox Signal 12:489–509

Gong H, Jiao Y, Hu WW, Pua EC (2005) Expression of glutathione-S-transferase and its role in plant growth and development in vivo and shoot morphogenesis in vitro. Plant Mol Biol 57:53–66

Green RM, Graham M, O'Donovan MR, Chipman JK, Hodges NJ (2006) Subcellular compartmentalization of glutathione: correlations with parameters of oxidative stress related to genotoxicity. Mutagenesis 21:383–390

Großkinsky DK, Koffler BE, Roitsch T, Maier R, Zechmann B (2012) Compartment-specific antioxidative defense in Arabidopsis against virulent and avirulent *Pseudomonas syringae*. Phytopathology 102(7):662–673

Gullner G, Tóbiás I, Fodor J, Kőmíves T (1999) Elevation of glutathione level and activation of glutathione-related enzymes affect virus infection in tobacco. Free Radic Res 31:S155–S161

Gutscher M, Pauleau AL, Marty L, Brach T, Wabnitz GH, Samstag Y, Meyer AJ, Dick TP (2008) Real-time imaging of the intracellular glutathione redox potential. Nat Methods 5:553–559

Han Y, Chaouch S, Mhamdi A, Queval G, Zechmann B, Noctor G (2013) Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H_2O_2 to activation of salicylic acid accumulation and signaling. Antioxid Redox Signal 18:2106–2121

Hernández JA, Ferrer MA, Jiménez A, Ros-Barceló A, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of *Pisum sativum* L. leaves: its relation with NaCl-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Hernández JA, Rubio M, Olmos E, Ros-Barceló A, Martínez-Gómez P (2004) Oxidative stress induced by long-term Plum pox virus infection in peach (*Prunus persica* L. cv GF305). Physiol Plant 122:486–495

Hernández JA, Diaz-Vivancos P, Rubio M, Olmos E, Ros-Barceló A, Martínez-Gómez P (2006) Long-term PPV infection produces an oxidative stress in a susceptible apricot cultivar but not in a resistant cultivar. Physiol Plant 126:140–152

Hernández JA, Gullner G, Clemente-Moreno MJ, Künstler A, Juhász C, Diaz-Vivancos P, Király L (2016) Oxidative stress and antioxidative responses in plant-virus interactions. Physiol Mol Plant Pathol 94:134–148

Horemans N, Foyer CH, Asard H (2000) Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci 5(6):263–267

Hakmaoui A, Pérez-Bueno ML, García-Fontana B, Camejo D, Jiménez A, Sevilla F, Barón M (2012) Analysis of the antioxidant response of *Nicotiana benthamiana* to infection with two strains of Pepper mild mottle virus. J Exp Bot 63: 5487–5496

Jiménez A, Hernández JA, del Río LA, Sevilla F (1997) Evidence for the presence of the ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol 114:275–284

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV (2005) Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the Arabidopsis oxidative stress response to ozone. Plant Cell 17(3):957–970

Király L, Künstler A, Höller K, Fattinger M, Juhász C, Müller M, Gullner G, Zechmann B (2012) Sulfate supply influences compartment specific glutathione metabolism and confers enhanced resistance to tobacco mosaic virus during a hypersensitive response. Plant Physiol Biochem 59:44–54

Kopriva S, Rennenberg H (2004) Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. J ExpBot 55:1831–1842

Krueger S, Niehl A, Lopez Martin MC, Steinhauser D, Donath A, Hildebrandt T, Romero LC, Hoefgen R, Gotor C, Hesse H (2009) Analysis of cytosolic and plastidic serine acetyltransferase mutants and subcellular metabolite distributions suggests interplay of the cellular compartments for cysteine biosynthesis in Arabidopsis. Plant Cell Environ 32:349–367

Kuźniak E (2010) The ascorbate–gluathione cycle and related redox signals in plant–pathogen interactions. In: Anjum NA et al (eds) Ascorbate-glutathione pathway and stress tolerance in plants. Springer, Dordrecht, pp 115–136

Kuźniak E, Skłodowska M (1999) The effect of *Botrytis cinerea* infection on ascorbate-glutathione cycle in tomato leaves. Plant Sci 148:69–76

Kuźniak E, Sklodowska A (2001) Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. Plant Sci 160:723–731

Kużniak E, Sklodowska M (2004) Differential implication of glutathione, glutathione-metabolizing enzymes and ascorbate in tomato resistance to *Pseudomonas syringae*. J Phytopathol 152:529–536

Kuźniak E, Skłodowska M (2005) Compartment-specific role of the ascorbate-glutathione cycle in the response of tomato leaf cells to *Botrytis cinerea* infection. J Exp Bot 56(413):921–933

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol 48:251–275

Matern S, Peskan-Berghoefer T, Gromes R, Vazquez Kiesel R, Rausch T (2015) Imposed glutathione-mediated redox switch modulates the tobacco wound-induced protein kinase and salicylic acid-induced protein kinase activation state and impacts on defence against *Pseudomonas syringae*. J Exp Bot 66:1935–1950

May MJ, Parker JE, Daniels MJ, Leaver CJ, Cobbett CS (1996) An Arabidopsis mutant depleted in glutathione shows unaltered responses to fungal and bacterial pathogens. Mol Plant-Microbe Interact 9:349–356

Meyer AJ, Fricker MD (2000) Direct measurement of glutathione in epidermal cells of intact Arabidopsis roots by two-photon laser scanning microscopy. J Microsc 198:174–181

Meyer AJ, Brach T, Marty L, Kreye S, Rouhier N, Jacquot JP, Hell R (2007) Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. Plant J 52:973–986

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

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulates NPR1 function through redox changes. Cell 113:935–944

Mukaihara T, Hatanaka T, Nakano M, Oda K (2016) *Ralstonia solanacearum* type III effector RipAY is a glutathione-degrading enzyme that is activated by plant cytosolic thioredoxins and suppresses plant immunity. mBio 7(2):e00359-16

Müller M, Zellnig G, Urbanek A, Zechmann B (2005) Recent developments in methods intracellulary localizing glutathione within plant tissues and cells (a minireview). Phyton 45:45–55

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279

Ohkama-Ohtsu N, Radwan S, Peterson A, Zhao P, Badr AF, Xiang C, Oliver DJ (2007) Characterization of the extracellular gamma-glutamyltranspeptidases, GGT1 and GGT2, in Arabidopsis. Plant J 49(5):865–877

Parisy V, Poinssot B, Owsianowski L, Buchala A, Glazebrook J, Mauch F (2007) Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J 49:159–172

Porcel R, Barea JM, Ruiz-Lozano JM (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157:135–143

Schnaubelt D, Queval G, Dong Y, Diaz-Vivancos P, Makgopa ME, Howell G, De Simone A, Bai J, Hannah MA, Foyer CH (2015) Low glutathione regulates gene expression and the redox potentials of the nucleus and cytosol in *Arabidopsis thaliana*. Plant Cell Environ 38:266–279

Simon UK, Polanschütz LM, Koffler BE, Zechmann B (2013) High resolution imaging of temporal and spatial changes of subcellular ascorbate, glutathione and H_2O_2 distribution during *Botrytis cinerea* infection in Arabidopsis. PLoS ONE 8(6):e65811

Sklodowska M, Gajewka E, Kuźniak E, Wielanek M, Mikiciński A, Sobixzewski P (2011) Antioxidant profile and polyphenol oxidase activities in apple leaves after *Erwinia amylovora* infection and pretreatment with a benzothiadiazole-type resistance inducer (BTH). J Phytopathol 159:495–504

Song XS, Wang YJ, Mao WH, Shi K, Zhou YH (2009) Effect of cucumber mosaic virus infection on electron transport and antioxidant system in chloroplasts and mitochondria of cucumber and tomato leaves. Physiol Plant 135:246–257

Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R (2011) Respiratory burst oxidases: the engines of ROS signaling. Curr Opin Plant Biol 14:691–699

Terrer C, Vicca S, Hungate BA, Phillips RP, Prentice IC (2016) Mycorrhizal association as a primary control of the CO_2 fertilization effect. Science 353(6294):72–74

Tiedemann AV (1997) Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. Physiol Mol Plant Pathol 50:151–166

Tolin S, Arrigoni G, Trentin AR, Veljovic-Jovanovic S, Pivato M, Zechmann B, Masi A (2013) Biochemical and quantitative proteomics investigations in *Arabidopsis* ggt1 mutant leaves reveal a role for the gamma-glutamylcyclein plant's adaptation to environment. Proteomics 13:2031–2045

Torres MA, Dangl JL, Jones JDG (2002) Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci U S A 99:517–522

Vahisalu T, Puzõrjova I, Brosché M, Valk E, Lepiku M, Moldau H, Pechter P, Wang YS, Lindgren O, Salojärvi J, Loog M, Kangasjärvi J, Kollist H (2010) Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. Plant J 62(3):442–453

Vanacker H, Carver TL, Foyer CH (1998) Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol 117(3):1103–1114

Vanacker H, Carver TL, Foyer CH (2000) Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. Plant Physiol 123(4):1289–1300

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughen SC, Cobbett CS, Van Montagu M, Inzé D (2000) The *ROOT MERISTEMLESS1/CADMIUM SENSITIVE 2* gene

defines a glutathione dependent pathway involved in the initiation and maintenance of cell division during postembryonic root development. Plant Cell 12:97–110

Vianello A, Zancani M, Peresson C, Petrussa E, Casolo V, Krajňáková J, Patui S, Braidot E, Macrì F (2007) Plant mitochondrial pathway leading to programmed cell death. Physiol Plant 129:242–252

Wingate VPM, Lawton MA, Lamb CJ (1988) Glutathione causes a massive and selective induction of plant defense genes. Plant Physiol 87:206–210

Wojtaszek P (1997) Oxidative burst: an early plant response to pathogen infection. Biochem J 322(Pt 3):681–692

Zechmann B, Mauch F, Sticher L, Müller M (2008) Subcellular immunocytochemical analysis detects the highest concentrations of glutathione in mitochondria and not in plastids. J Exp Bot 59(14):4017–4027

Zechmann B, Müller M (2010) Subcellular compartmentation of glutathione in dicotyledonous plants. Protoplasma 246(1-4):15–24

Zechmann B, Zellnig G, Müller M (2005) Changes in the subcellular distribution of glutathione during virus infection in *Cucurbita pepo* (L.) Plant Biol 7:49–57

Zechmann B, Zellnig G, Müller M (2007a) Virus-induced changes in the subcellular distribution of glutathione precursors in *Cucurbita pepo* (L.) Plant Biol 9:427–434

Zechmann B, Zellnig G, Urbanek-Krajnc A, Müller M (2007b) Artificial elevation of glutathione affects symptom development in ZYMV-infected *Cucurbita pepo* L. plants. ArchVirol 157:747–762