Glutathione Reductase: A lucky turn to remediate plant's oxidative stress

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Novelty statement:

glutathione reductase is an essential enzyme in plants that contributes to cellular redox homeostasis. Its activity is vital for maintaining the balance between oxidized and reduced glutathione and is also responsible for the regeneration of reduced glutathione (GSH) from its oxidized form, glutathione disulfide (GSSG). The primary function of glutathione reductase is to maintain the cellular pool of reduced glutathione, which is an essential antioxidant molecule. The enzyme is found in different subcellular compartments, including the cytosol, chloroplasts, mitochondria, and peroxisomes, reflecting its diverse functions within the plant cell. The activity of glutathione reductase in plants can vary depending on various factors such as developmental stage, tissue type, and environmental conditions. The present review gives a comprehensive overview of the importance of this enzyme.

Abstract

Abiotic conditions have a significant negative impact on the growth, maintenance, and ultimately yield of the plant, which causes significant losses in terms of food crisis. The excessive production of hazardous reactive oxygen species (ROS), such as superoxide ion, hydrogen peroxide, and hydroxyl radicals, is the cause of oxidative stress, which is connected to basically all abiotic stressful situations. As a result of these reactions, the plants suffer cellular harm and molecular deterioration, which ultimately results in plant death. in plants under stressful conditions, a series of reactions (enzymatic and non-enzymatic) has started that enhance plant resistance to such conditions. A major enzyme of the antioxidant system is glutathione reductase (GR)because it is essential for maintaining the sulfhydryl (-SH) group. It maintains the GSH depletion through the glutathione-ascorbate pathway. Reduced glutathione also involves the removal of reactive oxidants which are necessary byproducts of metabolic activities so it is recognized as one of the most critical cellular antioxidants. On the other hand, glutathione reductase primarily keeps the GSH pool in its reduced form. Changes in glutathione redox status can be caused by a variety of biotic or abiotic stressors that impact the rate of ROS formation and detoxification

Keywords: Glutathione reductase, Glutathione, ROS, abiotic stress

Due to photosynthesis, plants serve as a main producer in the food chain and as a result, Consumers benefit. These beneficial but static creature is facing environmental stresses. Plants have developed a variety of defence mechanisms against it. Two types of factors biotic such as diseases, pathogens and abiotic such as intense light, high temperature, cold, water stress, and salt stress factors affect plant growth and metabolism (Maksymiec, 2007).

Salinity, heavy metals, water stress, high temperature, cold, and UV-B radiation are examples of abiotic stress factors that can similarly alter the structure of plants. Positive (such as tolerance) or negative (such as decreased photosynthetic activity, growth inhibition, accelerated senescence, or damage to the plant parts) impacts can be seen depending on the severity of the stress (Maksymiec, 2007; Mittler, 2006). These effects are typically caused by redox imbalances and excessive ROS production in the cells. ROS ($O^{2\cdot-}$, $OH^{\cdot}$, $HO^{2\cdot}$, H_2O_2) are normally present in various cell compartments (Karuppanapandian et al., 2008; Mafakheri et al., 2010; Mittler, 2002; Mittler et al., 2004; Torres et al., 2002; Vellosillo et al., 2010) but under stress and as a result of the loss of redox homeostasis, they may build up and cause damage to a variety of molecules, including DNA, proteins, fats, photosynthetic pigments, and others (Arora et al., 2002; Dat et al., 2000; Jaleel et al., 2009).

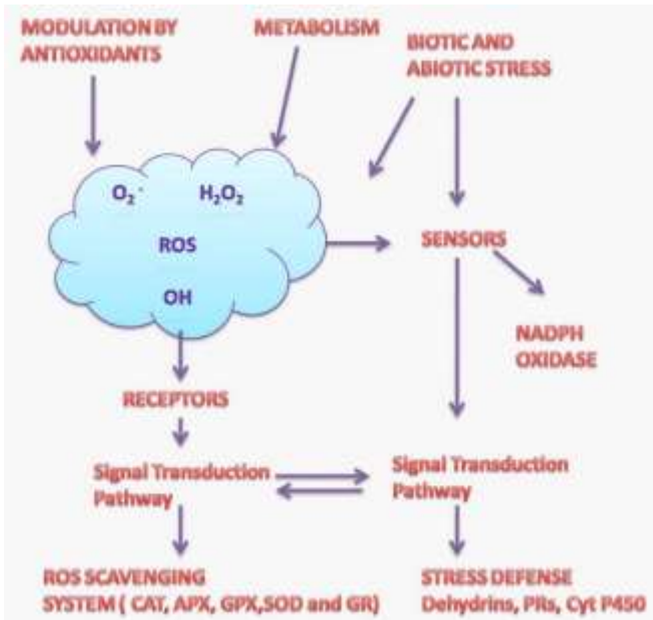

Figure 1. ROS scavenging by antioxidants in plants under biotic and abiotic stress.

It is believed that a vast gene network known as the "ROS gene network" controls ROS generation and scavenging, enabling this duality in function to exist in plants (Mittler et al., 2004). To generate the energy required for their developmental activities, plants need oxygen (O_2). Ground state oxygen is converted during typical cellular metabolism to water (H_2O) and reactive oxygen species, which include. $O^{2\cdot-}$, H_2O_2, $HO^{2\cdot}$, $OH^{\cdot}$ and $^1O_2$ (Halliwell, 2006; Mittler, 2002; Scandalios, 2005). It is assumed that 1- 2% of the O_2 absorbed by plants is diverted to the ROS formation in distinct intercellular sites (Blokhina et al., 2003)). ROS are produced from O_2 either through energy transfer or electron transfer processes. The reaction chain of ROS production initially requires an energy input, but the following phases are exothermic and spontaneous. Absorption of surplus energy by O_2 can also result in the production of $^1O_2$, which is a more reactive molecule than O_2 (Halliwell, 2006; Mittler, 2002). $^1O_2$ is formed in chloroplasts as a result of the photosensitization of chlorophyll (Chl) molecules under UV stress (Rao & Reddy, 2008).

$O^{2\cdot-}$ is produced by a single electron reduction of O_2 which is moderately reactive and transient ROS. As a result, $O2^{\cdot-}$ is unable to pass through biomembranes and easily dismutates into H_2O_2. Protonation in aqueous solutions converts $O^{2\cdot-}$ into $HO^{2\cdot}$. By removing hydrogen atoms from lipid hydroperoxides, $HO^{2\cdot}$ can pass across bio-membranes and start auto-oxidation of lipids (Halliwell & Gutteridge, 2015). H_2O_2 is a moderately reactive, long-lasting molecule that can permeate short distances away from its source. Enzymes can be inactivated by H_2O_2 by oxidizing their thiol groups. By oxidizing the thiol groups on enzymes, H_2O_2 can render them inactive. H_2O_2 might freely pass through membranes, allowing it to disperse harm and perhaps work as a secondary messenger in the plant's hormonal response to stress (Halliwell, 2006; Møller et al., 2007). This shows that H_2O_2 (ROS) is dual in its nature. H_2O_2 is one of the radicals that start lipid peroxidation(LP) since it can also produce $OH^{\cdot}$, the ROS family member that is highly reactive (Halliwell & Gutteridge, 2015; Lee et al., 2007).

Stressful situations have the potential to change the equilibrium between ROS generation and removal in plant cell organelles, even though reactive oxygen species are a byproduct of regular cellular metabolism (Apel & Hirt, 2004; Vellosillo et al., 2010). Superoxide radical ($O2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical ($HO2^{\cdot}$), hydrogen peroxide (H2O2), alkoxy radical ($RO^{\cdot}$) proxy radical ($ROO^{\cdot}$), singlet oxygen (1O2) and excited carbonyl (RO*) are among the reactive oxygen species that are detrimental to plants (Dismukes et al., 2001; Karuppanapandian et al., 2008; Manoharan et al., 2005; Vellosillo et al., 2010).

All biomolecules, severely harming biological components, Genome instability and abnormalities are targeted by ROS and usually lead to cell death and permanent metabolic losses (Karuppanapandian et al., 2011). As in (figure.2) severe oxidative stress can cause cells to go through fatal response pathways such as apoptosis, necrosis, and possibly other types of cell death pathways that can eventually result in apoptosis (Awasthi et al., 2015)

Additionally, ROS can serve as a supplementary messenger for controlling the number of biological and developmental processes as well as in pathogenic resistance i.e., the HR: hypersensitivity response in plants (Foyer & Noctor, 2005; Guan & Scandalios, 2000; Mittler et al., 2004; Pei et al., 2000).

It is not expected that $OH^{\cdot}$ radical itself act as a signaling molecule. However, the results of $OH^{\cdot}$ reactions can trigger signaling procedures. Cells effectively avoid $OH^{\cdot}$ by sequestrating the catalytic metals into metallochaperones (small proteins responsible for transporting metals) (Halliwell, 2006; Møller et al., 2007). Hydroxide ($OH^{\cdot}$)cano react with all biological compounds, including pigments, proteins, fats, and DNA, as well as nearly every cell element. Because these extremely reactive ROS cannot be scavenging by pscavenged, so their excessiventhesis leads to programmed cell death (PCD) (Karuppanapandian et al., 2011; Manoharan et al., 2005; Vranová et al., 2002).

The reactive oxygen species are removed by numerous antioxidant defence mechanisms in stable circumstances (Foyer & Noctor, 2005; Navrot et al., 2007). In plant cells, both the production of reactive oxygen species and their scavenging are controlled processes, and the plant's reaction is determined by the balance of oxidative and antioxidative capacity. Under a stress-free environment, the antioxidant defence system provides enough protection against ROS; but, under stress conditions, ROS generation overcomes plant scavenging capacity, which causes environmental stress (Apel & Hirt, 2004).

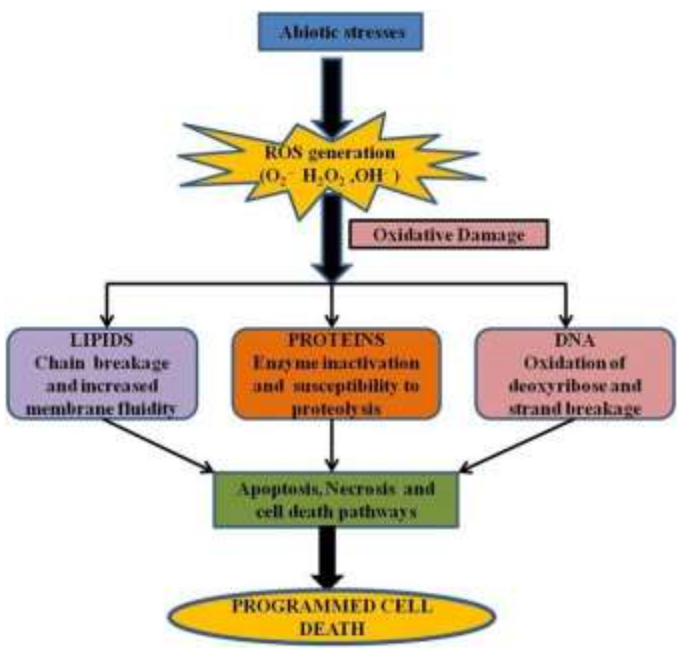

Figure 2. Reactive oxygen species (ROS) generation under abiotic stress (Awasthi et al., 2015)

Like all living organisms, Plants produce a variety of organic substances and follow strategies to regulate biological ROS production or the increased ROS levels under stressful conditions (Yousuf et al., 2012). These molecules, which are termed antioxidants, are present in almost all cellular organelles and function in a systematic manner (Gill & Tuteja, 2010). Antioxidants are crucial for the best optimal functioning of plant cells as they serve as the first protective barrier against damage from oxidants (Bartels & Sunkar, 2005; Gill & Tuteja, 2010; Miller et al., 2010; Rajput et al., 2016). Antioxidants have a role in the scavenging pathways of ROS as like the Halliwell-Asada (or ascorbate-glutathione) cycle in the chloroplast, the water-water cycle in the mitochondria, the peroxisomes, the apoplast, the cytosol and the cycle of peroxisomal glutathione peroxidase. Because of their inert nature, plants have developed a complex network of cellular antioxidants made up of both enzymatic and non-enzymatic components that are essential for defending against a range of stressors (Rajput et al., 2021).

The antioxidant defence mechanism prevents oxidants from harming other biological elements such as enzymes, nucleic acids, and unsaturated proteins and lipids. As a result, the scientific community has been showing a lot of interest in the defence mechanism of plants (Alscher et al., 1997; Dumont & Rivoal, 2019). Non-enzymatic components like free amino acids, α-tocopherols, alkaloids, flavonoids, carotenoids, phenolic compounds, ascorbic acid (AA), and glutathione (GSH). Enzymatic substances are ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), peroxidases (POX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferees (GST), and dehydroascorbate reductase (DHAR) (Figure.4) (Bhardwaj et al., 2021; Maximiano & Franco, 2022; Rajput et al., 2015). Glutathione reductase includes in the second line of defence mechanism. The term "scavenging antioxidants" is frequently used to describe this class of antioxidants. They disrupt chain propagation reactions and scavenge active radicals to prevent chain start. By giving free radicals an electron, they can neutralize or scavenge them in the process to transform into new but less harmful free radicals. By using other antioxidants of this class, these "new radicals" are quickly neutralized and rendered completely harmless (Ighodaro & Akinloye, 2018)

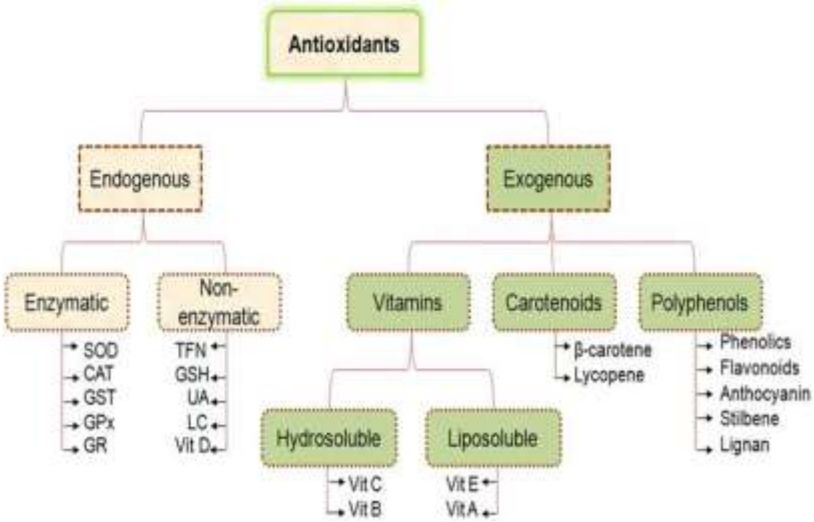

Figure 3. Schematic representation of general antioxidants (Bhardwaj et al., 2021)

Antioxidant enzymes perform their functions in plants under stress conditions through a cascade manner. SOD dismutates oxygen (O^{-1}) during stressful settings into O^2 and H_2O_2, CAT converts the H_2O_2 into water and molecular oxygen (O_2), and POX works in the extracellular space to scavenge H_2O_2. Plant GPX catalyses the conversion of H_2O_2 and HO^{-2} to water and lipid alcohols, respectively, by using thioredoxin as an electron donor. The conversion of oxidised glutathione (GSSG; dimeric) to reduced glutathione (monomeric form) is catalyzed by glutathione reductase (GR) (Rajput et al., 2021). At this stage, glutathione reductase plays an important role by maintaining the GSH/GSSG ratio and provides GSH to glutathione peroxidase (GPX). The GSH pool (GSH/GSSG ratio) and reducing environment in the cell are maintained by GR, which is important for the active operation of proteins (Creissen et al., 1994; Edwards et al., 1990).

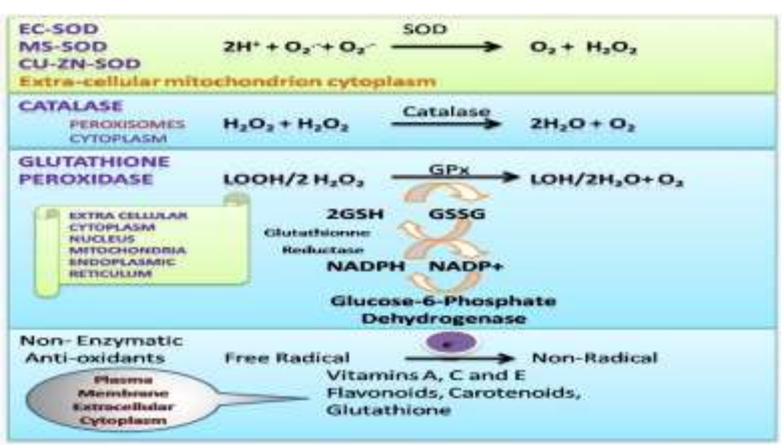

Figure 4. Removal of ROS species by the activation of the antioxidant cascade.

These enzymes also play a crucial role in the development and germination of plants by regulating cellular and subcellular processes like mitosis, morphogenesis, ageing, and apoptosis. They also serve to protect various cell constituents from harm. Additionally, they play a role in various mechanisms, including cell differentiation, growth and division, regulation of senescence and sulphate transport, detoxification of xenobiotic, metabolites complexation, control of enzymes metabolic activity, synthesis of proteins and nucleotides, phytochelatins, and genes functioning that are responsive to stress (Abdel Latif, 2011; Liu et al., 2014; Maximiano & Franco, 2022; Mullineaux & Rausch, 2005; Sairam et al., 2011).

Glutathione is an oxide-reductase where NADPH is used as a cofactor that conducts both the oxidation and reduction processes. Because glutathione reductase is found in chloroplasts, cytosol, and mitochondria, it has been demonstrated that in the photosynthetic tissue of plants, the isoform of chloroplast is responsible for more than 80% of its activity (Ashraf, 2009; Edwards et al., 1990; Romero-Puertas et al., 2006; Stevens et al., 2000). Glutathione reductase generates a homodimer that is linked to Flavin adenine dinucleotide (FAD). The majority of glutathione reductases in plants are homodimers with one FAD per monomer and molecular weights between 100 and 150 kDa. When thiols are not present, GR generally forms tetramers and larger forms. Generally, glutathione reductase forms tetramers and bigger forms in the absence of thiols. Under cellular circumstances, the enzyme's product GSH keeps the enzyme in its dimeric state despite the catalytic activity of these larger forms (Yousuf et al., 2012). Instead of reagents or products, both pH and temperature determine the composition of the higher form, thus GR dimers can be converted into tetramers or either greater constituting states. GR catalytic activity is regulated by this mechanism (Rao & Reddy, 2008). Glutathione reductase (GR) is a part of the antioxidant defence mechanisms of plants because it participates in both enzymatic and non-enzymatic oxidation reduction activities within the cell.

Utilizing NADPH as a cofactor, glutathione reductase changes oxidised glutathione (GSSG) into reduced glutathione (GSH) (Edwards et al., 1990; Romero-Puertas et al., 2006; Stevens et al., 2000). One mole of NADPH is needed to turn one mole of GSSG into one mole of GSH. The catalytic process consists of two steps: first, NADPH is used to reduce the flavin group, and an oxidative disulfide bond is reduced to produce a cysteine and a thiolate anion after the flavin is first oxidised. Thiol disulfide interchange reactions are used to minimize GSSG in the second stage (Ghisla & Massey, 1989). If GSSG does not reoxidize the reduced enzyme, there can be a reversible inactivation. GR functions in a ping-pong manner during the reduction of GSSG to GSH, NADPH binds to FAD, transfers a hydride to it, and then dissociates before glutathione can bind (Rao & Reddy, 2008). Furthermore, GR controls the proportion of reduced to oxidized glutathione and provides GSH with glutathione peroxidase and dehydroascorbate reductase. NADPH, H^+ provides the reduction power for GR, but GR dissipates this power, which raises the $NADP^+$/NADPH, H^+ ratio (Hasanuzzaman et al., 2012). In cells where GSH builds up, plants become more tolerant to stress and their glutathione reductase activity rises. In terms of GR catalytic processes, it is important to note that the amount of available substrate heavily influences the GR redox interconversion, in contrast to the reduced GR form, the oxidised GR form exhibits better stability because it can withstand divalent metal ions (Rao & Reddy, 2008). GR, which also significantly contributes to reactive oxygen species detoxification and GSH regeneration, helps in the tolerance to environmental stress conditions in plants (Hasanuzzaman et al., 2012; Mirza et al., 2010). In addition to providing a resistance to stress, enhanced GR activity has the capability of changing the redox condition of the essential transport system of electron constituents. The maintenance of oxidized to reduced glutathione ratio in the cells of the plants and GSH recycling play a significant role in GR's ability to resist stress (Pang & Wang, 2010; Rao & Reddy, 2008).

Many physiological processes depend on the regulation of a large GSH/GSSG ratio by glutathione reductase (GR), and a decline in this ratio could be used as an indication of osmotic stress. Additionally, the reduced to-oxidized glutathione ratio is essential for controlling several procedures related to plant growth and signalling pathways. GSH content and subcellular distribution, in addition to redox status, are important elements in the regulation of redox signalling and homoeostasis (Sabetta et al., 2017). Plant homeostasis is disturbed due to ROS production in excess under stressful climate change situations.

Glutathione (GSH) is a common thiol tripeptide with a low molecular weight and antioxidant molecule that contains Sulphur which is essential for managing plant growth, productivity and tolerance to stress both biotically and abiotically. Under stress conditions, glutathione readily develops in plant cells. Oxidized (GSSG) and reduced (GSH) are the two types of glutathione. under ideal circumstances, glutathione is mostly found in its reduced form (GSH), which has a free thiol group. Disulfide glutathione(GSSG) is created when two molecules of reduced glutathione (GSH) form a disulfide bond(Sabetta et al., 2017). As reduced glutathione can remove reactive oxidants which are necessary byproducts of metabolic activities so it is recognized as one of the most critical cellular antioxidants. As a result, in both environmental and biological stresses in plants, GSH play an important role, where its function is to eliminate ROS and hence reduce the amount of oxidative damage (Foyer & Noctor, 2005). Moreover, GSH is involved in the amplification of ROS signals in plants via interactions with stress hormones (Han et al., 2013).

The abundant metabolite GSH in plants is known to have a role in signal transduction and directly scavenges $OH^{\bullet}$ and $1O^2$. It may also protect enzyme thiol groups (Foyer & Noctor, 2005). GSSG accumulation in plants frequently occurs under stressful conditions and is associated with a rise in the total glutathione pool, which appears to be mostly brought on by GSSG accumulation (Mhamdi et al., 2010; Smith et al., 1984; Willekens et al., 1997). under stress conditions, oxidative activities overcome glutathione reductases (GR) ability to reduce glutathione. Increased glutathione disulfide (GSSG) concentration activates -glutamylcysteine synthetase (-ECS), resulting in a rise in the total glutathione pool (Figure.1). Moreover, the cytoplasm and nucleus, which are delicate subcellular regions, are protected from excessive GSSG buildup by the compartmentalization of GSSG in vacuoles. In response to oxidative stress, GSH biosynthesis can rise because thiol and GSH formation are triggered at the translational and post-synthetic levels in response to oxidative stress, GSH biosynthesis may increase (Gromes et al., 2008; Hicks et al., 2007; Queval et al., 2009). Many GSH functions are controlled by these reversible redox processes. GSH synthesis depends on glutamylcysteine synthetase and glutathione synthetase both of these are dependent on ATP, which are respectively encoded by the nucleus genes having GSH1 and GSH2. Plastids are the first stage in the synthesis of reduced glutathione. The second step, on the other hand, can occur in either the plastids or the cytosol (Sabetta et al., 2017).

The reduced to oxidized glutathione ratio in different cell constituents can be used as a reliable indicator of oxidative stress and this is the reason for increase in oxidized glutathione in some portions, such as the vacuole (Noctor et al., 2013). In plants under normal conditions, GSH and GR play the most important function in the H_2O_2 scavenging pathway in chloroplasts (Halliwell & Foyer, 1978). The high reduction status of the cellular pool of GSH is an essential factor. Glutathione reductase primarily keeps the GSH pool in its reduced form, the activities of which are dependent on the primary electron carrier and NADPH (Edwards et al., 1990; Halliwell & Foyer, 1978). On the other hand, tolerance level to any kind of stress is increased due to the presence of glutathione reductase and reduced glutathione. The reduced to oxidized glutathione proportion as well as the overall GSH level have an impact on the redox capacity of glutathione and on balancing of interaction between targets for sensitive proteins and oxidative signals (Meyer et al., 2007). Due to the high sulfhydryl (SH) concentration of GSH, it guards against cellular component damage by scavenging free radicals. As a result, it acts as a sensitive indicator of cell growth and function (Tanwir et al., 2021). Different circumstances where oxidant generation takes place can cause glutathione to deviate from its highly reduced state that impacts the rate of ROS formation and detoxification (Gómez et al., 2004; Gupta et al., 1991; Vanacker et al., 2000).

GSH functions as a scavenger, limiting severe cellular oxidation. GSH can also generate mixed disulfides when it reacts with various thiols. Many GSH functions are controlled by these reversible redox processes. Glutathione is involved in cell cycle regulation, redox signalling, enzymatic activity and also sense changes in cellular reduced and oxidized glutathione levels. As GSH's antioxidant and signaling roles are linked so it needs an enzyme like glutathione reductase (GR) (Sabetta et al., 2017). Glutathione reductase not only regulates the subcellular redox environment but is also involve in plant reproduction which is crucial for cells to survive (Trivedi et al., 2013).

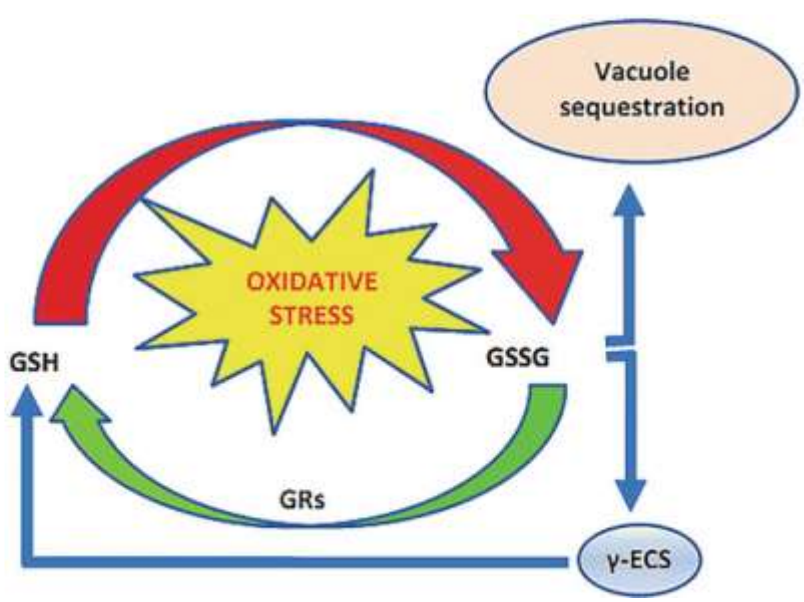

Figure 5. Glutathione content and redox state changes as a result of oxidative stress (Sabetta et al., 2017).

The glutathione redox potential, which regulates the interactions between oxidant indicators and sensitive protein receptors, can be influenced not only by variations in the reduced-to-oxidized glutathione ratio but also by variations in the overall reduced glutathione content (Meyer et al., 2007). Under oxidative stress condition in plants if the glutathione redox pool is not maintained properly then plants undergoes oxidative damage which leads to the death of the plant. Therefore, regulation of the GSH pool and tolerance to oxidative stress for plants is impossible without glutathione reductase (GR).

Reference

Abdel Latef, A. A. H. (2011). Ameliorative effect of calcium chloride on growth, antioxidant enzymes, protein patterns and some metabolic activities of canola (Brassica napus L.) under seawater stress. *Journal of plant Nutrition*, *34*(9), 1303-1320.

Alscher, R. G., Donahue, J. L., & Cramer, C. L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum*, *100*(2), 224-233.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signaling transduction. *Annual review of plant biology*, *55*, 373.

Arora, A., Sairam, R., & Srivastava, G. (2002). Oxidative stress and antioxidative system in plants. *Current science*, 1227-1238.

Ashraf, M. (2009). The biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology advances*, *27*(1), 84-93.

Awasthi, R., Bhandari, K., & Nayyar, H. (2015). Temperature stress and redox homeostasis in crops. *Frontiers in Environmental Science*, *3*, 11.

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical reviews in plant sciences*, *24*(1), 23-58.

Bhardwaj, J. K., Panchal, H., & Saraf, P. (2021). Ameliorating effects of natural antioxidant compounds on female infertility: a review. *Reproductive sciences*, *28*(5), 1227-1256.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany*, *91*(2), 179-194.

Creissen, G., Broadbent, P., Kular, B., Reynolds, H., Wellburn, A., & Mullineaux, P. (1994). Manipulation of glutathione reductase in transgenic plants: implications for plants' responses to environmental stress. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, *102*, 167-175.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., & Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences CMLS*, *57*(5), 779-795.

Dismukes, G., Klimov, V., Baranov, S., Kozlov, Y. N., DasGupta, J., & Tyryshkin, A. (2001). The origin of atmospheric oxygen on Earth: the innovation of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences*, *98*(5), 2170-2175.

Dumont, S., & Rivoal, J. (2019). Consequences of oxidative stress on plant glycolytic and respiratory metabolism. *Frontiers in Plant Science*, *10*, 166.

Edwards, E. A., Rawsthorne, S., & Mullineaux, P. M. (1990). Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (Pisum sativum L.). *Planta*, *180*(2), 278-284.

Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, *28*(8), 1056-1071.

Ghisla, S., & Massey, V. (1989). Mechanisms of flavoprotein-catalyzed reactions. *EJB Reviews 1989*, 29-45.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909-930.

Gómez, L. D., Vanacker, H., Buchner, P., Noctor, G., & Foyer, C. H. (2004). Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. *Plant Physiology*, *134*(4), 1662-1671.

Gromes, R., Hothorn, M., Lenherr, E. D., Rybin, V., Scheffzek, K., & Rausch, T. (2008). The redox switch of γ-glutamylcysteine ligase via a reversible monomer–dimer transition is a mechanism unique to plants. *The Plant Journal*, *54*(6), 1063-1075.

Guan, L. M., & Scandalios, J. G. (2000). Hydrogen peroxide-mediated catalase gene expression in response to wounding. *Free Radical Biology and Medicine*, *28*(8), 1182-1190.

Gupta, A. S., Alscher, R. G., & McCune, D. (1991). Response of photosynthesis and cellular antioxidants to ozone in Populus leaves. *Plant Physiology*, *96*(2), 650-655.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry*, *97*(6), 1634-1658.

Halliwell, B., & Foyer, C. (1978). Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography. *Planta*, *139*(1), 9-17.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Han, Y., Mhamdi, A., Chaouch, S., & Noctor, G. (2013). Regulation of basal and oxidative stress-triggered jasmonic acid-related gene expression by glutathione. *Plant, Cell & Environment*, *36*(6), 1135-1146.

Hasanuzzaman, M., Hossain, M. A., Silva, J. A., & Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defence is a key factor. In *Crop stress and its management: perspectives and strategies* (pp. 261-315). Springer.

Hicks, L. M., Cahoon, R. E., Bonner, E. R., Rivard, R. S., Sheffield, J., & Jez, J. M. (2007). Thiol-based regulation of redox-active glutamate-cysteine ligase from Arabidopsis thaliana. *The Plant Cell*, *19*(8), 2653-2661.

Ighodaro, O., & Akinloye, O. (2018). First-line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, *54*(4), 287-293.

Jaleel, C. A., Riadh, K., Gopi, R., Manivannan, P., Ines, J., Al-Juburi, H. J., Chang-Xing, Z., Hong-Bo, S., & Panneerselvam, R. (2009). Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. *Acta Physiologiae Plantarum*, *31*(3), 427-436.

Karuppanapandian, T., Saranyadevi, A., Jeyalakshmi, K., & Manoharan, K. (2008). Mechanism, control and regulation of leaf senescence in plants. *J Plant Biol*, *35*, 141-155.

Karuppanapandian, T., Wang, H. W., Prabakaran, N., Jeyalakshmi, K., Kwon, M., Manoharan, K., & Kim, W. (2011). 2, 4-dichlorophenoxyacetic acid-induced leaf senescence in mung bean (Vigna radiata L. Wilczek) and senescence inhibition by co-treatment with silver nanoparticles. *Plant Physiology and Biochemistry*, *49*(2), 168-177.

Lee, S.-H., Ahsan, N., Lee, K.-W., Kim, D.-H., Lee, D.-G., Kwak, S.-S., Kwon, S.-Y., Kim, T.-H., & Lee, B.-H. (2007). Simultaneous overexpression of both CuZn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. *Journal of plant physiology*, *164*(12), 1626-1638.

Liu, N., Lin, Z., Guan, L., Gaughan, G., & Lin, G. (2014). Antioxidant enzymes regulate reactive oxygen species during pod elongation in Pisum sativum and Brassica chinensis. *PloS one*, *9*(2), e87588.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P., & Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian journal of crop science*, *4*(8), 580-585.

Maksymiec, W. (2007). Signaling responses in plants to heavy metal stress. *Acta Physiologiae Plantarum*, *29*(3), 177-187.

Manoharan, K., Karuppanapandian, T., Sinha, P. B., & Prasad, R. (2005). Membrane degradation, accumulation of phosphatidic acid, stimulation of catalase activity and nuclear DNA fragmentation during 2, 4-D-induced leaf senescence in mustard. *Journal of Plant Biology*, *48*(4), 394-403.

Maximiano, M. R., & Franco, O. L. (2022). CRISPR/Cas: The New Frontier in Plant Improvement. *ACS Agricultural Science & Technology*, *2*(2), 202-214.

Meyer, A. J., Brach, T., Marty, L., Kreye, S., Rouhier, N., Jacquot, J. P., & Hell, R. (2007). Redox-sensitive GFP in Arabidopsis thaliana is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. *The Plant Journal*, *52*(5), 973-986.

Mhamdi, A., Hager, J., Chaouch, S., Queval, G., Han, Y., Taconnat, L., Saindrenan, P., Gouia, H., Issakidis-Bourguet, E., & Renou, J.-P. (2010). Arabidopsis GLUTATHIONE REDUCTASE1 plays a crucial role in leaf responses to intracellular hydrogen peroxide and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. *Plant Physiology*, *153*(3), 1144-1160.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment*, *33*(4), 453-467.

Mirza, H., Hossain, M., & Fujita, M. (2010). Physiological and biochemical mechanisms of nitric oxide induced abiotic stress tolerance in plants. *American Journal of Plant Physiology*, *5*(6), 295-324.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, *7*(9), 405-410.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in plant science*, *11*(1), 15-19.

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in plant science*, *9*(10), 490-498.

Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.*, *58*, 459-481.

Mullineaux, P. M., & Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. *Photosynthesis research*, *86*(3), 459-474.

Navrot, N., Rouhier, N., Gelhaye, E., & Jacquot, J. P. (2007). Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiologia Plantarum*, *129*(1), 185-195.

Noctor, G., Mhamdi, A., Queval, G., & Foyer, C. H. (2013). Regulating the redox gatekeeper: vacuolar sequestration puts glutathione disulfide in its place. *Plant Physiology*, *163*(2), 665-671.

Pang, C.-H., & Wang, B.-S. (2010). Role of ascorbate peroxidase and glutathione reductase in ascorbate–glutathione cycle and stress tolerance in plants. *Ascorbate-glutathione pathway and stress tolerance in plants*, 91-113.

Pei, Z.-M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G. J., Grill, E., & Schroeder, J. I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature*, *406*(6797), 731-734.

Queval, G., Thominet, D., Vanacker, H., Miginiac-Maslow, M., Gakière, B., & Noctor, G. (2009). H2O2-activated up-regulation of glutathione in Arabidopsis involves induction of genes encoding enzymes involved in cysteine synthesis in the chloroplast. *Molecular Plant*, *2*(2), 344-356.

Rajput, V., Chen, Y., & Ayup, M. (2015). Effects of high salinity on physiological and anatomical indices in the early stages of Populus euphratica growth. *Russian Journal of Plant Physiology*, *62*(2), 229-236.

Rajput, V. D., Minkina, T., Yaning, C., Sushkova, S., Chapligin, V. A., & Mandzhieva, S. (2016). A review on salinity adaptation mechanism and characteristics of Populus euphratica, a boon for arid ecosystems. *Acta Ecológica Sinica*, *36*(6), 497-503.

Rajput, V. D., Singh, R. K., Verma, K. K., Sharma, L., Quiroz-Figueroa, F. R., Meena, M., Gour, V. S., Minkina, T., Sushkova, S., & Mandzhieva, S. (2021). Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology*, *10*(4), 267.

Rao, A., & Reddy, A. R. (2008). Glutathione reductase: a putative redox regulatory system in plant cells. In *Sulfur assimilation and abiotic stress in plants* (pp. 111-147). Springer.

Romero-Puertas, M. C., Corpas, F. J., Sandalio, L. M., Leterrier, M., Rodríguez-Serrano, M., Del Río, L. A., & Palma, J. M. (2006). Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. *New phytologist*, *170*(1), 43-52.

Sabetta, W., Paradiso, A., Paciolla, C., & Pinto, M. C. d. (2017). Chemistry, biosynthesis, and antioxidative function of glutathione in plants. In *Glutathione in plant growth, development, and stress tolerance* (pp. 1-27). Springer.

Sairam, R. K., Vasanthan, B., & Arora, A. (2011). Calcium regulates Gladiolus flower senescence by influencing antioxidative enzymes activity. *Acta Physiologiae Plantarum*, *33*(5), 1897-1904.

Scandalios, J. (2005). Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazilian journal of medical and biological research*, *38*, 995-1014.

Smith, I., Kendall, A., Keys, A., Turner, J., & Lea, P. (1984). Increased levels of glutathione in a catalase-deficient mutant of barley (Hordeum vulgare L.). *Plant Science Letters*, *37*(1-2), 29-33.

Stevens, R. G., Creissen, G. P., & Mullineaux, P. M. (2000). Characterisation of pea cytosolic glutathione reductase expressed in transgenic tobacco. *Planta*, *211*(4), 537-545.

Tanwir, K., Javed, M. T., Shahid, M., Akram, M. S., & Ali, Q. (2021). Antioxidant defense systems in bioremediation of organic pollutants. In *Handbook of Bioremediation* (pp. 505-521). Elsevier.

Torres, M. A., Dangl, J. L., & Jones, J. D. (2002). Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proceedings of the National Academy of Sciences*, *99*(1), 517-522.

Trivedi, D. K., Gill, S. S., Yadav, S., & Tuteja, N. (2013). Genome-wide analysis of glutathione reductase (GR) genes from rice and Arabidopsis. *Plant signaling & behavior*, *8*(2), e23021.

Vanacker, H., Carver, T. L., & Foyer, C. H. (2000). Early H2O2 accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. *Plant Physiology*, *123*(4), 1289-1300.

Vellosillo, T., Vicente, J., Kulasekaran, S., Hamberg, M., & Castresana, C. (2010). Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. *Plant Physiology*, *154*(2), 444-448.

Vranová, E., Inzé, D., & Van Breusegem, F. (2002). Signal transduction during oxidative stress. *Journal of experimental botany*, *53*(372), 1227-1236.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D., & Van Camp, W. (1997). Catalase is a sink for H2O2 and is indispensable for stress defence in C3 plants. *The EMBO journal*, *16*(16), 4806-4816.

Yousuf, P. Y., Hakeem, K. U. R., Chandna, R., & Ahmad, P. (2012). Role of glutathione reductase in plant abiotic stress. In *Abiotic stress responses in plants* (pp. 149-158). Springer.