Chapter 16
Glyoxalase Pathway and Drought Stress Tolerance in Plants

Mohammad Rokebul Hasan, Ajit Ghosh, Charanpreet Kaur, Ashwani Pareek, and Sneh Lata Singla-Pareek

16.1 Introduction

Crop plants are constantly exposed to a broad range of environmental stresses. Of which, drought is the most devastating one that barriers agroecosystem productivity (Lambers et al. 2008; Farooq et al. 2011). It adversely affects plant metabolism, growth, development, and survival, and thus, is a constraint for plant productivity worldwide (Ahuja et al. 2010; Hasanuzzaman and Fujita 2011; Hasanuzzaman et al. 2012). In addition, climate prediction models indicate more severe and frequent droughts in future, thereby drastically impacting global crop production (IPCC 2008; Manavalan et al. 2009). Being sessile and sensitive organisms, plants have evolved a wide range of molecular programs to readily sense, respond, and cope with changing environments in order to protect themselves from these unforeseen

M.R. Hasan • S.L. Singla-Pareek (✉)
Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi 110 067, India
e-mail: sneh@icgeb.res.in; snehpareek@gmail.com

A. Ghosh
Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi 110 067, India

Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh

C. Kaur
Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi 110 067, India

Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

A. Pareek
Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

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variations (Ahuja et al. 2010). Response mechanisms to drought stress involve changes at morphological, physiological, and biochemical levels (Zhu 2001). Re-programming in gene expression occurs under stress conditions causing alterations in plant biochemical, transcriptomic, and proteomic machinery (Cohen et al. 2010; Ahuja et al. 2010). In such situations, tolerance to stress can be achieved through modulation of several genes or by organizing the action of different genes from various cellular biochemical pathways (Sasaki-Sekimoto et al. 2005).

As a common phenomenon, stress leads to excessive production of certain deleterious chemical entities such as reactive oxygen species (ROS) and methylglyoxal (MG) in plants (Yadav et al. 2007; Hossain and Fujita 2010; Hossain et al. 2011a). MG is a ubiquitous metabolite generated as a concomitant of intracellular metabolism and, therefore, exists in all cells during normal physiological growth and development conditions and accumulates to higher concentrations under many environmental stresses (Yadav et al. 2008). It is responsible for oxidative stress either through increased production of ROS or by forming advanced glycation end products (AGEs) with macromolecules (Kalapos 2008; Sousa Silva et al. 2013). As it accumulates at higher concentrations under stress conditions, plants have evolved several detoxification mechanisms to combat the so-called dicarbonyl and oxidative stress caused by MG. The primary route for MG detoxification is the thiol-dependent glyoxalase system which catalyzes the conversion of cytotoxic MG (2-oxopropanal) to D-lactic acid via S-D-lactoylglutathione (SLG) (Fig. 16.1). The presence of the

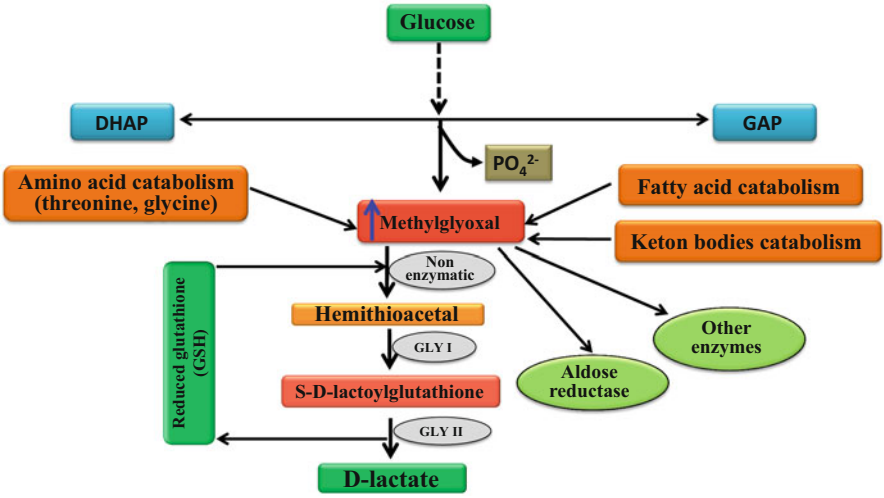

Fig. 16.1 Different routes of methylglyoxal formation and detoxification system in plants. Nonenzymatic generation of MG through β-elimination of phosphate group from enediolate phosphate intermediate is the central route of MG synthesis in plants. Besides, metabolism of amino acids, fatty acids, and ketone bodies contribute to MG formation; first enzyme GLY I converts hemithioacetal formed from spontaneous combination of MG and GSH into S-D-lactoylglutathione which is then converted to D-lactate by GLY II, regenerating GSH in the system. *DHAP* dihydroxyacetone phosphate, *GAP* D-glyceraldehyde-3-phosphate

glyoxalase pathway has been reported in several plant species and involves two enzymes, GLY I and GLY II, which have been purified as well as physiologically and biochemically characterized and functionally validated from various plant species (Yadav et al. 2007; Hoque et al. 2007; Hasanuzzaman and Fujita 2011; Hossain et al. 2014). The efficient role of this pathway in stress management has been extensively studied in various living organisms, including prokaryotes to eukaryotes, and has been shown to be associated with abiotic stress adaptation (Kaur et al. 2014a). Here, we discuss basic molecular programs suggested to confer tolerance to drought stress alongside their envisaged approaches. Special emphasis will be given on molecular mechanisms of glyoxalase pathway mediated drought stress tolerance in plants.

16.2 Effects of Drought on Plant Health

Drought is harmful for the plant growth and development with varying effects based on the severity of the stress. The plants also display a variety of responses on exposure to drought conditions causing alterations at both morphological and molecular levels (Farooq et al. 2009). Drought condition in plants results in alterations in relative water content, water and nutrient relations, photosynthesis, assimilate partitioning and respiration thereby, limiting economic yield (Farooq et al. 2009). Siddique et al. (2001) reported that the relative water content, transpiration rate of wheat and rice under drought stress was lower than control ones. Nutrient contents such as P and PO_4^{3-} in the plant tissue decreased significantly under drought conditions, because of lowered PO_4^{3-} mobility as a result of lower water availability (Peuke and Rennenberg 2004). Drought negatively affects plant photosynthetic efficiency caused by a reduction in leaf expansion, hampered photosynthetic machinery, and early leaf senescence (Wahid and Rasul 2005). The metabolism of carbohydrate, concentration of sucrose in leaves and their export rate decreased due to an increase in the acid invertase activity caused by drought stress (Kim et al. 2000). Liu and Li (2005) observed that the biomass of shoot and root, photosynthesis, and respiration rate of root reduced sharply in wheat exposed to severe drought conditions. Drought-induced yield reduction has been reported in pigeon pea also where a 40–55 % decrease in seed yield was observed at the flowering stage (Nam et al. 2001).

Environmental factors activate a variety of plant responses to drought stress, from altered gene expression and cellular metabolism to adjustment in proper growth and development, thus enabling them to survive under such conditions (Yamaguchi-Shinozaki and Shinozaki 2006; Rampino et al. 2006; Perera et al. 2008; Oh et al. 2009; Wilson et al. 2009). Under drought conditions, gene expression related to various processes such as signaling which includes transcription factors (like NAC family genes, basic leucine zippers, MYB-type transcription factors, zinc fingers, and ethylene-responsive factors) and protein kinases (like calcium-dependent protein kinase and CBL-interacting protein kinase); osmolyte biosynthesis (e.g., trehalose biosynthesis); accumulation of antioxidants (like Mn-superoxide

Table 16.1 Loss in plant yield due to drought stress in some important field crops

Sl No.	Crop	Growth stage	Yield reduction (%)	References
1	Rice	Reproductive (severe stress)	48–94	Lafitte et al. (2007)
2	Rice	Grain filling (severe stress)	60	Basnayake et al. (2006)
3	Wheat	Stem elongation + anthesis	22	Akram et al. (2011)
4	Barley	Seed filling	49–57	Samarah (2005)
5	Maize	Grain filling	79–81	Monneveux et al. (2006)
6	Sunflower	Reproductive	60	Mazahery-Laghab et al. (2003)
7	Soybean	Reproductive	46–71	Samarah et al. (2006)
8	Chickpea	Reproductive	45–69	Nayyar et al. (2006)

dismutase); and several other processes are known to be affected (Sahoo et al. 2013). It has been reported that the severity as well as duration of the drought stress is determinate for economic yield reduction in many commercial field crop species (Table 16.1). In order to survive under stressful conditions plants must upregulate MG and ROS detoxification processes to avoid cellular damage and also to maintain steady state in different plant physiological processes. In this article we shall discuss the effect of drought stress at biochemical and molecular levels only.

16.3 Methylglyoxal Synthesis, Toxicity, and Accumulation Under Drought Conditions

MG is unavoidably produced during metabolism even under normal physiological conditions (Yadav et al. 2005; Hossain et al. 2009). The generation rate of MG varies depending upon the organism, tissue, cell, and physiological conditions (Yadav et al. 2005) and is formed via different nonenzymatic and enzymatic pathways (Richard 1993). In plants, spontaneous synthesis of MG by nonenzymatic mechanisms is considered to be the central route for its generation under normal and stress circumstances (Fig. 16.2). The nonenzymatic formation of MG occurs via removal of phosphoryl group through β-elimination from 1,2-enediolate of triose sugars, dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GAP), during glycolysis (Phillips and Thornalley 1993; Richard 1993). Under stress, in order to maintain metabolic homeostasis, the glycolysis increases resulting in disproportion in the pathway. As a result, excessive MG is inevitably produced as a byproduct of glycolysis during such conditions. Apart from glycolysis, several other sources for MG generation have also been reported and include oxidation of aminoacetone (Lyles and Chalmers 1992), ketone bodies (Aleksandrovskii 1992), and acetone (Casazza et al. 1984; Koop and Casazza 1985) (Fig. 16.1).

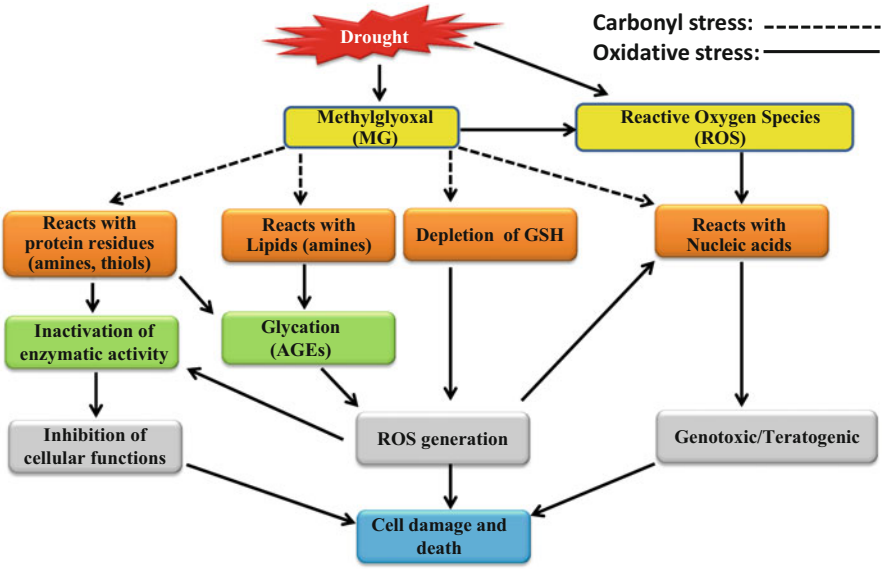

Fig. 16.2 Correlation between MG and ROS generation and their effects on cellular functions during drought stress in plants. MG exhibits direct inhibitory effects on proteins, lipids, and nucleic acids, resulting in carbonyl stress. Generation of ROS and depletion of glutathione is an indirect effect, causing cell damage or death and has been referred to as oxidative stress

Besides these, Maillard (Thornalley et al. 1999) and lipoperoxidation (Esterbauer et al. 1982) reactions also contribute to nonenzymatic sources of MG.

Excessive MG is toxic to the cell inhibiting cell proliferation (Ray et al. 1994). It can easily react with amine groups of proteins, nucleic acids, and lipids in an irreversible manner and form methylglyoxal-derived Advance Glycation End Products (MAGE). MG forms hydroimidazolone derivate (three related structural isomers; MG-H1, MG-H2 and MG-H3), argpyrimidine and tetrahydropyrimidine (THP) with arginine residues (Gomes et al. 2006) and also with lysine residues forming CEL [Nε-(carboxyethyl)lysine] and MOLDs (methylglyoxal–lysine dimers) (Gomes et al. 2006), and upon reaction with nucleic acids it generates MGdG {3-(2-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-b] purine-9(8)-one} and CEdG [*N*2-(1-carboxyethyl)-deoxyguanosine] adducts (Thornalley 2003a). In addition, amine-containing basic phospholipids (phosphatidylethanolamine and phosphatidylserine) react with MG and form lipid linked AGEs (carboxymethylethanolamine) (Brown et al. 2005). Furthermore, MG has also been shown to induce ROS formation and apoptosis by activation of signal-regulating kinase (ASK1) (Du et al. 2001). The toxicity of MG is also evident from its ability to cause increased sister chromatin exchange, endoreduplication, DNA strand breaks as well as inducing point mutations (Chaplen 1998). Moreover, it is associated with inhibition of normal growth and development (Hoque et al. 2012c) and results in a number of diverse detrimental effects including the formation of

advanced glycation end products (AGEs) and influencing the antioxidant defense system (Wu and Juurlink 2002; Hoque et al. 2010). MG levels rise to toxic concentrations in plants on exposure to drought stress. In rice, MG concentration at physiological conditions is about 27.5 ± 1.2 and 62.3 ± 3.2 μmol/g fresh weight in root and shoot, respectively, which increase two- to sixfold in response to drought (Yadav et al. 2005). In another study, MG concentration is reported to increase 1.63-fold as compared to control condition after 24 h of drought stress in pumpkin seedlings (Hossain et al. 2009).

16.4 Methylglyoxal Detoxification Pathways

Methylglyoxal (MG) is a physiological highly reactive genotoxic and cytogenic α-oxoaldehyde compound. Due to highly reactive properties of MG, its concentrations must be kept below the threshold levels to sustain cellular homeostasis. Whatever route through which MG is produced, it is primarily detoxified by the ubiquitous glyoxalase pathway (Thornalley 1993). Recent investigations in plants have demonstrated the involvement of the glyoxalase system in drought stress tolerance (Hossain et al. 2009; Hasanuzzaman and Fujita 2011). Apart from glyoxalase pathway, there are other enzymes involved in the detoxification process as well (Kalapos 1999).

16.4.1 Glyoxalase Pathway

The glyoxalase pathway is a ubiquitous mechanism for cellular metabolism of MG in the living systems and operates in the cytoplasm of cells in both prokaryotes and eukaryotes. At the time of its discovery in 1913 (Neuberg 1913; Dakin and Dudley 1913), it was believed to be a single enzyme. Later in 1951, involvement of two enzymes for MG detoxification was reported (Racker 1951). The thiol-dependent glyoxalase system comprises two enzymes, glyoxalase I (GLY I; S-D lactoylglutathione lyase; EC 4.4.1.5) and glyoxalase II (GLY II; hydroxyacylglutathione hydrolase; EC 3.1.2.6). The first enzyme of the pathway, GLY I, catalyzes the conversion of MG to S-D-lactoylglutathione with the help of reduced glutathione (GSH), while the second enzyme, GLY II, converts S-D-lactoylglutathione to D-lactic acid and regenerates GSH back to the system (Racker 1951) (Fig. 16.1). MG detoxification is highly dependent on the availability and concentration of endogenous GSH and thus, insufficiency of cellular GSH leads to the accumulation of MG. The overexpression studies of glyoxalase enzymes have demonstrated that glyoxalases can prevent excessive accumulation of MG in plants under stress conditions, acting primarily by maintaining intracellular antioxidant pools (Singla-Pareek et al. 2003; Hoque et al. 2007; Hasanuzzaman and Fujita 2011; Hasanuzzaman et al. 2011; El-Shabrawi et al. 2010; Ghosh et al. 2014). Additional information on the

biological function of glyoxalase system comes from the molecular engineering studies of the corresponding genes. Several investigations provide a potential framework for understanding the physiological roles of the glyoxalase system in higher plants in response to various stresses. However, underexpression of glyoxalase I in tobacco showed increased levels of MG leading to cytotoxicity resulting in failure of seed germination (Yadav et al. 2005). In addition, it was reported that glyoxalase enzymes increased the tolerance of plants to drought-induced oxidative damage by maintaining the GSH/GSSG ratio (Hasanuzzaman and Fujita 2011). Further, upregulation of GLY I and GLY II can confer stress tolerance to plants. It was reported that drought stress enhanced GLY II transcript expression in *Brassica* and rice (Yadav et al. 2007; Saxena et al. 2005).

16.4.2 Non-glyoxalase Pathways

In addition to glyoxalases, there are other ways in which MG can be detoxified in the plant system. Since MG contains both ketone and aldehyde groups, it can readily undergo oxidation or reduction reactions (Kalapos 1999; Yadav et al. 2008). Consequently, the enzymes which are involved in oxido-reduction can catalyze the conversion of MG to either acetol or lactaldehyde. Enzymes such as aldo-reductases and dehydrogenases catalyze such reactions (Fig. 16.1). ALR1 (Alcohol; NADP-oxido-reductase, EC. 1.1.1.2), ALR2 (alditol: NAD poxido-reductase, EC. 1.1.1.21), and ALR3 (carbonyl reductase; EC. 1.1.1.184) are representatives of reductase family involved in MG detoxification. These ALRs have been shown to possess broad substrate specificity and are potentially involved in MG detoxification in the plants. Overexpression of aldose/aldehyde reductase (*ALR*) in tobacco plants has been shown to confer tolerance against drought stress. The transgenic plants exhibited reduced loss of photosynthetic efficiency and decreased lipid peroxidation, thiobarbituric acid reactive species (TBRS) and H_2O_2 accumulation as compared to non-transgenic plants (Hideg et al. 2003). Further, pyruvate dehydrogenases are found in abundance in plants and have also been shown to catalyze MG detoxification (Baggetto and Lehninger 1987). Therefore, efficient detoxification of MG might be a sustainable strategy for tolerance against various stresses (Hasanuzzaman and Fujita 2011).

16.5 Correlation Between MG and ROS Production

In plants, stress is generally associated with increased levels of MG and ROS such as superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Van Breusegem et al. 2001; Chaves et al. 2003; Reddy et al. 2004). Being a potent and highly reactive glycating agent, it accelerates inactivation of antioxidant defense mechanism (Martins et al. 2001; Thornalley 2003b). MG is

Table 16.2 Correlation between MG and ROS generation

Sl No.	Reaction	Catalyst	Reference
1	$\text{Aminoacetone} + O_2 \rightarrow \text{MG} + NH_4 + H_2O_2$	Semicarbazide sensitive amine oxidase (SSAO)	Yu et al. (2003)
2	$\text{Aminoacetone} + O_2 \rightarrow \text{MG} + \text{NH4} + O_2^-$	Fe^{2+}	Dutra et al. (2001)
3	$\text{Acetol} + O_2 \rightarrow \text{MG} + H_2O_2$	Galactose oxidase	Johnson et al. (1985)
4	$\text{MG} + H_2O + O_2 \rightarrow \text{Pyruvate} + H_2O_2$	Glyoxal oxidase	Kersten and Kirk (1987)
5	$\text{MG} + e^- \rightarrow \text{MG}^- + O_2 \rightarrow \text{MG} + O_2^-$	PSI	Saito et al. (2011)

interlinked with ROS, as evident from the generation of ROS during both formation and decomposition of MG in different cellular reactions (Table 16.2). In plants, MG hampers normal physiological metabolic functions either directly or indirectly through generation of ROS in cells (Hoque et al. 2012a, c) (Fig. 16.2). ROS can readily react with various biologically important macromolecules such as proteins, lipids, and DNA, resulting in oxidative damage and impedes the normal cellular metabolic functions (Apel and Hirt 2004; Foyer and Noctor 2005). Prolonged drought stress accelerates overproduction of MG as well as ROS resulting in oxidative damage (Yadav et al. 2005; Smirnoff 1993). Thus, excessive accumulation of ROS can overcome the antioxidant defense system and results in alteration in metabolic processes, reduction in photosynthesis, interruptions in cellular coordination leading to growth retardation, reduced fertility, causes premature senescence and death of plants (Hossain et al. 2011b; Saito et al. 2011; Krasensky and Jonak 2012). Therefore, ROS should be regulated in plants through the synchronization of ROS production and ROS scavenging systems to withstand oxidative damage by homeostatic regulation of signaling events (Foyer and Noctor 2005).

16.6 Impairment of Cellular Functions by MG and ROS

MG and ROS are generated during the course of metabolism in vivo and are highly reactive glycation agents. The probable involvement of ROS in reactions between MG and macromolecules was first reported by Szent-Györgyi in the 1960s (Szent-Györgyi 1968). Later in the 1970s, the interaction between protein amines and MG was investigated (Kon and Szent-Györgyi 1973). Moreover, the ROS generating ability of MG was also reported (Kalapos et al. 1993). Currently, little information is available regarding the role of MG and ROS under drought stress conditions in plants. However, relationship of MG and ROS with cellular macromolecules had been studied over the course of time and can be utilized to study the emerging role of glyoxalase in plant drought tolerance. This section concentrates on understanding the mechanism of MG and ROS toxicity in the plants.

16.6.1 MG-Mediated Disruption in Cellular Functioning

Being a strong electrophile, MG can readily modify functional groups of various macromolecules and thus, influences their biological activity (Kalapos 1994). It disturbs cellular metabolism upon excessive accumulation and is directly involved in imposing carbonyl stress (Fig. 16.2), when MG levels supersede detoxification capability of glyoxalase I and other related enzymes, then carbonyls bind to protein, lipids, and other macromolecules, thereby leading to ROS generation and that advanced to apoptosis or to malfunction (Kalapos 2008). It is also inhibiting the activity of various important cellular enzymes, including glycolytic enzymes, intramitochondrial enzymes, Na^+-K^+-ATPase, transport proteins and enzymes participating in cell defense (Leoncini et al. 1980; Kun 1950; Mira et al. 1991; Ferguson et al. 1998; Vander Jagt et al. 1997; Amicarelli et al. 2003). Further, it is also capable of reacting with nucleic acids, and is suggested to be a carcinogenic, mutagenic, and teratogenic agent (Hasegawa et al. 1995; Sugimura and Sato 1983; Chaplen 1998; Brambilla et al. 1985). GSH is a well-known intracellular antioxidant agent involved in the protection of cells from oxidative stress (Sen 1997). It may be trapped as S-2-hydroxyacylglutathione at excessive accumulation of MG and subsequently causing GSH depletion (Kalapos et al. 1992). However, MG can act as directly as cytotoxic agent affecting various cellular machineries or it can reduce GSH concentration under stress condition. It is reported that a significant decrease in GSH levels occur in the presence of various concentrations of MG (Kalapos et al. 1992). Additionally, MG also decreased the thiol containing proteins level in isolated mitochondria (Kun 1950). Finally, MG inhibits the activity of several enzymes (Kalapos 1994) and also depletes GSH levels both in vivo and in vitro (Amicarelli et al. 2003).

16.6.2 ROS-Mediated Disruption in Cellular Functioning

Despite their toxic nature, ROS actually have a double role in vivo depending on their concentration, duration and site of action, preceding encounter to stress, etc. (Miller et al. 2010). In general, low doses are treated as signals that mediate at least some part of the responses towards stress while at certain levels of phytotoxicity, they cause a great threat that may in due course lead to programmed cell death (Gechev and Hille 2005). When the cellular ROS concentration exceeds beyond the threshold levels, then living systems can be said to be in a state of "oxidative stress" (Fig. 16.2). Abiotic stress such as drought leads to excessive accumulation of ROS due to imbalance in cellular homeostasis (Sharma and Dubey 2005). They can pose cellular damage by triggering oxidation of proteins, peroxidation of lipids, damage to nucleic acids, inhibition of enzyme activities, activation of programmed cell death (PCD) eventually leading to death of the cells (Reddy et al. 2004; Sharma and Dubey 2005; de Carvalho 2008; Ahuja et al. 2010; Karuppanapandian et al. 2011).

16.7 Drought Induced Alteration in Expression of Glyoxalase Genes

The role of glyoxalase genes has been demonstrated under abiotic stress conditions through various transcriptomic studies. Stress-induced alterations in glyoxalase gene expression clearly suggest a direct role of glyoxalase genes in stress adaptation and acclimation pathway. Upon mannitol treatment, a two- to threefold upregulation in GLY I expression has been observed in different tissues such roots, stems, and leaves (Espartero et al. 1995). GLY I preferentially accumulates in the phloem sieve elements as revealed through immunohistochemical localization analysis. Further, a dose-dependent GLY I transcript analysis has also been performed in *Brassica juncea* in response to salt, drought, and heavy metal stresses (Veena and Sopory 1999). A significant two- to threefold enhancement in the level of GLY I transcript was observed in response to 400 mM mannitol. In order to identify novel genes involved in desiccation tolerance in the foliage of the grass *Sporobolus stapfianus*, Blomstedt et al. 1998 prepared a cDNA library from the desiccated leaf tissue. After differential screening, six clones including GLY I have been identified that show increased transcript abundance and thus might be associated with desiccation tolerance. Northern blot analysis showed a threefold increase in GLY I transcript in response to dehydration as compared to the fully hydrated tissue and a twofold increase in response to subsequent drying. In *S. stapfianus*, GLY I transcripts are also induced by 1.6-fold after treatment with ABA. Moreover, microarray analysis of transgenic plants overexpressing NAC transcription factor genes shows upregulation of several stress-inducible genes including GLY I and resulting transgenic plants show significant tolerance towards drought stress (Tran et al. 2004). Further, a sharp fourfold upregulation in GLY I expression has been observed after transcriptome profiling of wild type and co-suppressed MSI1 (chromatin assembly factor 1) *Arabidopsis* lines (Alexandre et al. 2009). Apart from activation of GLY I transcripts, co-suppressed MSI1 plants have increased levels of free proline and showed enhanced tolerance towards drought. A noticeable increase in the GLY I transcript was also observed in pumpkin seedlings in response to different stresses including drought, salinity, heavy metal, and heat (Hossain et al. 2009). Moreover, genome wide expression analysis of *Arabidopsis* and rice using microarray data identified several glyoxalase members with altered expression in response to drought stress (Mustafiz et al. 2011). An upregulation in expression of *AtGLYI3*, *AtGLYI6*, and *AtGLYI7* genes occurs in a time-dependent manner under drought conditions in Arabidopsis seedlings, whereas *AtGLYI2*, *AtGLYI4*, and *AtGLYI9* are downregulated under such conditions. Similarly, rice glyoxalase genes, *OsGLYI2, OsGLYI6*, and *OsGLYI11*, are induced, but *OsGLYI5* and *OsGLYI10* are downregulated in response to drought stress in the rice seedlings. Expression of rice GLY I transcripts were further analyzed in the 2 weeks rice seedlings in response to different abiotic stresses such as heat, cold, dehydration, wounding, MG, salt, and oxidative stress by qRT-PCR (Kaur et al. 2013). A 4.5-fold upregulation in *OsGLYI-11.2* expression was observed, followed by *OsGLYI-7.1* under drought conditions; while

other members *OsGLYI-2*, *OsGLYI-8*, and *OsGLYI-11.3* showed sharp decline in gene expression. Furthermore, differential gene expression studies in soybean leaf tissues revealed upregulation of GLY I family members along with other regulatory and functional genes under drought stress (Le et al. 2012).

Like GLY I, expression of GLY II transcript was also found to vary under different stresses. Expression of rice GLY II gene was analyzed in response to various abiotic stresses such as desiccation, salinity, heat, cold, and ABA and SA treatment (Yadav et al. 2005). Significant accumulation of GLY II transcript was found in response to all stress agents. Desiccation stress resulted in the accumulation of GLY II transcript in a short duration of 15 min followed by gradual increase in accumulation with time till 2 h (Yadav et al. 2007). Genome wide transcript analysis of rice GLY II transcripts showed strong induction of all GLY II members in response to drought stress (Mustafiz et al. 2011). Amongst the Arabidopsis GLY II genes, the expression of *AtGLYII1* and *AtGLYII2* was found to be highly upregulated in response to drought stress in both shoot and root tissues (Mustafiz et al. 2011). However *AtGLYII3*, *AtGLYII4*, and *AtGLYII5* were downregulated in response to drought stress in both shoot and root tissues in Arabidopsis.

16.8 Drought Induced Alteration in Levels of Glyoxalase Proteins

Proteins are vital components of living organisms that are directly involved in various physiological and metabolic pathways of cells. Hence, studying variations in levels of glyoxalase proteins or their enzyme activities will give more precision in understanding the role of these enzymes in stress adaptation and in efficient monitoring of the stress response. Activity of glyoxalase has been monitored by various research groups under different environmental stimuli. Initial reports have revealed an increase in GLY I activity during cell division (Deswal et al. 1993) and proliferative callus cultures of groundnut (*Arachis hypogaea* L.cv. JL24) (Jain et al. 2002). To identify the altered proteins during drought stress, functional proteome studies have been performed and have secured an important place in the era of comparative and functional genomics. To investigate the mechanism of plants' osmotic stress response, rice protein profiles were monitored from mannitol-treated plants using proteomics approach (Zang and Komatsu 2007). Proteins from the basal part of leaf sheaths showed strong induction in levels of GLY I protein in response to stress. To study the changes in wheat grain proteome in response to drought, two-dimensional gel electrophoresis among three wheat genotypes with different genetic background was performed under well-watered and drought conditions (Hajheidari et al. 2007). The overall effect of drought was highly significant and about 650 spots were reproducibly detected and analyzed. Mass spectrometry analysis using MALDITOF/TOF led to the identification of 57 proteins with significant alteration. A significant downregulation (twofold) in GLY I protein levels was observed in the susceptible genotypes, with no or insignificant changes in the tolerant counterpart.

Further, GLY I protein was also identified in a two-dimensional gel electrophoresis experiment carried out in two distinct sunflower genotypes in response to drought (Castillejo et al. 2008). The susceptible genotype showed a decrease in the intensity of the 17 spots out of 28 altered proteins. The proteins that showed a decline in their levels included a GLY I protein, along with some other important proteins such as photosystem II oxygen-evolving complex protein 1, carbonic anhydrase, RubisCO large and small subunits, ferredoxin-$NADP^+$ reductase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, aldolase and superoxide dismutase. Furthermore, comparative proteomic analysis of differentially expressed chickpea and rice extracellular matrix proteins also led to the identification of a GLY I protein during dehydration stress (Bhushan et al. 2007; Pandey et al. 2010). GLY I protein was also found to be significantly upregulated in the nuclear fraction of chickpea in response to dehydration stress (Pandey et al. 2008). In addition, analysis of drought responsive leaf proteome of a C3 xerophyte, *Citrullus lanatus* also revealed alteration in levels of GLY I protein (Akashi et al. 2011).

Significant increase in levels of GLY I protein and GLY I activity was observed in onion bulb in response to various stress treatments (Hossain et al. 2007). An induction of 1.3- to 1.4-fold was observed in both the levels of GLY I protein and activity in response to drought stress. A sharp increase in GLY I activity (1.27-fold) was observed after 24 h of drought stress in pumpkin seedlings (Hossain et al. 2009). A similar pattern of induction was observed in GLY II enzyme activity in response to drought. The potential role of various chemical compounds in increasing drought tolerance by enhancing glyoxalase enzyme activity has been determined by different studies (Hasanuzzaman and Fujita 2011; Alam et al. 2013). For instance, drought stress induced oxidative damage of rapeseed seedlings could be reversed by the pretreatment of selenium that enhances the activities of antioxidant and MG detoxifying enzymes (Hasanuzzaman and Fujita 2011). Selenium pretreated rapeseed seedlings exposed to various degrees of drought stress showed a sharp rise in their ascorbic acid level, reduced glutathione content, and maintained a high GSH/GSSG ratio as compared with the drought-stressed plants without selenium treatment. It has been reported that pretreatment with 25 mM of selenium resulted in a 23 % increase in GLY I activity and also a significant increase in GLY II activity in rapeseed seedlings as compared to control. A similar study showed that exogenous addition of salicylic acid in mustard seedlings mediates short-term tolerance against drought stress by upregulating the antioxidant defense and glyoxalase pathway (Alam et al. 2013). Drought stress resulted in a sharp decline in the level of ascorbate, relative water content, and chlorophyll content in the mustard seedlings, but increased their proline, malondialdehyde, and H_2O_2 levels. However, salicylic acid supplementation in the drought stressed seedlings enhanced ascorbate, reduced glutathione, chlorophyll, and relative water content, as well as decreased the GSSG level to maintain the ratio of GSH/GSSG. Salicylic acid supplemented drought stressed seedlings also enhanced the enzyme activities of GLY I, GLY II, and different antioxidant enzymes as compared to drought-stressed plants without salicylic acid supplementation. Moreover, temperature (either heat or cold)-shock positively modulates the oxidative protection in salinity and drought stressed mustard

(*Brassica campestris* L.) seedlings in a very similar mechanism by increasing glyoxalase activity (Hossain et al. 2013a, b). Seedlings pre-exposed to either heat-shock or cold-shock conditions positively modulate the activities of GLY I and GLY II, and maintain lower levels of GSSG, H_2O_2, and malondialdehyde as compared to the control as well as non-treated drought stressed seedlings.

16.9 Signaling Roles of MG in Regulation of Stomatal Closure and Stress Responsive Gene Expression

Despite having inhibitory effects on cell growth, MG has been shown to possess signaling roles in bacteria (Campbell et al. 2007), humans (Kang et al. 1996; Akhland et al. 2001), and yeast (Maeta et al. 2005; Takatsume et al. 2006). However in plants, role of MG in signal transduction is less studied. Nonetheless, it has been reported that MG induces ROS formation (Hoque et al. 2012a) and that ROS mediates abscisic acid (ABA) and methyl jasmonate (MeJA) signaling pathways in guard cells related to stomatal regulation (Munemasa et al. 2007). Hoque and coworkers have shown that MG induces stomatal closure in a reversible manner and also induces generation of ROS in *Arabidopsis* (Hoque et al. 2012a). It was found that MG induced significant accumulation of ROS and also increased cytosolic Ca^{2+} oscillations in the guard cells which were suppressed by pretreatment with SHAM (salicylhydroxamic acid). SHAM-sensitive peroxidases diffuse extracellular oxidative burst into the intracellular space contributing to intracellular ROS accumulation in the guard cells and trigger stomatal closure via a Ca^{2+}-dependent pathway (Hoque et al. 2012a). Additionally, it was also observed that MG was also engaged in inhibiting light-induced stomatal opening via the modification of C-terminal region of KAT1, an inward-rectifying potassium channel thereby inhibiting K^+ influx into the guard cells (Hoque et al. 2012b). The involvement of MG in regulation of stomatal movements indicates towards its role in signal transduction pathways in drought stress adaptation. Because of closure of stomata is the primary response of almost all plants to drought to prevent transpirational water loss (Mansfield and Atkinson 1990). Regulation of stomata may result in response to decrease in leaf turgor or low humidity atmosphere (Ludlow and Muchow 1990; Maroco et al. 1997). In response to drought, MG levels have been reported to increase up to sixfold depending upon the crop species (Yadav et al. 2005).

Further, MG is capable of altering expression of genes known to be involved in drought stress adaptation. For instance, MG was found to affect the transcript levels of ABA-dependent genes, RD29B and RAB18, which are generally induced in response to dehydration. MG could significantly induce RD29B (fivefold) and RAB18 (threefold) gene expression that too in a concentration-dependent manner (Hoque et al. 2012c). In addition, MG has also been shown to enhance expression of triose phosphate isomerase (*OscTPI*) and *OsETHE1* in rice (Sharma et al. 2012; Kaur et al. 2014b). Moreover, global gene expression profiles in rice in response to exogenous MG showed its involvement in signal transduction. MG affected the

expression of various genes involved in stress-induced signal transduction cascades such as protein kinases (mitogen-activated protein kinase, calcium/calmodulin-dependent protein kinases, Ser/Thr protein kinase, histidine kinase, and receptor-like kinase) and transcription factors (bZIP, AP2 domain-containing protein, NAM, WRKY, and zinc finger proteins), which were significantly represented in the perturbed transcriptomes, indicating an interlink between MG and stress-responsive signal transduction pathways (Kaur et al. 2015). Collectively, MG plays a significant role in signal transduction possibly acting as a stress signal molecule in plants, where it conveys signals to the cellular machinery to maintain the cellular homeostasis towards adaptation in drought stress.

16.10 Conclusion and Future Perspective

The pathways involved in drought stress adaptation in plants are regulated at both physiological and molecular levels. Molecular information of response and tolerance mechanisms is likely to pave way for engineering plants that could make them withstand drought stress. Many achievements have been made over the last few years in understanding the protective role of glyoxalases in MG detoxification under drought conditions (Fig. 16.3). Drought stress leads to increased accumulation of MG and MG-derived ROS. It is now well known that MG has deleterious effects on

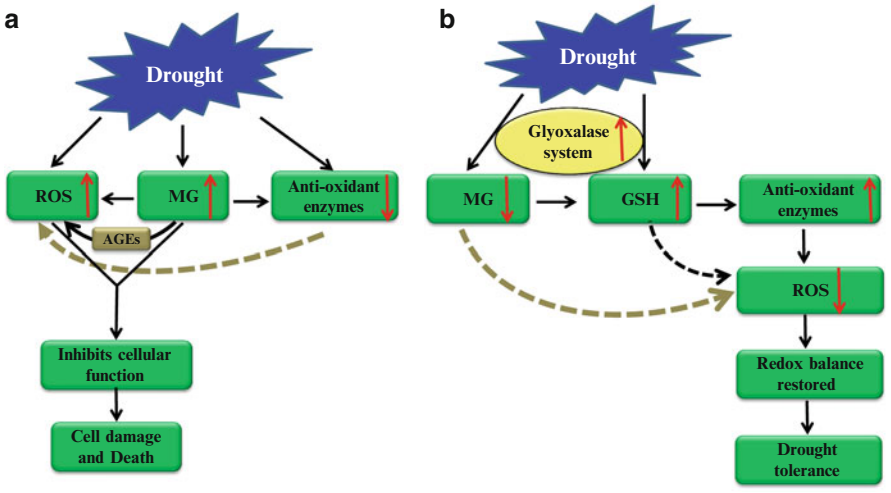

Fig. 16.3 Role of glyoxalase pathway in drought stress adaptation. During drought, MG and ROS levels increase which then impair the redox balance of cell. MG levels also induce ROS generation through the formation of AGEs, resulting in ROS-mediated cellular injury and death (**a**). Increase in glyoxalase activity through overexpression helps in maintaining cellular redox homoeostasis under drought stress by reducing MG levels and regenerating GSH back into the system, thereby decreasing ROS generation which leads to improved drought tolerance (**b**)

plant growth and development and that glyoxalase pathway serves an important detoxification role in the living systems. Several transcriptome and proteome studies carried out to identify genes involved in drought stress response have revealed a link between glyoxalases and drought stress adaptation indicating glyoxalase pathway to be a crucial intracellular component of plant stress response. Further, MG transmits signals to the cellular machinery for inducing changes in plant transcriptome, transcription factors, protein kinase as well as regulation of stomatal movements for adaptation to drought stress conditions. However, the specific role of MG as a signal molecule itself or as a component in signaling cascade in plants needs further investigation for deeper understanding of its role in stress response and tolerance.

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