

## Targeting Glycinebetaine for Abiotic Stress Tolerance in Crop Plants: Physiological Mechanism, Molecular Interaction and Signaling

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**Abstract:** In the era of climate change, abiotic stresses (e.g., salinity, drought, extreme temperature, flooding, metal/metalloid(s), UV radiation, ozone, etc.) are considered as one of the most complex environmental constraints that restricts crop production worldwide. Introduction of stress-tolerant crop cultivars is the most auspicious way of surviving this constraint, and to produce these types of tolerant crops. Several bioengineering mechanisms involved in stress signaling are being adopted in this regard. One example of this kind of manipulation is the osmotic adjustment. The quarternary ammonium compound glycinebetaine (GB), also originally referred to as betaine is a methylated glycine derivative. Among the betaines, GB is the most abundant one in plants, which is mostly produced in response to dehydration caused by different abiotic stresses like drought, salinity, and extreme temperature. Glycinebetaine helps in decreased accumulation and detoxification of ROS, thereby restoring photosynthesis and reducing oxidative stress. It takes part in stabilizing membranes and macromolecules. It is also involved in the stabilization and protection of photosynthetic components, such as ribulose-1, 5-bisphosphate carboxylase/oxygenase, photosystem II and quarternary enzyme and protein complex structures under environmental stresses. Glycinebetaine was found to perform in chaperone-induced protein disaggregation. In addition, GB can confer stress tolerance in very low concentrations, and it acts in activating defense responsive genes with stress protection. Recently, field application of GB has also shown protective effects against environmental adversities increasing crop yield and quality. In this review, we will focus on the role of GB in conferring abiotic stress tolerance and the possible ways to engineer GB biosynthesis in plants.

**Keywords:** Osmolytes; compatible solutes; biostimulants; proline; amino acids; stress signaling

### 1 Introduction

From the prehistoric era, natural calamities came in the form of abiotic stress and hampered the growth and productivity of plants. As a sessile organism, plants frequently face a glut of abiotic stresses such as salinity, drought, flooding and submergence, low and high temperature, UV radiation, ozone, nutrient deficiency and metal toxicity [1]. With the present global climatic change, these abiotic stress factors are

taking place more frequently than earlier times leading to the vulnerability of crop productivity, and creating challenges for the farming community to feed the ever-growing population of this universe. Facing this fact, plant biologists are trying to develop new technologies towards sustaining crop productivity.

One of the most and best-documented stress-responsive mechanisms of plants is the synthesis and accumulation of compatible solutes. Compatible solutes are small organic metabolites, soluble in water, which are membrane-impermeable and can accumulate enormously with high concentrations (0.2 M) in cytoplasm corresponding to stress response [2]. Compatible osmolytes were discovered in many prokaryotic and eukaryotic cells, ranging from bacteria to higher organisms-plants and animals, and their chemical diversity is very extensive among organisms [3]. Researchers have identified many compatible solutes in plant cellular systems, for example, amino acids (proline, glutamate, glutamine and alanine) and their derivatives (ectoine and hydroxyectoine), quaternary amines (betaine, polyamines and dimethyl sulfoniopropionate), sugars (trehalose) and polyols including sugar alcohols (mannitol, sorbitol, pinitol, glycerol and galactinol) [4-6].

These osmoprotectors have wide-spectrum functions including scavenging of ROS, balancing of cell redox status, acting as osmoprotectants, stabilization of cytosolic pH, proteins, enzymes and membranes, as well as acting as a potential source of carbon and nitrogen for plants both during stress events, and the subsequent recovery episodes [6]. Among these, one of the most efficient compatible solutes is glycinebetaine (GB), which can bestow tolerance to plants at the time of abiotic stresses. Furthermore, GB is reported to be non-toxic even at higher concentrations, which allow the cells retain water and permit normal physiological process at the time of abiotic stresses [7-9].

Glycinebetaine is a low molecular weight compound, possesses high solubility and low viscosity, and can accumulate in the photosynthetic machinery-chloroplasts and plastids at a high level during abiotic stresses [10]. Hence, these extraordinary properties make GB as one of the most efficient osmoprotectants [2,7]. Reports suggest that the GB accumulation are in general correlated to the degree of stress tolerance [11]. In contrary, many species do not accumulate GB under normal or stress situations [12]. Among many of the compatible solutes, GB, in particular, is reported to be an effective plant protectant against multifaceted abiotic stresses [13-15]. Both the exogenous GB application to plants as well as the transgenic approaches in plants can increase the abiotic stress tolerance of plants. When applied exogenously, via nutrient or foliar spray, GB is readily uptaken by root or leaf tissue [16-17] and efficiently stabilize the quaternary structure of proteins and enzymes, oxygen-evolving photosystem II (PSII) and photosynthetic machinery components such as ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO), can protect the complex membrane structures at the time of unfavorable physiological temperature regimes and at higher cellular salt concentrations [18-19]. This mechanism protects plants and confers tolerance against abiotic stress.

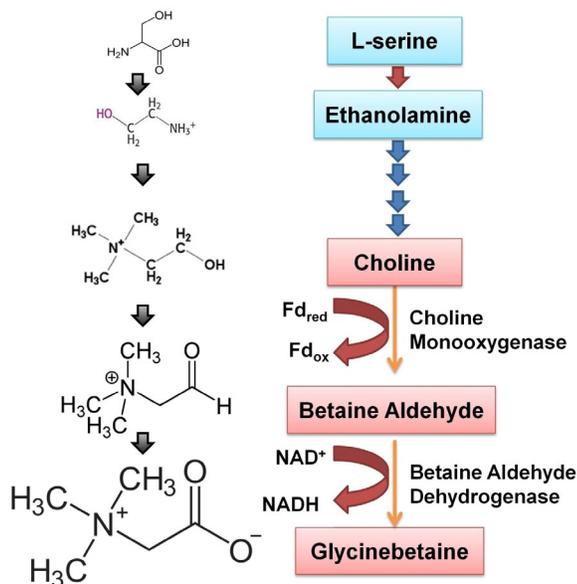
Oxidative stress is another consequence of most of the abiotic stress caused by overproduction of reactive oxygen species (ROS) [20]. Researchers discovered the beneficial role of GB in mitigating the oxidative damage due to abiotic stresses in plants. Alcázar et al. [21] reported that the application of exogenous hydroxyl radicals ( $\text{OH}^\bullet$ ) in *Arabidopsis* roots resulted in a massive, dose-dependent efflux of  $\text{K}^+$  ions from epidermal cells in the elongation zone. However, the presence of GB at 5 mM concentration in the incubation medium significantly reduced this efflux of  $\text{K}^+$  ions. Furthermore, in tomato plants, exogenously applied GB significantly reduced the chilling-induced production of  $\text{H}_2\text{O}_2$  [22], and reduce cell membrane damage in cotton [23]. Although GB does not scavenge ROS directly, it mitigates the damaging effects of oxidative stress by other means, for example by activating or stabilizing ROS-scavenging enzymes and/or repressing the production of ROS by other mechanisms. However, few researchers observed the signaling role of ROS at lower cellular concentrations, thus regulating plant growth and development, as well as various features conferring stress tolerance [24]. The remarkable feature of plants is that they evolved excellent cellular repair mechanisms that maintain cellular redox state and reduce the oxidized macromolecules to their previous reduced states [25], which involve the antioxidant system, comprising of non-enzymatic and enzymatic components, that scavenge or detoxify ROS [1,26].

Therefore, in this review, we present information highlighting the vital roles of GB. Moreover, the physiological and biochemical mechanisms of GB-induced abiotic stress tolerance and the signal

transduction mechanisms towards cellular responses for conferring abiotic stress tolerance are the focal points of this review.

## 2 Glycinebetaine Biosynthesis and Metabolism in Plants

Glycinebetaine is a naturally occurring quaternary ammonium compound (N, N, N-trimethylglycine), which is dipolar yet electrically neutral at physiological pH [27]. The biosynthesis of GB is usually characterized by the double step oxidation of choline using the toxic intermediate, betaine aldehyde. The enzymes participating in this process are usually localized in the stroma of the chloroplast. These are the ferredoxin-dependent soluble Rieske-type protein, choline monooxygenase (CMO) and the soluble NAD<sup>+</sup>-dependent betaine aldehyde dehydrogenase 1 (BADH1) [28]. Choline formed from the L-serine-ethanolamine pathway is reduced to the toxic betaine aldehyde via the action of CMO. Betaine aldehyde dehydrogenase 1 uses NAD<sup>+</sup> as the proton acceptor and oxidizes betaine aldehyde into GB (Fig. 1) [29]. Ladyman et al. [30] identified GB as an inert end product in barley plants, which accumulate in the young shoots exposed to stress but does not get degraded even after the stress cues are removed. However, radiotracers showed that the distribution of GB among the plant tissues changed. Although GB produced in mature leaves during stress, but is translocated to new expanding leaves through the phloem under well-watered conditions [30].



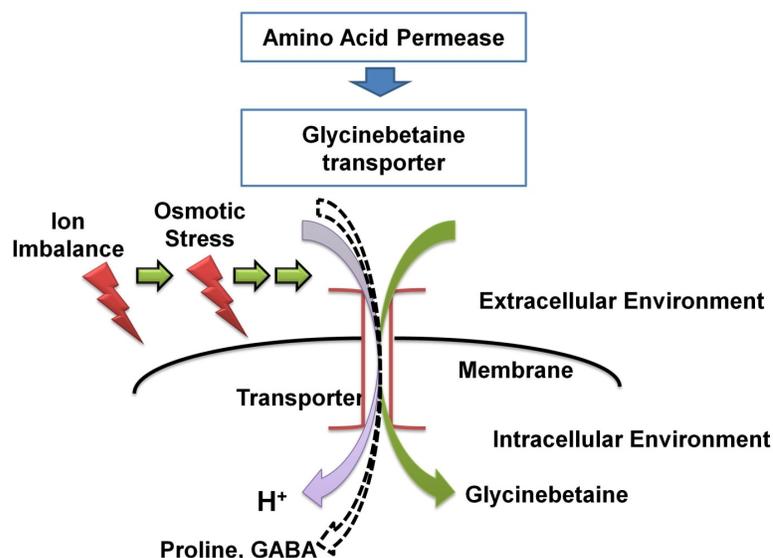
**Figure 1:** The biosynthesis of GB involves two-step catalysis of choline formed from the serine-ethanolamine pathway. Choline is acted upon by choline monooxygenase and reduced to betaine aldehyde along with simultaneous oxidation of ferredoxin (Fd<sub>ox</sub>). The betaine aldehyde formed is again oxidized to GB via reduction of NAD<sup>+</sup> to NADH by betaine aldehyde dehydrogenase

## 3 Glycinebetaine Transport and Trafficking in Plants

Glycinebetaine is a crucial osmolyte, and its transport and optimum distribution throughout the cell is essential for maintaining the cellular osmotic pressure and generating abiotic stress tolerance [31]. Considerable structural specificity has been observed in case of GB transport in *Escherichia coli*, where the kinetics of GB entry could be associated with the Michaelis-Menten relationship reproducing an affinity (K<sub>m</sub>) of 35 μM and a maximum velocity (V<sub>max</sub>) of 42 nmol min<sup>-1</sup> mg<sup>-1</sup> protein [32]. It was also observed that the GB transport is critically regulated by an active and specific system dependent on the osmotic strength of the growth medium [32]. Lai et al. [33] also identified a high-affinity and high-

specificity GB transporter in the halophilic archaeon, *Methanohalophilus portucalensis*, which allowed GB uptake at an observed  $V_{\max}$  of 8 nmol min<sup>-1</sup> mg<sup>-1</sup> proteins. The Gram-negative bacterium, *Azospirillum brasilense*, plays an important role in nitrogen fixation in association with the roots of graminaceous plants. Addition of GB in the medium during salt stress enabled trafficking of the osmolyte within the bacterial cells due to the presence of a periplasmic GB-binding protein. This stimulated the nitrogen fixation activity even under salt stress [34]. The mechanism of GB transport in plants is not yet elaborately understood. However, some reports indicate the presence of a strictly operating transporter, which is responsive to sub-optimal conditions and extracellular ionic imbalances [35].

Two full-length GB transporter genes (*AmT1* and *AmT2*) were cloned from the mangrove species, *Avicennia marina* [35]. These genes exhibited high homology with the proline (Pro) transporters of *Arabidopsis* and tomato. When *E. coli* cells were complemented with GB and Pro transporters, it resulted in efficient uptake of GB and Pro. Interestingly, mutations of Thr290-Thr291-Ser292 in the GB transporters to Arg290-Gly291-Arg292 resulted in a constitutively active form, independent of ionic concentrations. These results indicate that incorporation of the positive amino acids yields a positive conformational change in the quaternary structure of the transporters [35]. In another study, Schwacke et al. [36] identified Pro transporter 1 (ProT1) in tomato pollen, which transported Pro and  $\gamma$ -aminobutyric acid (GABA) with low affinity and GB with high affinity. This membrane protein was observed to be the member of the amino acid permeases (AAPs), which ensure proton-coupled uptake of diverse molecules ranging from amino acids to compatible solutes [36]. Fig. 2 proposes a general mechanism of GB trafficking within plant cells.



**Figure 2:** Glycinebetaine transport into the cell is induced by ionic imbalance due to osmotic stress. This protein belongs to the class amino acid permeases. The GB transporter co-transporters H<sup>+</sup> ions and is also permeable to Pro and  $\gamma$ -amino butyric acid to a lower extent (dotted arrow)

#### 4 Variation of Glycinebetaine Content in Plants Under Different Stress

Glycinebetaine, the most important organic osmolytes, show variation in their accumulation in diverse plant species due to the imposition of different environmental stresses such as salinity, drought, high temperatures, heavy metals, chilling, UV radiation, etc. [8,37,38]. It is involved in the protection of many plant species against a range of oxidative stress under stressful conditions, such as, salinity [39-42], drought [43-46], metal/metalloid stress [47-49], high temperature [45,50] and chilling [23,51,52]. A lot of research

findings confirmed the positive relationship between accumulation of GB and plant stress tolerance. Glycinebetaine accumulation also greatly varied depending on the stress type and plant species (Tab. 1).

**Table 1:** Variation of glycinebetaine content in plants under different abiotic stress

Plant species and genotypes	Stress type	Change in GB content	References
<i>Triticum aestivum</i> L. S-24 and MH-97	Salt; 15 dSm <sup>-1</sup> NaCl	Increased in both cultivars	Raza et al. [53]
<i>Atriplex halimus</i> , Montasir and Sbikha	Salt; 160 mM NaCl	Increased by 712 and 326% in Montasir and Sbikha genotype, respectively	Hassine et al. [44]
<i>Zea mays</i> L. Navjot and PEHM 3	Salt; 150, 75, 75 mM of NaCl, Na <sub>2</sub> SO <sub>4</sub> and CaCl <sub>2</sub> , respectively	Increased by 20 and 83% in Navjot and PEHM 3 genotypes, respectively	Kholová et al. [54]
	Salt; 100, 50, 50 mM NaCl, Na <sub>2</sub> SO <sub>4</sub> and CaCl <sub>2</sub> , respectively	Increased by 39 and 70% in Navjot and PEHM 3 genotypes, respectively	
	Salt; 50, 25, 25 mM NaCl, Na <sub>2</sub> SO <sub>4</sub> and CaCl <sub>2</sub> , respectively	Increased by 9 and 20% in Navjot and PEHM 3 genotypes, respectively	
<i>Solanum melongena</i> L. Bemisal and Dilnasheen	Salt; 100 mM NaCl	Increased significantly in both cultivars	Abbas et al. [55]
<i>T. aestivum</i> L. 711, PBW343, 3765 and WH542	Salt; 100 mM NaCl	Increased by 45.4, 33.3, 22.2 and 14.3% in 711, PBW343, 3765 and WH542 cultivars, respectively	Khan et al. [56]
<i>Vigna radiata</i> L.	Salt; 100 mM NaCl	Increased by 31.9%	Khan et al. [40]
<i>A. gmelini</i>	Salt; 250 mM NaCl	Increased considerably in bladder hairs	Tsutsumi et al. [41]
<i>S. lycopersicum</i> cv. 'Moneymaker'	Salt; 200 mM NaCl	Increased significantly	Wei et al. [42]
<i>T. aestivum</i> L. HF9703 and SN215953	Drought; withholding watering	Increased notably in both cultivars	Ma et al. [43]
<i>A. halimus</i> , Montasir and Sbikha	Drought; 15% PEG 10000	Increased GB by 559 and 216% in Montasir and Sbikha genotypes, respectively	Hassine et al. [44]
<i>T. aestivum</i> L. line T6 and its wild-type Shi4185	Drought; 30% PEG-6000	Increased significantly in both line	Wang et al. [45]
<i>Z. mays</i> , Agaiti-2002 and EV-1098	Drought; delaying irrigation (21 days interval)	Increased significantly in both genotypes	Ali and Ashraf [46]
<i>S. officinarum</i> L.	Drought; 5%, 7.5%, 10% and 12.5% PEG	Increased significantly with the increase of drought stress	Abbas et al. [37]
<i>Lemna gibba</i> L.	Cd; 0.5, 1.0 and 3.0 mM CdCl <sub>2</sub>	Increased by 26, 189 and 111% under 0.5, 1.0 and 3.0 mM CdCl <sub>2</sub> stress, respectively	Duman et al. [47]
<i>Lolium perenne</i> Lark	Cd; 0.5 mM CdCl <sub>2</sub>	Increased by 164% in shoots and 56% in roots	Lou et al. [48]
<i>Gossypium hirsutum</i> L. MNH 886	Cd; 1.0 and 5.0 μM CdCl <sub>2</sub>	Significantly increased in both leaves and roots with increasing Cd levels	Farooq et al. [49]
<i>T. aestivum</i> L. line T6 and its wild-type	Heat; 40°C	Increased significantly in both line	Wang et al. [45]

Plant species and genotypes	Stress type	Change in GB content	References
Shi4185			
<i>Saccharum</i> sp. cv. HSF-240	Heat; 42°C	Increased considerably	Rasheed et al. [57]
<i>Tagetes erecta</i> , Narai Yellow, Bali Gold and Columbus Orange	Heat; 39°C/29°C day/night temperature	Increased extensively in all cultivars	Sorwong and Sakhonwasee [58]
<i>T. aestivum</i> L. cv. Shi 4185	Chilling; 2/0°C day/night temperature	Increased significantly	Zhang et al. [59]
<i>Saccharum</i> sp. HSF-240	Chilling; 15°C	Increased considerably	Rasheed et al. [52]
<i>G. hirsutum</i> L. cv. Lumianyan19	Chilling; 5°C	Increased by 25.6%	Cheng et al. [23]

Raza et al. [53] checked the GB-induced modulation of antioxidant enzyme activities and ion accumulation in two wheat cultivars differing in salt tolerance. They observed that the salt-tolerant cv. S-24 accumulated significantly higher GB in the leaves than that in cv. MH-97 (salt-sensitive MH-97 under saline conditions). Two Tunisian populations of the Mediterranean shrub, *Atriplex halimus* L., [plants originating from a salt-affected coastal site (Monastir) or a non-saline semi-arid area (Sbikha)] were exposed to a nutrient solution containing 160 mM NaCl or drought-inducing 15% polyethylene glycol (PEG). Both drought and salt stress condition drastically increased GB content of the two plants, but higher GB content was observed under salt stress condition and in Montasir genotypes [44]. Under salt stress condition, the increase of GB content was 712 and 326% higher than the control in Montasir and Sbikha genotypes, respectively, whereas it was 559 and 216% higher in Montasir and Sbikha genotypes, respectively under drought condition. Kholová et al. [54] observed the effect of long-term soil salinity [S0 (0, 0, 0 mM), S1 (50, 25, 25 mM), S2 (100, 50, 50 mM) and S3 (150, 75, 75 mM) NaCl, Na<sub>2</sub>SO<sub>4</sub> and CaCl<sub>2</sub>, respectively] on two maize (*Z. mays* L.) genotypes, PEHM 3 (comparatively tolerant) and Navjot (susceptible) at vegetative and anthesis stages during summer/rainy season. Compared with control, salinity stress increased GB content by 9, 39, 20% in Navjot genotypes and 20, 70, 83% in PEHM 3 genotypes under S1, S2, and S3 stress condition, respectively. Two eggplant (*Solanum melongena* L.) cultivars, Dilnasheen and Bemisal, showed a similar result under salt stress (100 mM NaCl). Glycinebetaine accumulation in the leaves of both eggplant cultivars was considerably enhanced due to NaCl stress; GB accumulation in cv. Dilnasheen was higher than that in cv. Bemisal under salt stress [55]. Khan et al. [56] screened out four wheat (*Triticum aestivum* L.) cultivars 711, PBW343, 3765 and WH542 for checking variations in GB concentration under 100 mmol L<sup>-1</sup> NaCl stress. Salt-treated wheat plants showed greater accretion of GB than control plants. Maximum accumulation of GB (45.4%) was observed in wheat cultivar 711, whereas minimum accumulation was found in cultivar WH542 (14.3%) in contrast to control. The other two cultivars, PBW343 and 3765 accumulated 33.3% and 22.2% GB, respectively, under salt stress, compared to their respective control. Khan et al. [40] checked the effect of salt stress in mungbean (*Vigna radiata* L.) under saline condition (100 mM NaCl) and found that GB content increased by 31.9% in salt-treated plants in comparison with control plants. Tsutsumi et al. [41] treated *Atriplex gmelini* plants with three levels of NaCl (0, 50 or 250 mM). They observed that all salinity levels increased GB content in both bladder hairs and leaf laminae, but high salinity (250 mM NaCl) conditions showed a significant increase of GB content in bladder hairs than leaf laminae. Wei et al. [42] used a transgenic line and wild-type tomato (*S. lycopersicum* cv. 'Moneymaker') plants in a salinity experiment. They showed GB content increased in both wild-type and transgenic lines under 200 mM NaCl; the GB content in the transgenic lines was significantly higher than that in wild-type plants.

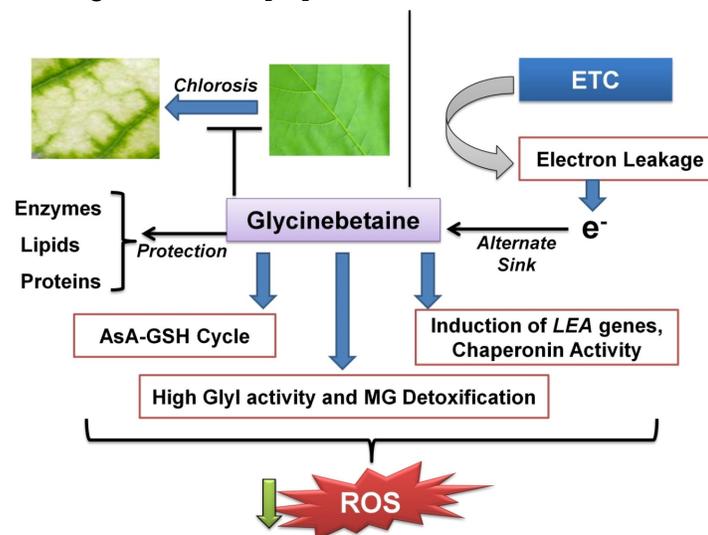
Drought stress also increased the endogenous level of GB. Ma et al. [43] checked the performance of two *T. aestivum* cultivars, HF9703 (drought-tolerant) and SN215953 (drought-sensitive) under drought stress condition and found that GB content of wheat plant increased with the increase of drought stress in HF9703 cultivar; however, in SN215953, it first increased and then declined. Wang et al. [45] used a transgenic *T. aestivum* line T6 and its wild-type Shi4185 for checking the GB-induced tolerance of the photosynthetic apparatus to drought (30% PEG-6000) and heat (40°C) stress. Stress-induced GB levels increased significantly in the leaves of the two lines, especially in T6, showing that the accumulation of GB sensitized the wheat plants to these stresses. Two maize cultivars, Agaiti-2002 (drought tolerant) and EV-1098 (relatively drought sensitive), were also exposed to water deficit conditions. As a result, endogenous GB content considerably increased in both leaf and seed. The cultivars did not differ significantly in leaf GB contents under both water stress and non-stress conditions; however, in case of seed, slightly higher GB contents were observed in cv. Agaiti-2002 than those of EV-1098 [60]. Basu et al. [61] subjected three rice cultivars to PEG-induced dehydration stress and have shown that GB level was maximally triggered after stress imposition in Pokkali, the salt-tolerant cultivar, while it was induced to a lesser extent in both the salt-sensitive cultivars, IR-29 and Pusa Basmati. Later, Abbas et al. [37] tested thirteen genotypes of sugarcane (*Saccharum officinarum* L.) under drought stress (5%, 7.5%, 10% and 12.5% PEG) and found that GB content increased significantly with the increase of drought severity.

Duman et al. [47] exposed an aquatic plant (*Lemna gibba* L.) to different levels of cadmium (Cd) and found that in contrast to control, GB content increased by 26, 189 and 111% under 0.5, 1.0 and 3.0 mM CdCl<sub>2</sub> stress, respectively. Glycinebetaine content also increased in perennial ryegrass under Cd stress condition [48]. In contrast to control, Cd stress (0.5 mM CdCl<sub>2</sub>) increased GB content by 164% in shoots and 56% in roots of perennial ryegrass. Farooq et al. [49] experimented with a cotton genotype MNH 886 under Cd stress (1.0 and 5.0 µM CdCl<sub>2</sub>). They observed GB concentrations significantly increased in both leaves and roots with increasing Cd levels in the growth medium in contrast to control. The up-regulation of *BADHI* transcript has been noted in the rice varieties, IR-64 and Nonabokra, during 1.5 mM CdCl<sub>2</sub> stress that highlights a direct correlation between Cd-tolerant capacity of each variety and *BADHI* gene expression level and hence GB level [62].

Sugarcane (*Saccharum* sp. cv. HSF-240) plant showed higher GB content under heat stress condition (42°C) in contrast to control plants [57]. Sorwong and Sakhonwasee [58] checked the effects of GB under heat stress conditions. They exposed three marigold cultivars, namely, 'Narai Yellow', 'Bali Gold', and 'Columbus Orange' to heat stress (39°C/29°C day/night temperature). Heat stress conditions caused an increase of endogenous GB content. Zhang et al. [59] used wild-type *T. aestivum* cv. Shi 4185 and three transgenic lines (T1, T4, and T6) expressing the *BADH* gene isolated from *A. hortensis* L. under cold stress. Following two days of cold treatment (2/0°C day/night temperature), the GB content increased significantly in all the plants. The increase of GB content was higher in the transgenic lines than the wild-type. Nayyar et al. [63] recorded that chilling stress (12-14/3-5°C day/night temperatures) in chickpea (*Cicer arietinum* L.) increased the GB content more than two-folds on the 4th day of stress in comparison with the 1st day of stress, but it started to quickly decline subsequently. Xing and Rajashekar [51] experimented with *A. thaliana* (Columbia) under cold stress (4/2°C (day/night, 4 weeks and GB (10 mM) was applied exogenously to the plants as a foliar spray for increasing stress tolerance. The endogenous level of GB in the leaves augmented noticeably throughout the cold acclimation treatment. During the first day of cold acclimation, the endogenous GB level was increased by three-folds and following three days of cold acclimation, the endogenous GB content reached at the maximum. Later, fluctuation in GB content was observed during the whole stress period. Rasheed et al. [52] cultivated sugarcane (HSF-240) bud under chilling stress for 2 d. They maintained stress treatments by imposing temperature in a growth chamber. Under chilling stress, the GB accumulation of plant notably increased. A similar increase of GB content was observed in cotton cv. Lumianyan19 after 3 d of cold stress [5°C; 23].

## 5 Glycinebetaine-Induced Improvement in the Photosynthetic Machinery

The chloroplast is the site of GB production. The final steps of GB biosynthesis are localized in the stroma. Synthesis of this zwitter ionic osmolyte leads to improved photosynthetic efficiency during abiotic stresses [64]. Glycinebetaine protects the soluble stromal and luminal enzymes, lipids and proteins involved in photosynthesis [2; Fig. 3]. Abiotic stresses induce the generation of excess electrons from photosynthesis. Glycinebetaine reportedly acts as an additional or alternative biochemical sink for quenching the excess electrons and prevents the photosynthetic apparatus from over-reduction. Thus, GB reverses photosynthetic inhibition [65]. Glycinebetaine accumulation under sub-optimal conditions enhances the photosynthetic CO<sub>2</sub> fixation, stomatal conductance and water-use efficiency [66]. In addition, GB inhibits the stress-induced reduction in the energy trapping efficiency by protecting the oxygen-evolving center of photosystem II (PSII) and maintaining the PSII activity. Moreover, GB protects the photosynthetic apparatus from chronic photo-inhibition by preventing over-reduction of Q<sub>A</sub> [31]. Ohnishi and Murata [67] reported the abilities of GB to repair stress-induced photo damages of PSII. The inhibition of the PSII reaction core protein, D1, and its degradation was also reversed by GB [67]. Glycinebetaine also counteracts abiotic stresses by inducing RuBisCO activase and promoting CO<sub>2</sub> fixation [10].



**Figure 3:** Glycinebetaine acts as an alternate sink for absorbing the excess electrons leaked from the electron transport chains (ETCs) of mitochondria and chloroplast. GB inhibits chlorosis and protects lipids and proteins. It also exhibits chaperonin activity and preserves enzyme structures during oxidative stress. GB recharges the antioxidant machinery by activating the enzymes involved in the ascorbate-glutathione (AsA-GSH) cycle and glyoxylase I (GlyI). The latter enables efficient detoxification of the toxic methylglyoxal (MG). GB also induces the expression of downstream stress-inducible genes encoding late embryogenesis abundant (LEA) proteins

## 6 Glycinebetaine-Induced Antioxidant Defense

Being an osmolyte, GB acts as a potential scavenger of the toxic ROS produced during abiotic stress [68]. The ROS consists of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide (O<sub>2</sub><sup>•-</sup>) and hydroxyl radicals (OH<sup>•</sup>), which are produced when electrons from the mitochondrial and chloroplastic electron transport systems leak and interact with O<sub>2</sub> in the dearth of conventional electron acceptors. The ROS degrades the membrane systems by inducing uncontrolled lipid peroxidation and triggers distortions in protein and nucleic acid structures [69]. Almost all kinds of abiotic stresses result in a burst in the production of ROS. Effective ROS scavenging by non-enzymatic antioxidants like Pro, polyamines (PAs), ascorbate (AsA), glutathione (GSH), etc. and enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase

(GR), etc. is crucial for maintaining the physiological processes under adverse situations [68]. The cellular accumulation of GB recharges the antioxidant machinery and preserves the optimum osmotic pressure during desiccation [43]. Interestingly, GB itself does not exhibit antioxidant activity but induces the ROS-defense machinery [70]. Exogenous application of GB in tobacco cell cultures induced the activities of the enzymes like APX, dehydroascorbate reductase (DHAR) and GR participating in the AsA-GSH cycle [71]. However, such GB-mediated induction was visible only under salt stress and not under control conditions, indicating towards the fact that GB regulates ROS detoxification by sensing its level within the cell [71]. Increased GB accumulation reduces cellular ROS production and stabilizes the rate of CO<sub>2</sub> assimilation in plants grown under unfavorable conditions [72].

The glycolytic metabolism produces methylglyoxal (MG) as a toxic by-product, which severely inhibits cell growth in plants. The MG content steeply increases during abiotic stresses and is required to be neutralized in order to maintain the cellular physiology [73]. Banu et al. [74] reported the exogenous application of GB to decrease the MG and H<sub>2</sub>O<sub>2</sub> content in tobacco cells cultured in a saline medium. The cells exhibited increased transcript accumulation of *GPX*. Hoque et al. [75] reported that application of GB reduced protein carbonylation in tobacco cell cultures in a saline medium. The treated cells also exhibited increased GSH redox state and activities of GPX, glutathione *S*-transferase (GST) and glyoxalase I (Gly I) during salinization. This effectively indicated that GB activates the integrated systems involved in antioxidant defense and MG detoxification [75; Fig. 3].

## 7 Glycinebetaine-Induced Abiotic Stress Tolerance in Plants

Glycinebetaine is a compatible solute capable of performing multiple functions as a non-enzymatic antioxidant. From our previous discussion, it is clear that GB crucially maintains normal cellular physiology under harsh environmental conditions. Such properties of this osmolyte have enabled its diverse participation in generating abiotic stress tolerance across plant species.

### 7.1 Drought

Water deficit or physical drought stress is one of the most prevalent types of edaphic stress, which severely limits the growth and yield of susceptible plants [64,76]. Some plant species have developed evolutionary strategies to tackle such stressful situations by triggering the accumulation of protective compatible solutes and antioxidative enzymes [77,78]. Several plant studies showed that GB protects the plants against drought stress (Tab. 2). Ibrahim et al. [79] compared the level of osmolyte accumulation in two cotton genotypes, Zhongmian 23 (stress tolerant) and Zhongmian 41 (sensitive). It was observed that the former exhibited its tolerance due to enhanced accumulation of GB, soluble sugar and K<sup>+</sup> under both drought (4% soil moisture) and salt stress (200 mM NaCl). Zhongmian 23 also displayed higher activities of SOD, POD, CAT, APX, and Na<sup>+</sup>/K<sup>+</sup>-ATPase [79]. In another recent study, it was observed that exposure to drought stress increased GB, Pro and soluble sugar content in the flag leaves of wheat plants [80]. Further, proteomic investigations using two-dimensional difference gel electrophoresis (2D-DIGE) identified 66 and 105 differentially accumulated protein (DAP) spots in the flag leaves and grains during drought stress. These proteins were mainly associated with oxidative stress responses, photosynthesis, starch, and energy metabolism [80].

Drought priming of wheat seeds promoted drought tolerance in the progenies of the treated seeds [81]. Successive application of drought priming in three generations resulted in improved photosynthetic rate and antioxidant capacity in the offspring plants. The physiological basis of such drought tolerance was attributed to higher BADH and  $\Delta^1$ -pyrroline-5-carboxylate synthase (P5CS) activities, leading to enhanced accumulation of GB and Pro in the offspring seedlings of the treated plants. Thus, GB acts as a crucial osmolyte, which promotes parental priming-induced drought tolerance [81]. Hydropriming and osmopriming of bread wheat seeds with 1.5% CaCl<sub>2</sub> promoted trans-generational drought memory and improved salt tolerance due to increased accumulation of leaf GB and Pro. These osmolytes reduced

malondialdehyde (MDA) and Na<sup>+</sup> contents and improved leaf area and water relation parameters in the offspring plants exposed to stress [82].

**Table 2:** Recent instances of GB promoting protection against drought and salinity

Species	Stress	Effects	Reference
<i>Gossypium hirsutum</i> genotype Zhongmian 23	Drought/Salt	Tolerance due to enhanced GB accumulation and activities of antioxidant enzymes	Ibrahim et al. [79]
<i>Triticum aestivum</i>	Drought	Glycinebetaine accumulation, together with enhanced photosynthesis, starch and energy metabolism	Deng et al. [80]
<i>T. aestivum</i>	Drought	Parental drought priming induced GB accumulation and drought tolerance in offspring seedlings	Wang et al. [81]
<i>T. aestivum</i>	Drought/Salt	Osmoprimering with CaCl <sub>2</sub> promoted GB accumulation and trans-generational drought memory and salt tolerance	Tabassum et al. [82]
<i>Nerium oleander</i>	Drought/Salt	Accumulation of GB recharged antioxidant machinery, inhibited transport of toxic ions from roots to shoots	Kumar et al. [83]
<i>Spinacea oleracea</i>	Drought	H <sub>2</sub> S-induced accumulation of GB and trehalose	Chen et al. [84]
<i>Zea mays</i>	Salt	Foliar sprays of Cu and Zn mitigated salt stress by promoting GB, Pro, amino acid and sugar accumulation	Iqbal et al. [85]
<i>Solanum lycopersicum</i>	Salt	Symbiosis with <i>Piriformospora indica</i> induced GB accumulation	Ghorbani et al. [86]
<i>Pistacia vera</i>	Salt	Accumulation of GB and associated osmolytes enhanced Na <sup>+</sup> and Cl <sup>-</sup> exclusion across cultivars	Akbari et al. [87]
<i>Suaeda aegyptiaca</i>	Salt	Enhanced GB content under stress conferred the halophytic character	Sabzalian et al. [88]
<i>Spiribacter salinus</i>	Salt	Enhanced GB content under stress conferred the halophytic character	Leon et al. [89]
<i>Lycium ruthenium</i>	Salt	Induction of <i>AMADH1</i> during salt stress triggered GB accumulation	Liu et al. [90]

Analysis of the upstream promoter of *phosphoethanolamine N-methyltransferase (PEAMT)* gene (involved in choline and hence GB biosynthesis) led to the identification of stress-inducible *cis*-acting elements like abscisic acid (ABA)-responsive elements (ABREs) involved in salt and drought stress responses [91]. The promoter assay using  $\beta$ -glucuronidase (GUS) fusion displayed significant GUS expression under drought, salt, and oxidative stresses. This indicates the inducible status of this gene in accelerating GB accumulation under abiotic stress [91]. In another study, Kumar et al. [83] showed the involvement of GB in conferring drought and salt tolerance in ornamental plant *Nerium oleander*. The stressed plants also accumulated soluble carbohydrates and exhibited mechanisms, which inhibited the transport of toxic ions from the roots to the aerial biomass [83].

## 7.2 Salt

Salinity is regarded as the physiological drought stress, where Na<sup>+</sup> ions present in the soil at a toxic level infiltrate the cell membrane and disrupt endogenous osmotic pressure. A large array of enzymes and proteins are inhibited in the presence of excess Na<sup>+</sup> ions [92]. Being a potential osmoprotectant, GB mitigates salt-induced injuries by maintaining the cellular turgor, protecting biomacromolecules and inducing antioxidant levels (Tab. 2).

Foliar treatment of zinc (Zn) and copper (Cu) mitigated the injurious effects of salt by enhancing the contents of GB, Pro, total free amino acids and sugars in *Z. mays* seedlings [85]. Ghorbani et al. [86]

recently reported that inoculation of tomato plants with the symbiotic fungus, *Piriformospora indica*, improved the growth parameters under salt stress. Symbiotic association triggered the accumulation of GB and Pro, which efficiently protected the chlorophyll (chl) pigments, and hence, the plants could maintain enhanced photosynthetic efficiency under sub-optimal edaphic conditions. This finding opens a new avenue, where GB is associated with symbiosis to regulate abiotic stress tolerance in plants. Glycinebetaine, Pro, total phenolics and soluble carbohydrate content sharply increased in the rootstock and leaves of pistachio plants on exposure to salt stress [87]. The accumulation of the osmolytes also facilitated exclusion of  $\text{Na}^+$  and  $\text{Cl}^-$  in the rootstocks of the assayed cultivars grown under saline conditions [87].

Kageyama et al. [93] recently identified a novel methyltransferase, TpGSDMT in the marine diatom, *Thalassiosira pseudonana* that catalyzed a three-step synthesis of GB. Due to the induction of this gene during salt stress, it was proposed to be involved in salt tolerance in *T. pseudonana* cells [93]. In an interesting study, Carillo [94] reported the accumulation of GABA and not GB in durum wheat plants exposed to salt stress. It has been proposed that the stress associated metabolomic plasticity favors the GABA shunt instead of GB production in salt-stressed durum wheat seedlings [94]. Sabzalian et al. [88] observed that the GB content did not markedly increase in the halophytic plant species *Distichlis spicata* on exposure to salt stress. This might be due to the fact that the species being a halophyte maintains high endogenous levels of GB, which efficiently maintains cellular osmotic pressure under stress. However, the GB content steeply increased under salt stress in another halophytic species, *Suaeda aegyptiaca* indicating towards the stress-induced metabolic plasticity existing across plant species [88]. Leon et al. [89] also reported the effectiveness of GB and arsenobetaine in ameliorating salt-induced damages in the halophile, *Spiribacter salinus*. The radiolabeled GB was used to study its transport, and it was observed that the GB pool was intricately associated with the presence of salt in the growth medium. Recently, the salt-induced *aminoaldehyde dehydrogenase (AMADH)* similar to the conventional *BADH* gene was characterized in the halophyte *Lycium ruthenicum* Murr. [90]. Induction of *AMADH1* upon salinization led to significant GB accumulation in the seedlings as well as in fruits. On the contrary, virus-mediated *AMADH1* silencing interfered with GB accumulation and reduced the salt tolerating capacity of the plants [90]. This indicates the promising roles of GB in conferring salt tolerance traits across plants.

### 7.3 Toxic Metals/Metalloids

Over increasing population of the world has accelerated industrialization and urbanization resulting in accumulation and release of excessive amounts of different toxic metal/metalloids to the environment [95]. Excess concentrations of toxic metal/metalloids impose severe threats on both flora and fauna. Most of the toxic metal/metalloids are not bio-degradable and can bio-accumulate in living organisms; consequently, they can contaminate the entire food chain [96]. They affect the surroundings by destroying soil, water, and air properties, which reduce the quality and quantity of crop and eventually destroy human health [95]. Toxic metal/metalloids in the plant-growing media facilitate their entry to the plant cell, which causes oxidative damage due to overproduction of ROS. Every organism has well established antioxidant defense systems to defend its cells against ROS at a certain level. Moreover, plant cell increases different osmolyte synthesis to reduce ROS-induced oxidative damage [95]. Glycinebetaine is one of the most abundant osmolytes produced in higher plants under stressful environments and involved in the protection of plants against different toxic metal/metalloids stress [12,97,98; Tab. 3]. However, the natural manufacture of GB in cellular level is not sufficient to defend plants from severe stress. Under these circumstances, exogenous application of GB gradually becomes a familiar strategy to overcome abiotic stresses in plants including toxic metal/metalloids stress. However, its response differs with plant genotypes and stress type and intensity [12]. In most of the cases, exogenous application of GB decreased the accumulation of toxic metal/metalloids in the plant [49,98,99]. However, Duman et al. [47] showed the reverse result. They suggested that the addition of GB in the growing media under stress decreased the accumulation of Cd in the plant. In general, the increase or decrease of metal accumulation depends on the dose of both stress and GB. As GB scavenges a range of

ROS, promotes osmotic adjustment, stabilizes proteins and protects membrane integrity in plants under adverse conditions, it plays a vital role to confer tolerance in plants against different metal/metalloids toxicity [100]. Islam et al. [101] exposed suspension-cultured cells of *Nicotiana tabacum* L. to 100 mM CdCl<sub>2</sub> with or without 1 mM and 10 mM GB. They recorded that exogenous GB at 10 mM notably decreased the amount of MDA under Cd stress, but the application of 1 mM GB did not result in a noteworthy decrease of MDA content. Both the doses of GB decreased the Cd accumulation of plant. A similar reduction of MDA due to supplementation of GB was recorded in Cd-affected mung bean seedlings [102]. Duman et al. [47] treated *Lemna gibba* plant with different doses of Cd (0.5, 1, and 3 mM CdSO<sub>4</sub>.7H<sub>2</sub>O) and GB (0.5, 1, 2, and 5 mM). Exposure to 0.5 mM GB reduced the Pro content, but an increase was observed for 2 and 5 mM GB. Significant MDA content reduction was observed under 0.5 mM GB treatment in Cd affected plants. Later Bharwana et al. [99] checked the protective role of GB in cotton (MNH 886) plant against two levels of lead (Pb) stress [50 µM and 100 µM Pb(NO<sub>3</sub>)<sub>2</sub>]. They observed that supplementation of 1 mM GB in Pb-affected plant reduce the adverse effects of Pb stress in cotton plants. Excessive electrolyte leakage (EL) and MDA production due to Pb stress notably decreased in both leaf and root of the cotton plant because of GB treatment. Moreover, transpiration rate (TR) and water use efficiency (WUE) significantly increased in the Pb-affected plant due to GB supplementation, which resulted in improved plant stress tolerance. Rasheed et al. [103] conducted an experiment with two wheat cultivars, viz., Millat-2011 and Punjab-2011. They exposed two-week old wheat plants to Cd stress. After one week of Cd treatment, they sprayed GB on the leaves of Cd-affected wheat plants. Two weeks later, they observed that spraying of GB showed tolerance to Cd toxicity. They recorded that MDA content sharply decreased in the Cd-treated plants after GB application. In another experiment, 53 day-old perennial ryegrass (*Lolium perenne*) seedlings were exposed to seven different doses of Cd (0 and 0.5 mM CdCl<sub>2</sub>) and GB (0, 20, and 50 mM) separately or in combination in hydroponic culture for seven days [48]. Cadmium stress considerably decreased the normalized relative transpiration (NRT) and increased EL and MDA content. Exogenous GB ameliorated the adverse effects of Cd stress on perennial ryegrass by increasing NRT and decreasing EL and MDA content in Cd-stressed plants. Furthermore, perennial ryegrass with 20 mM GB application suppressed the Cd accumulation in both shoots and roots. Their findings concluded that exogenous application of GB with 20 mM is the best strategy to ameliorate the detrimental impacts of Cd stress on perennial ryegrass. Application of GB contributes to decreased Cr concentrations in wheat plants and plays different biochemical function for detoxification of heavy metals [98]. Farooq et al. [49] treated four-weeks-old cotton genotype (MNH 886) with different Cd levels (0 µM, 1.0 µM, and 5.0 µM CdCl<sub>2</sub>) and GB (0 and 1 mM GB) for six weeks. Cadmium treatments significantly decreased TR and WUE but increased the MDA content and EL in both leaves and roots as compared to control in a dose-dependent manner. On the other hand, GB application in Cd-stressed plants considerably increased the TR and WUE [except (1.0 µM CdCl<sub>2</sub> + 1 mM GB) treatments] but decreased the levels of MDA and EL in both leaves and roots in contrast to respective only-Cd treatments. Radicle bearing seeds of *Pennisetum typhoideum* were treated with Ni, GB and aspirin separately or in combination, where Ni increased the level of MDA, 4-hydroxy-2-nonenal and lipoxygenase (LOX), whereas decreased the membrane stability index (MSI). Supplementation of GB with Ni reversed the above-mentioned parameters [104]. Exogenous GB increased MSI by 122% and decreased the level of MDA, 4-hydroxy-2-nonenal and LOX by 37, 17 and 23% in *P. typhoideum* plants.

**Table 3:** Summary of the GB-induced metal/metalloid, extreme temperatures and waterlogging tolerance in plants

Plant species and genotypes	Dose and duration of stress	Dose and duration of GB	Effect of GB	References
<i>Vigna radiata</i> cv. Binamoog-1	Cd; 1 mM, 2 d	5 mM, 2 d	<ul style="list-style-type: none"> <li>Decreased MDA content by 30%</li> </ul>	Hossain et al. [102]
<i>Gossypium hirsutum</i> L. MNH 886	Pb; 50 $\mu$ M Pb(NO <sub>3</sub> ) <sub>2</sub> , 42 d	1 mM, 42 d	<ul style="list-style-type: none"> <li>Decreased electric leakage (EL) by 39% in both leaf and root</li> <li>Decreased MDA content by 37% in leaf and 34% in root</li> <li>Increased transpiration rate</li> <li>Increased water use efficiency</li> <li>Decreased the Pb accumulation in the plant parts</li> </ul>	Bharwana et al. [99]
<i>Triticum aestivum</i> , Millat-2011 and Punjab-2011	Cd; 200 $\mu$ M, 21 d	20 mM, 14 d	<ul style="list-style-type: none"> <li>Considerably decreased MDA content</li> </ul>	Rasheed et al. [103]
<i>Lemna gibba</i> L.	Cd; 0.5 CdSO <sub>4</sub> .7H <sub>2</sub> O, 6 d	5 mM, 14 d	<ul style="list-style-type: none"> <li>Increased Pro content</li> <li>Decreased MDA content</li> </ul>	Duman et al. [47]
<i>Lolium perenne</i>	Cd; 0.5 mM CdCl <sub>2</sub> , 7 d	50 mM, 7 d	<ul style="list-style-type: none"> <li>Decreased MDA content by 28%</li> <li>Decreased electric conductivity by 13%</li> <li>Increased normalized relative transpiration (NRT) by 85%</li> </ul>	Lou et al. [48]
<i>G. hirsutum</i> L. MNH 886	Cd; 5.0 $\mu$ M CdCl <sub>2</sub> , 42 d	1 mM, 42 d	<ul style="list-style-type: none"> <li>Increased transpiration rate by 44%</li> <li>Increased water use efficiency by 27%</li> <li>Decreased MDA content</li> <li>Decreased electrolyte leakage</li> <li>Decreased Cd content in leaf, stem, and root</li> </ul>	Farooq et al. [49]
<i>Pennisetum typhoideum</i>	Ni; 135 ppm NiCl <sub>2</sub> , 5 d	100 $\mu$ M, 5 d	<ul style="list-style-type: none"> <li>Membrane stability index (MSI) increased by 122%</li> <li>Malondialdehyde level decreased by 37%</li> <li>4-hydroxy-2-nonenal level decreased by 17%</li> <li>Lipoxygenase activity decreased by 23%</li> </ul>	Xalxo et al. [104]
<i>Hordeum vulgare</i> L. cv. Haider-93	Heat; 40 $\pm$ 1/32 $\pm$ 1°C day/night temperature, 10 d	20 mM, 24 h	<ul style="list-style-type: none"> <li>Increased water potential</li> <li>Decreased relative membrane permeability</li> <li>Decreased leakage of important ions (Ca<sup>2+</sup>, K<sup>+</sup>, and NO<sub>3</sub><sup>-</sup>)</li> </ul>	Wahid and Shabbir [50]
<i>Saccharum</i> sp. cv. HSF-240	Heat; 42°C, 21 d	20 mM, 8 h	<ul style="list-style-type: none"> <li>Improved the accumulation of soluble sugars</li> <li>Protected the developing tissues</li> </ul>	Rasheed et al. [57]
<i>Tagetes erecta</i> , Narai Yellow, Bali Gold and	Heat; 39°C/29°C day/night temperature, 15 d	0.5 and 1 mM, 15 d	<ul style="list-style-type: none"> <li>Increased transpiration rate</li> <li>Decreased production of MDA</li> </ul>	Sorwong and Sakhonwasee [58]

Plant species and genotypes	Dose and duration of stress	Dose and duration of GB	Effect of GB	References
Columbus Orange			<ul style="list-style-type: none"> <li>• Decreased cell death</li> </ul>	
<i>Saccharum</i> sp. cv. HSF-240	Chilling; 15°C, 2 d	10 mM GB, 8 h	<ul style="list-style-type: none"> <li>• Improved the K<sup>+</sup> and Ca<sup>2+</sup> nutrition</li> <li>• Increased free Pro and GB content</li> <li>• Enhanced levels of soluble sugars</li> </ul>	Rasheed et al. [52]
<i>G. hirsutum</i> cv. Lumianyan19	Chilling; 5°C, 3 d	400 µg/ml, 14 h	<ul style="list-style-type: none"> <li>• The increased relative water content</li> <li>• Decreased cell membrane damage</li> </ul>	Cheng et al. [23]
<i>Solanum lycopersicum</i> L. cv. Roma	Waterlogging; 25 d	50 mM, 25 d	<ul style="list-style-type: none"> <li>• Decreased MDA content by 27%</li> <li>• Decreased membrane injury by 12 and 27% in leaf and root, respectively</li> <li>• Decreased Na accumulation</li> <li>• Increased K and Ca accumulation</li> </ul>	Rasheed et al. [105]

#### 7.4 Extreme Temperature

Due to the global climate change, extreme temperature (both high and low) is becoming the most important concern for the plant scientists, worldwide [106]. Every biochemical process of the plant is governed by a crucial temperature range. Where the rise or fall of temperature is beyond the critical threshold for a considerable period, it causes irreversible damage to plant growth and development [106]. In many research, both endogenous and exogenous GB confirmed its positive interaction with improving plant stress tolerance (Tab. 3).

Heat stress adversely affects plant physiology and biochemistry, while GB plays a protective role under stressful conditions. For confirmation, Wahid and Shabbir [50] pretreated the seeds of *Hordeum vulgare* L. cv. Haider-93 with different doses (10, 20, 30, 40 and 50 mM) of GB for 24 h. The seedlings were next exposed to heat stress ( $40 \pm 1/32 \pm 1^\circ\text{C}$  day/night temperature). They observed that 20 mM GB increased water potential and reduced relative membrane permeability and leakage of important ions including Ca<sup>2+</sup>, K<sup>+</sup> and NO<sub>3</sub><sup>-</sup> under heat stress condition. Similarly, GB improved the accumulation of soluble sugars and protected the developing tissues from heat stress effect in sugarcane (*Saccharum* sp. cv. HSF-240; [57]). Addition of GB to the germination medium or imbibition of seeds in a solution of GB enhanced the tolerance of wild-type tomato (*S. lycopersicum* L. cv. 'Moneymaker') seeds to high temperature. The seeds treated with exogenous GB also expressed heat-shock genes at elevated levels and accumulated more HSP70 than controls [107]. Sorwong and Sakhonwasee [58] checked the effects of GB under heat stress conditions. They exposed three marigold cultivars, namely, 'Narai Yellow', 'Bali Gold', and 'Columbus Orange' to heat stress (39°C/29°C day/night temperature). Heat stress conditions caused photoinhibition and low levels of CO<sub>2</sub> assimilation rate, stomatal conductance, and transpiration rate in all marigold cultivars in comparison with their respective control plants. Foliar applications of GB at 0.5 and 1 mM alleviated the above-mentioned negative impacts, improved water status and lowered the production of MDA as well as increased stress tolerance.

Nayyar et al. [63] grew *C. arietinum* plants under chilling stress (12-14/3-5°C day/night temperatures) in combination with exogenous GB (1 mM). They observed that GB reduced the chilling-induced damages of the plant. Importantly, GB reduced EL by 63% and increased cellular respiration by 69%. Xing and Rajashekar [51] experimented with *A. thaliana* (Columbia) under cold stress (4/2°C day/night temperature) up to four weeks. They recorded that exogenous GB (10 mM) increased freezing stress tolerance in *A.*

*thaliana* (Columbia) plant. Sugarcane (HSF-240) bud, pretreated with 10 mM GB for 8 h, showed improvement in chilling stress tolerance [52]. Pretreatment by GB improved the K<sup>+</sup> and Ca<sup>2+</sup> nutrition, increased free Pro and GB content and levels of soluble sugars, which finally developed chilling stress (15°C for 2 d) tolerance in sugarcane. Recently, Cheng et al. [23] demonstrated that exogenous GB treatment of seeds increases the chilling tolerance of cotton at the seedling stage. Moreover, GB decreases the membrane damage and improves the antioxidant defense system in the cotton plant.

### **7.5 Waterlogging and UV Radiation**

Waterlogging/flooding stress inflicts serious hazard to plant survival by upsetting the nutritional balance and obstructing diverse physiological and biochemical activities of the plant, which results in a disturbance in growth and development, and ultimately yield loss [1]. Use of different exogenous protectant to overcome waterlogging/flooding is common in plant science, but the potential use of GB in alleviating the effects of waterlogging/flooding stress in plants is very rare. Rasheed et al. [105] investigated the effect of GB on the growth and physiology of tomato plants under waterlogged conditions (Tab. 3). They imposed waterlogging at 15 day-old tomato seedlings (cv. Roma) for 25 days. This was achieved by blocking the drainage holes and supplying water to form a three cm film of stagnant water at the surface of each pot. Glycinebetaine (50 mM) was applied 10-day after waterlogging imposition and continued up to 50 d old plant. After 15-day, they observed different data. Waterlogging enhanced MDA content and deteriorated membrane integrity. It also increased the concentration of Na and reduced the concentrations of K and Ca (root only) in the different parts of the plant. On the other hand, GB application in waterlogged plant decreased MDA content; improved membrane integrity and nutritional status of the plant, which resulted in waterlogging stress tolerance.

Due to depletion of stratospheric ozone layer, ultraviolet radiation (UV) can easily enter to the ground surface of the earth and hamper the living organism. Ultraviolet radiation is capable of negatively influencing plant growth and developmental processes [26,108]. Different research work is going on to increase tolerance to UV-induced stress. Moussa and Jaleel [109] studied the effect of GB on Fenugreek plant (cv. Giza 2) under UV-induced stress. They exposed dry seeds to different doses of irradiation of 0.0, 25, 50, 100, and 150 Gray (1 Gray = 100 rad), using a gamma source, <sup>60</sup>Co, with the strength of 500 Ci and dose rate of 0.54 Gy·min<sup>-1</sup>. Then, they soaked the irradiated and non-irradiated seeds in 50 mM of GB for 24 h. Finally, plants were grown for 30 days from the seeds to check the effect of both radiation and GB. They observed that gamma radiation treatment alone decreased the accumulation of reducing, non-reducing and total soluble sugars of the plant, but GB-soaked gamma irradiated plants showed an increase in the above sugar contents. Hence, GB protection was more pronounced against gamma radiation stress at lower doses.

## **8 Interaction of GB with Phytohormones and Other Signaling Networks**

Phytohormones are the chief signaling molecules in plants, which critically regulate abiotic stress responses across plant species. Stress hormones like ABA, ethylene, salicylic acid (SA) and other traditional plant growth regulators (PGRs) are essentially involved in the signalosome regulation in plants [110]. Other molecules like the gasotransmitter nitric oxide (NO), charged PAs and even plant nutrients equilibrate the cellular environment during responses against sub-optimal conditions [111]. Glycinebetaine induces the antioxidant machinery in order to detoxify ROS molecules and stabilize the cellular osmoticum during abiotic stresses. This process demands intricate interactions between the molecular factors involved in signaling.

## 8.1 Phytohormones

### 8.1.1 Abscisic Acid

Abscisic acid is regarded as the universal stress phytohormone involved in myriad physiological regulations under abiotic stresses. Plant tissues accumulate high ABA contents in response to stress [112]. This allows rapid stomatal closure and protects the plants from unwanted desiccation and water loss [113]. Application of exogenous ABA stimulated GB accumulation in barley plants exposed to salt, drought and low-temperature stress [114]. According to the report of Ishitani et al. [115], it can be suggested that such ABA-induced GB accumulation was due to increased *BADH* transcription in the leaves and roots of barley plants. In an interesting study, it was observed that exogenous application of either ABA or GB increased freezing tolerance in *Arabidopsis*. However, treatment with only ABA stimulated GB production, indicating the fact that ABA possibly acts upstream of the GB biosynthetic pathway [51].

The inter-connection between ABA and GB synthesis has been reported [116]. Goggin et al. [117] showed that treatment of plants with fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl] phenyl]-4[1H]-pyridinone) inhibited ABA biosynthesis by blocking the carotenoid pathway. Interestingly, application of fluridone suppressed ABA levels and as well as *BADH* transcription in *Sorghum bicolor* [116]. Thus, reduction in endogenous ABA content also suppressed the endogenous GB level. Similar fluridone-mediated inhibition of ABA and GB was also reported by Zhang et al. [118] in corn plants. In a study involving the temporal regulation between ABA and GB, it was observed that drought stress initially increased the endogenous ABA content followed by *BADH* transcription and then GB content within the cells of the *Z. mays* seedlings [118].

Accumulation of ABA upon desiccation stress effectively induces stomatal closure, which could lead to reduced photosynthetic activities. However, in previous sections, we have discussed regarding the roles of GB in promoting the photosynthetic efficiency during abiotic stresses. Since ABA positively regulates the production of GB, the functions of ABA and GB might seem to be contradictory. However, Franks and Farquhar [119] reported that exogenous ABA application reduced stomatal conductance without affecting the photosynthetic capacity in *Tradescantia virginiana*. This inference probably highlights the deeply correlated functioning of ABA and GB in response to abiotic stresses.

### 8.1.2 Salicylic Acid

Endogenous SA accumulation is also associated with abiotic stress tolerant phenotype in plants. Salicylic acid triggers the production of enzymatic and non-enzymatic antioxidants, which efficiently scavenge ROS and confer protection against oxidative stress [120]. Exogenous application of SA has also been useful in generating tolerance against heavy metal toxicity, salt, drought and extremes of temperatures [121]. Aldesuquy et al. [122] showed that co-application of SA and GB further increased the yield in drought-stressed wheat plants compared to the amount of grain production in the stressed plants treated individually with SA or GB.

### 8.1.3 Ethylene

The gaseous hormone ethylene is constitutively required for optimal plant growth and development [2]. Ethylene also promotes tolerance to moderately intense forms of freezing, etc. by promoting stem elongation, expansion of leaf area, stem radial growth and leaf thickness [123]. Ethylene and GB biosynthesis are intricately linked with each other due to the involvement of the common mediator molecule, choline. Ethylene biosynthesis is facilitated by the transfer of a methyl group from *S*-adenosyl methionine (SAM) to choline. Thus, ethylene production might occur at the expense of GB synthesis or vice versa. Plants subjected to severe abiotic stress accumulate endogenous GB for ensuring growth and survival [28]. This possibly occurs at the expense of ethylene production due to channelization of choline towards GB synthesis. High ethylene accumulating species like tobacco and tomato did not accumulate

GB under cold or other forms of stress. It was also observed that the species with low ethylene accumulation exhibited increased GB synthesis in response to abiotic stresses [123].

#### 8.1.4 Traditional PGRs

Traditional PGRs consist of auxins, gibberellic acids (GAs) and cytokinins (CKs), which are involved in growth, development, stem elongation, polar transport of solutes, phototropism and gravitropism, cell division and reproductive initiation [124,125]. The antagonistic relationship between ABA and GA is known. Increase in the endogenous ABA content suppresses GA synthesis during abiotic stresses [112]. Since ABA accumulation triggers GB synthesis, it can be inferred that GB and GA are negatively associated with each other. In line with this proposition, Bao et al. [126] observed lower GA<sub>1</sub> levels in the transgenic *L. perenne* plants overexpressing the *CMO* and *BADH* genes from spinach chloroplasts. Cytokinin and GB accumulation are also negatively correlated. Kathuria et al. [127] observed that transgenic rice plants overexpressing the *codA* gene exhibited increased expression of *CK dehydrogenase 1 (CKX1)*, which encodes a CK degrading enzyme. The plants accumulated high amounts of GB and low concentration of CKs. Auxin also manifested negative regulation of GB synthesis. The exogenous application of the synthetic auxin, dicamba reduced *CMO* expression and hence GB accumulation in *Kochia scoparia* plants exposed to salt stress [128].

#### 8.2 Gasotransmitters

Hydrogen sulfide (H<sub>2</sub>S) and nitric oxide (NO) are gaseous molecules involved in myriad signaling processes in plants. These gasotransmitters are also involved in promoting plant tolerance against multiple abiotic stresses [78,129]. They act as crucial concentration-dependent redox-related signaling moieties.

Exogenous application of sodium hydrosulfide (NaHS) in *S. oleracea* seedlings increased the cellular production of H<sub>2</sub>S. As a result, the content of GB and trehalose steeply increased. The transcription of *BADH* and *CMO* genes were also up-regulated upon NaHS treatment. The H<sub>2</sub>S-induced accumulation of GB programmed the antioxidant machinery and promoted drought tolerance in the treated seedlings [84]. These reports pointed towards unknown signaling interactions between H<sub>2</sub>S and GB, which have not been exhaustively investigated. Recently, Zanganeh et al. [130] also showed that seed priming with SA and NaHS triggered the accumulation of GB and NO in *Z. mays* seedlings grown under Pb stress. The treated plants also exhibited lowered expression of the ethylene biosynthetic gene, *1-aminocyclopropane-1-carboxylate synthase 6 (ACS6)*. This was due to channelization of choline towards GB synthesis instead of ethylene production. The reduction of PA biosynthesis gene, *SAM decarboxylase (SAMDC)* and accumulation of NO under Pb stress indicate that arginine metabolism is probably channelized towards NO production instead of PA synthesis [130]. Thus, H<sub>2</sub>S, NO, and GB altogether positively interact to confer Pb tolerance in maize plants. The positive regulatory association between NO and GB was observed in maize seedlings exposed to oxidative stress [131]. Seedlings treated with nitric oxide synthase (NOS) inhibitor exhibited reduced *BADH* expression and hence GB accumulation. Due to reduced GB accumulation, the activity of the antioxidant enzymes was also suppressed. The *CAT* expression was reduced leading to elevated H<sub>2</sub>O<sub>2</sub> accumulation in the NOS-inhibitor-treated seedlings [131].

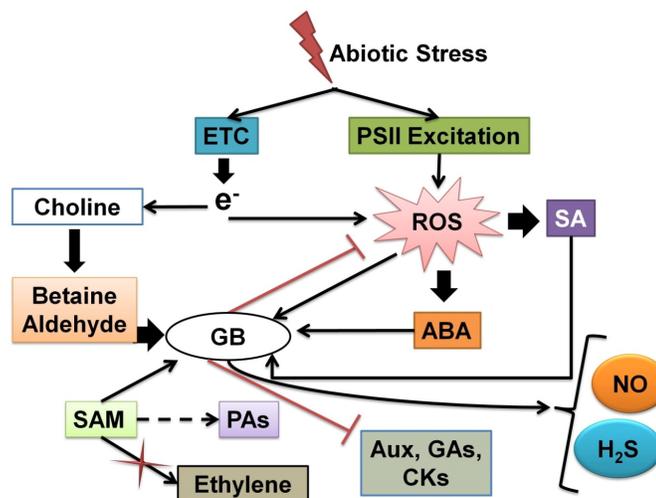
#### 8.3 Polyamines

Polyamines and GB are compatible solutes, which together regulate the cellular osmoticum during abiotic stresses [132,133]. Polyamines like putrescine, spermidine and spermine perform similar functions as GB and hence are correlated with the latter. Seedlings primed with spermidine and spermine, prior to salt treatment, showed *BADHI* expression to be higher in the shoots of salt-tolerant rice cultivar, Nonabokra than the sensitive rice cultivar, IR-64, as compared to non-primed seedlings under stress. On the contrary, in case of roots, priming with spermidine and spermine increased *BADHI* transcript accumulation in Nonabokra, while it decreased gene expression in IR-64 roots exposed to 75 mM NaCl

[134]. Polyamine priming of salt stressed-seedlings likewise enhanced *BADH1* expression in the shoots and roots of another salt-sensitive, aromatic rice cultivar, Gobindohog [135]. All these results clearly show the cross-talk between PAs and GB-biosynthesis machinery in rice under salt stress. Overexpression of *aldehyde dehydrogenase 10A8* (*ALDH10A8*) and *ALDH10A9* (encoding functional BADH) in *Arabidopsis* increased GB content under salt stress. The transgenic plants also exhibited an increase in the levels of free PAs like agmatine, cadaverine, and tyramine along with a reduction in the putrescine and spermidine content upon salinization [136]. Rippa et al. [137] reported the ability of BADHs to oxidize 3-aminopropanal (an intermediate compound in PA catabolism). Oxidation of spermine by polyamine oxidase (PAO) produced 3-aminopropanal, which was utilized as a substrate by ALDH10A8 and ALDH10A9 [136]. This shows that BADH isoforms are directly involved in the catabolism of spermine. Choudhary et al. [138] observed that exogenous application of epibrassinolide (an active brassinosteroid) and spermidine remarkably increased the titers of cellular antioxidants like GB, Pro, AsA, GSH and total phenolics in *Raphanus sativus* seedlings exposed to Cr stress. As a result, excess ROS was efficiently scavenged, and stress tolerance was conferred. This evidently illustrates the tripartite regulation of PAs, GB, and brassinosteroid in generating stress-induced responses. Synthetic PA (diamino hydroxyl ethyl phospho mineral and diamino hexanoic mineral amino ethanoic acid) supplementation of the perennial medicinal herb, *Stevia rebaudiana*, increased the endogenous GB and Pro contents on exposure to cold stress [139].

### 9 A model for GB-Induced Cross-Tolerance Under Abiotic Stress

In previous discussions, we revealed the diverse roles of GB in generating abiotic stress tolerance in plants. Since any kind of abiotic stress generates oxidative damages, GB, being an osmolyte, protects the system from such injuries. It can be proposed that increasing the endogenous accumulation of GB against one type of stress can also promote cross-tolerance against other abiotic stresses. In this section, we have proposed an outline of interactions between GB, phytohormones, photosynthetic efficiency and amelioration of ROS-induced injuries (Fig. 4) in order to suggest an interactive model on GB-induced cross-tolerance against abiotic stresses.



**Figure 4:** The physiological interactive model of GB-mediated stress tolerance in plants. The excess electrons, leaked from electron transport chains (ETCs) of mitochondria and chloroplast, are utilized in the process of choline generation and formation of betaine aldehyde. Over-excitation of photosystem II and electron leakage under abiotic stress induces uncontrolled production of reactive oxygen species (ROS). This promotes synthesis of stress phytohormones like salicylic acid and abscisic acid, which in turn stimulates the biosynthesis of GB. Gasotransmitters like nitric oxide (NO) and hydrogen sulphide (H<sub>2</sub>S) co-operate with GB to promote abiotic stress tolerance in plants. High endogenous levels of GB inhibit plant growth regulators like auxins (Aux), gibberellic acids (GAs) and cytokinins (CKs). S-

adenosyl methionine (SAM) is the common precursor of ethylene, GB and polyamine (PA) pathways. Under abiotic stress, SAM is more channelized towards GB and PA biosynthesis, which results in reduced ethylene content in GB accumulating plants

Plants exposed to any abiotic stress experience an increase in the PSII excitation pressure, resulting in imbalances in the electron transport chain (ETC) and the stromal redox equilibrium [2]. This initiates a burst in ROS production leading to extreme oxidative stress. In order to tackle uncontrolled damages, GB accumulation is stimulated via the channelization of SAM (common precursor of ethylene, PA and GB) towards the GB biosynthetic pathway [130]. Excess generated electrons are utilized in the process of choline oxidation to betaine aldehyde catalyzed by CMO. Thus, GB synthesis reduces PSII excitation pressure, restores the redox balance within the chloroplast and confers protection to the photosynthetic machinery [123]. The stress phytohormones like ABA and SA, which are synthesized in the majority of the abiotic stresses co-operatively stimulate the accumulation of GB within the cell [112,121]. The redox imbalance and ROS accumulation also signals the up-regulation of ABA biosynthetic genes and promotes ABA production, which again empowers the generation of GB and associated compatible solutes, dedicated towards physiological protection [2]. Under stress situations, the plant induces a systemic response, which channelizes the metabolic equivalents towards amelioration and protection rather than growth and development. In line with this theory, stress-triggered GB accumulation is accompanied by a reduction in the levels of traditional PGRs like auxins, GAs, and CKs [127]. Glycinebetaine also co-operates with gasotransmitters like NO and H<sub>2</sub>S via less studied interactive pathways to promote stress tolerance [130]. The model presents a compiled outcome of different observations across various plant species under diverse sub-optimal conditions. Thus, it can be stated that GB acts as a crucial compatible solute conferring protection against the basic photosynthetic damages and ROS-induced injuries.

## **10 Exogenous Use of GB: From Lab to the Field**

Many plant species naturally accumulate GB as major organic osmolytes, responsive to abiotic stresses and play adaptive roles in osmotic adjustment and protecting subcellular structures. However, these plants also show better performance when grown under normal conditions. Not all plants accumulate GB in a considerable amount to alleviate the adverse effect of stresses because of the inability of synthesizing or accumulating GB. Thus, extensive research has been undertaken to induce GB-mediated abiotic stress tolerance via genetic improvement as well as generating transgenic plants. However, all of these approaches have limitations and were unable to synthesize adequate amounts of GB. Therefore, the third and short-cut method for inducing GB-mediated tolerance, which has gained popularity is the exogenous GB application through seed treatment/priming, application through irrigation water and foliar spray [39]. Hence, this section of review article will highlight examples of the successful exogenous application of GB to improve growth and yield of plants under normal as well as stress conditions.

### ***10.1 Exogenous GB Application and Plant Growth Promotion***

Exogenous applications of GB to plants, as seed priming or pretreatment (before stress), co-treatment (during stress), and/or post-stress period, have been shown to increase the internal GB levels, thereby enhancing plant growth both under stressed or non-stressed conditions [39; Tab. 4]. However, the response differed greatly among plant species. The first thing to be considered is that not all species are responsive to the exogenous application and secondly, the effective dose varied from species to species, and sometimes over-dose may show toxic effects resulting in growth inhibition. Therefore, optimum concentrations and appropriate plant developmental stages must be carefully identified for the exogenous application.

**Table 4:** Summary of the effects of the exogenous glycinebetaine application on growth promotion of different plant species

Plant Species	Dose of exogenous GB	Growth promoting effect of GB	Reference
<i>O. sativa</i> cv. Pathumthani1	50 mM	Day dependent increase in plant height under non-stressed and salt-stressed conditions	Cha-um and Kirdmanee [140]
<i>O. sativa</i> cv. NIAB-IRRI-9 and Super Basmati	15 mM	Slightly effective in increasing root FW and DW, and biomass of NIAB-IRRI-9 rice under the non-saline condition, but efficiently improve the growth parameters under 60 mM and 120 mM salt in both cultivars	Shahbaz and Zia [141]
<i>O. sativa</i> cv. Pathumthani1	100 mM	Increased plant height, panicle length under normal and drought condition as well	Cha-um et al. [142]
<i>O. sativa</i> cv. Xiushui 63	100 $\mu$ M	Increased FW and DW of shoot and root. Furthermore improved growth retardation under 50 $\mu$ M Cd stress	Cao et al. [97]
<i>O. sativa</i> cv. MR 220 and MR 253	5 and 10 mM	Enhanced FW, DW and chlorophyll contents	Teh et al. [143]
<i>Triticum aestivum</i> L. cv. MH 97	50 mM	Improve shoot FW and DW, root FW and DW, shoot length and leaf area both under control and drought condition	Shahabaz et al. [144]
<i>T. aestivum</i> cv. Punjab-2011	20 mM	Decreased shoot length. Increased root length and shoot FW, but root DW remained unchanged under nonstressed condition. Straight opposite picture was shown under Cd toxicity (200 $\mu$ M)	Rasheed et al. [103]
<i>Zea mays</i> L.	250 mM	Plant height and leaf dry weight increased, but leaf area and stem dry weight decreased.	Reddy et al. [145]
<i>Gossypium hirsutum</i> L.	100 mM	Stem height, number of nodes, and internodal length increased under well irrigated, and drought stressed conditions	Noreen et al. [146]
<i>G. hirsutum</i> L.	100 mM	Increase germination rate and plant height under well irrigated and dehydrated condition.	Aziz et al. [147]
<i>Brassica napus</i> L.	10, 15 and 20 mM	Plant height, root length, and biomass increased under normal condition, while significant improvement was observed in growth parameters comparison with stress (75% and 50 % of Field capacity)	Dawood and Sadak [148]
<i>B. napus</i> <i>B. campestris</i>	5 and 10 mM	Increased plant height, shoot and root fresh and dry weight under control and saline (65 and 130 mM salt) conditions	Khan et al. [13]
<i>Cucumis sativas</i>	10 mM	Increased the plant length, leaf number, shoot FW and DW, root FW and DW, and leaf area under both normal and salt-stressed (50 mM salt) conditions	Youssef et al. [15]
<i>Allium cepa</i>	25 and 50 mM	Increased leaf area, shoot fresh and dry weight I controlled situations. But also effectively increase these parameters under salt (4.80 dS m <sup>-1</sup> ) stress	Rady et al. [14]
<i>Solanum lycopersicum</i> L.	10 mM	Improved plant height, leaf number, leaf area under well-watered condition (100 % of ET <sub>0</sub> )	Ragab et al. [149]
<i>S. lycopersicum</i>	50 mM	Increased shoot and root dry matter content	Rasheed et al. [105]
<i>Lactuca sativa</i> L.	25 mM	Stem diameter, shoot, and root FW and DW increased under the non-stressed condition, but no significant increase regarding plant height. But significant increase regarding all growth parameter was observed under salt stress (100 mM NaCl) conditions	Yildirim et al. [150]
<i>Pisum sativum</i> L. cv. Pea 09	5 and 10 mM	Increased plant height, root length, shoot FW and DW, and root FW under controlled condition	Nusrat et al. [151]
<i>P. sativum</i> L. cv. Master-B	4 mM	Increase leaf number and average leaf FW under irrigated as well as drought stress	Osman [152]
<i>Vigna unguiculata</i> L.	5 mM and 10 mM	Increased leaf FW and DW under both non-stressed and saline (50 mM) conditions	Manaf [153]
<i>Phaseolus vulgaris</i> L.	5 mM	Improved plant height, leaf number, leaf area, and plant FW under non-stressed condition as well as NaCl (35 mM) stress	Osman and Selim [154]
<i>Lolium perenne</i> L.	20 mM	Enhanced shoot FW under control condition whereas, a substantial increase in the shoot and root FW was observed under saline (250 mM NaCl) condition	Hu et al. [155]
<i>Viburnum lucidum</i> L. <i>Callistemon citrinus</i> (Curtis) Stapf.	2.5 mM	Increased plant height, number of leaves, leaf area, shoot and root DW, root-to-shoot ratio, along with apical and lateral shoot length, and number of lateral shoots under controlled as well as saline (200 mM) conditions	Cirillo et al. [156]
<i>Citrus sinensis</i> L. cv. Washington navel	50 mM	A significant increase in seedling height, leaf area, leaf number, DW shoot, and roots, while branches number unchanged	Abdullah et al. [157]

Exogenously applied GB was tested in *Brassica* under normal and saline conditions, where different growth parameters, viz., plant height, shoot and root fresh and dry weight were positively influenced by GB under normal growing condition. Moreover, GB efficiently reduced the deleterious effect of salinity [13]. Foliar application of GB at 25 and 50 mM on onion growth and physio-biochemical attributes was investigated under normal and 4.80 dS m<sup>-1</sup> salt stress, where GB showed significant increase in growth indices in terms of shoot length, leaf area per plant, shoot fresh weight (FW) and dry weight (DW) under non-stressed and stressed condition as well [14]. Foliar GB application resulted in significant increases in growth attributes of lettuce grown under 100 mM NaCl, compared with non GB treated plants, where 25 mM GB showed the best performance for mitigating salinity damage and growth improvement. Yet, 25 mM foliar- applied GB increased FW and DW of shoot by 54.9% and 80.5%, respectively, whereas it increased FW and DW of root by 40.0% and 64.3%, respectively under stress conditions [150]. Application of low levels of GB (15 mM) was slightly effective in increasing root fresh and dry biomasses of NIAB-IRRI-9 rice under non saline condition, but efficiently improved the growth parameters under 60 mM and 120 mM salt in both NIAB-IRRI-9 and Super Basmati variety of rice [141]. Glycinebetaine applied as foliar spray increased shoot DW and root FW in *Pisum sativum* L. cv. Pea 09, while decreased root FW and root length in *P. sativum* L. cv. Meteor Fsd under non-saline conditions [151]. Exogenous application of GB markedly mitigated the inhibitory effects of Cd on *T. aestivum* plant growth. In this context, foliar application of GB had higher shoot length and dry mass under control and Cd stress [103].

In *Vigna unguiculata*, 50 mM NaCl significantly reduced growth parameters as compared with the control (non-saline). However, under the saline condition, foliar application of GB showed significant increments in all growth parameters. As for dry weight, 10 mM GB without salinity increased significantly the dry matter compared to the control seedlings. There was no significant difference in dry weight with 5 mM GB in control and NaCl-treated seedlings. Moreover, GB treatments without salinity and under 50 mM NaCl increased leaf area as compared with the control [153]. Glycinebetaine treatments were efficient for improving the growth under water stress, as well as under well-irrigated conditions. Among seed treatment and foliar spray of GB, the foliar spray was more effective, and foliar application with 100 ppm GB (0.85 mM) was the best, contributing the highest seedling FW and DW in aromatic fine-grain rice cv. Super-Basmati rice [158]. Aziz et al. [147] tested the *G. hirsutum* to normal and drought stress condition in combination with GB. Their results indicated that only germination rate and plant height were positively altered by GB application. However, under drought condition, GB significantly effected the number of monopodial and sympodial branches, a number of main stem nodes, plant height, and the ratio of height to the node. A similar result was also postulated in cotton under dehydration stress [146].

### **10.2 Exogenous GB application and yield improvement**

In most of the crop plants, the reproductive stage is the most vulnerable period to abiotic stresses, which reduce the final yield. Hence, protection against abiotic stress at the reproductive stage is considered as the key to higher yields from crops in stress-prone areas globally. On the other hand, scientists are now targeting the yield gap in order to get higher yield using some biostimulants [159]. Therefore, exogenous GB application as a stimulant in crop plants appears to be an effective strategy for lessening the yield gap as well as improving abiotic stress tolerance. Exogenous application of GB to plants, as seed priming or pre treatment, co-treatment, and/or post-stress period, have been shown to increase the internal GB levels, thereby enhancing plant growth and final crop yield [158; Tab. 5]. However, the response differed greatly among plant species. The first thing to be considered is that not all species are responsive to the exogenous application and secondly, the effective dose varied from species to species, and sometimes over-dose may show toxic effects resulting in growth or yield reduction [39]. Therefore, optimum concentrations and appropriate plant developmental stages must be carefully identified for the exogenous application.

**Table 5:** Summary of the effects of the exogenous glycinebetaine application on yield attributes and yield improvement of different plant species

Plant Species	Dose of exogenous GB	Yield attributes and yield improving effects	Reference
<i>Oryza sativa</i> cv. Pathumthani1	100 mM	Panicle length and weight, fertility percentage, and 100-grain weight were improved	Cha-um et al. [142]
<i>O. sativa</i> cv. KS-282 and Basmati-385	100 mM	Increased yield attributes under both non-stressed and drought stresses (15% soil moisture)	Khan et al. [120]
<i>Triticum aestivum</i> L. cv. SARC-I and Inqlab-91	50 mM	Enhanced 100-grain weight and grain yield under control and drought condition	Shahabaz et al. [144]
<i>T. aestivum</i> L.	100 mM	Spike length, the number of spikelet spike <sup>-1</sup> , average number of grains spike <sup>-1</sup> , grain yield ha <sup>-1</sup> of wheat increased both in unstressed as well as drought condition	Raza et al. [160]
<i>T. aestivum</i> L. cv. MH 97	100 mM	Increase productive tillers m <sup>-2</sup> , number of grains spik <sup>-1</sup> , 1000 grain weight, biological yield, and grain yield under irrigated and drought condition	Gupta and Thind [161]
<i>G. max</i> L. Merr cv. DPX	10 Kg h <sup>-1</sup>	Improve pod number plant <sup>-1</sup> , grain number pod <sup>-1</sup> , 1000 grain yield under saline (11.1 dS m <sup>-1</sup> ) condition	Rezaei et al. [162]
<i>Pisum sativum</i> L. cv. Master-B	4 mM	Increase pod number, pod FW and green pod yield under irrigated as well as drought stress	Osman [152]
<i>Phaseolus vulgaris</i> L.	5 mM	Recorded highest flowers number plant <sup>-1</sup> , pods number plant <sup>-1</sup> , fruit number, harvest index, along with the lowest value of fruit abscission percentage in both under control (0 ppm NaCl) and salinity (2000 ppm NaCl)	Osman and Selim [154]
<i>L. esculentum</i> Mill.	10 mM	Enhanced number of flowers and of fruits per plant, and marketable yield. Increase fruit quality attributes viz. fruit weight, fruit diameter, and TSS under well-watered condition (100 % of ET <sub>0</sub> )	Ragab et al. [149]
<i>L. esculentum</i> Mill.	50 mM	Increased fruit quality attributes	Rasheed et al. [105]
<i>Brassica napus</i> L.	10, 15 and 20 mM	Significant increases in seed yield; oil, carbohydrate, and protein contents of the seeds under control and stress (75% and 50 % of Field capacity) situations	Dawood and Sadak [148]
<i>Helianthus annuus</i> L. cv. Hysun-33	100 mM	Increase head diameter, achenes head <sup>-1</sup> , 1000 achene weight and achene yield under irrigated and drought stressed condition	Hussain et al. [163]
<i>H. annuus</i> L.	50 mM	Slightly increased yield under the irrigated condition, but underwater deficiency spraying at vegetative stage substantially increase yield compared to stress treatment	Iqbal et al. [164]
<i>Cucumis sativas</i> L.	10 mM	Increased the number of fruit plant <sup>-1</sup> and yield plant <sup>-1</sup> under control as well as saline (50 mM and 100 mM NaCl) conditions	Youssef et al. [15]
<i>Allium cepa</i> L.	25 mM	Increased yield upto 2 fold under salinity stress (4.80 dS m <sup>-1</sup> )	Rady et al. [14]
	50 mM	Increased yield upto 3 fold under salinity stress (4.80 dS m <sup>-1</sup> )	
<i>Gossypium hirsutum</i> L.	100 mM	Biological yield, seed yield, number of balls plant <sup>-1</sup> , ball weight and lint percentage under well irrigated and drought stressed conditions	Noreen et al. [146]
<i>G. hirsutum</i> L.	100 mM	Increase number of open and unopen balls plant <sup>-1</sup> and ball weight, lint yield plant <sup>-1</sup> , seed yield plant <sup>-1</sup> and ginning out turn (GOT) percentage under well irrigated and dehydrated condition	Aziz et al. [147]

Glycinebetaine up-regulates the yield under normal or abiotic stress conditions due to its osmoprotective role on photosynthetic machinery and maintaining the ion homeostasis [160]. Moreover, it improves assimilation of CO<sub>2</sub> under drought stress [163] and biosynthesis and transport of hormones, for example, CKs, which have a positive role in the transportation of photoassimilates [165].

A field experiment was conducted to investigate the influence of exogenous application of 100 mM GB on the performance of wheat at maximum tillering and anthesis stage. Foliar-applied GB improved grain spike<sup>-1</sup> and thousand grain weight under control and stressed ones [161]. The response also suggested that the optimum level of GB is required for yield improvement under irrigated condition and drought, which varied among susceptible and tolerant genotypes [161,166,167]. Pre-soaking of seeds with GB was not significant in alleviating the adverse effects of water deficit on the yield of sunflower, but a positive effect was obtained by foliar spray under both water limited and well-irrigated conditions.

Severity of drought in *P. sativum* is due to drought at the vegetative and flowering stage, which is mainly owing to a reduction in pod number plant<sup>-1</sup> [168]. However, control (unstressed) plants produced the maximum pod number plant<sup>-1</sup> followed by drought-stressed one treated with GB at the vegetative or reproductive stage. When the drought-stressed plants were subjected to recovery, although the growth increased, pod development remained paused and did not alleviate the detrimental effect of drought stress. On the other hand, re-watering at the flowering stage resumed reproductive activity, but the new pods failed to reach maturity, which in turn reduced the pod number per plant. In this connection, exogenously supplied GB and Pro increased the pod number per plant either in well-watered plants or under all treatments of drought [152]. In this regard, Rezaei et al. [162] also reported maximum seed yield of soybean under control and salt stress due to foliar application of GB, which was associated with an increase in pod number. If the drought stress was imposed at the vegetative stage, foliar application of GB was more effective and enhanced the hundred achene weight of sunflower [164].

Exogenous GB treatment can significantly increase the seed yield, as well as the oil, carbohydrate, and protein contents in *B. napus* seeds in either unstressed plants or plants irrigated with 75% or 50% field capacity (FC). In this regard, 20 mM GB was effective in alleviating the detrimental effect of mild and severe drought stress, but more effective under severe drought than the mild stress [148]. Exogenous GB (100 mM) applications under irrigated and water stressed conditions improved grain yield and number of grains per spike by modulating the photosynthetic ability through maintaining turgor potential of leaf [160].

Foliar application of GB enhanced biomass production and significantly increased grain yield of maize and sorghum. It also resulted in more grains of maize, sorghum, and wheat, with interactions between water and GB treatments [169]. Exogenous GB recuperated the rice plants from water stress as indicated by greater plant height as well as an increase in the yield from GB pretreated plants compared to no-GB (untreated) ones [142]. Glycinebetaine pretreatment considerably increased hundred grain weight as well as total grain yield of wheat in contrast to GB untreated plant [160]. A similar result was also found in maize [170] and dicotyledonous species such as bean [154]. Moreover, researchers observed that pretreatment with GB maintained the healthy condition of the mature plants particularly at the reproductive stage, which facilitated more yield in crops.

Although a number of examples exist for successful GB application to improve plant growth and yield under normal and abiotic stress, the field application is yet to be practiced widely. To gain the maximum output along with ensuring economic feasibility of GB application, further investigations are required to determine the most effective dose and frequency of applications, along with the most responsive growth stage(s) of specific crop plants, which may vary from crop to crop. Moreover, a better understanding of the mode of action of exogenous GB may aid its effective utilization for the farming community.

## 11 Glycinebetaine-Induced Regulation of the Molecular Machinery

Glycinebetaine is postulated to be involved in the protection of the transcriptional and translational machinery via the induction of genes encoding osmoprotective proteins [10]. *In vitro* studies have revealed that GB lowers the melting temperature of double-stranded DNA. This enables GB to qualify as a candidate to activate transcription under high salt concentrations within the cell [171]. Cold responsive genes encoding a late embryogenesis abundant group of proteins, viz., wheat cold-regulated 410 (WCOR410), wheat cold specific 120 (WCS120) and WCS413 were found to be up-regulated in GB-treated wheat seedlings exposed to low-temperature stress [172]. Tomato plants exposed to chilling stress

and exogenously treated with GB exhibited high transcription of *cat1* gene encoding CAT [173]. Transgenic tomato plants overexpressing the GB biosynthetic gene *choline oxidase A (codA)* from soil bacterium *Arthrobacter* sp., resulted in the induction and repression of 30 and 29 genes, respectively in the flower buds due to GB accumulation [174]. These reports concomitantly propose the ability of GB to directly or indirectly regulate transcription and hence stress-induced gene regulation. Bourot et al. [175] also showed that GB behaves like a chaperon *in vivo*. Thus, GB might confer protection to the proteins and the translational machinery itself to prevent unwanted misfolding and aggregation under osmotic stress (Fig. 3).

## 12 Glycinebetaine Versus Proline: A Comparison on Their Mimicry

Proline is an amino acid, which acts as an osmolyte and compatible solute. Like GB, Pro also accumulates under almost all kind of abiotic stress [176]. The biosynthesis of GB and Pro can be related via arginine and the PA synthesizing pathway. Though principally formed from glutamate (Glu), Pro can also be produced from ornithine (Orn). The Orn aminotransferase (OAT) catalyzes the formation of  $\Delta^1$ -pyrroline-5-carboxylate (P5C) from Orn. P5C is then acted upon by P5C reductase (P5CR) to form Pro [8]. Orn decarboxylase (ODC) again catalyzes Orn to form putrescine. Contrary, the PA and GB biosynthetic pathways are connected to each other through the common precursor, SAM [130]. Hence, it seems that channelization of Orn towards Pro and siphoning of SAM towards GB synthesis might enable the simultaneous accumulation of both the osmolytes at the expense of PAs. The experiments to undermine the greater efficacy between Pro and GB are not available in the literature. Like GB, exogenous application of Pro or generating transgenics that overexpress Pro biosynthetic genes have also led to recharging of the entire antioxidant machinery along with up-regulation of several abiotic stress-inducible genes encoding late embryogenesis abundant proteins, heat shock proteins, etc. [176]. Hasanuzzaman et al. [39] reported that exogenous treatment of rice seedlings with Pro triggered GB accumulation under salt stress. In another study, Sobahan et al. [177] showed the co-operative action of GB and Pro in salt stress amelioration of sensitive rice seedlings. It was found that exogenous application of GB and Pro promoted stomatal closure and induced the production of vacuoles, which could store the excess  $\text{Na}^+$  ions [177]. A recent review by Murmu et al. [178] contains many more instances on the positive correlation between GB and Pro under abiotic stresses.

Zhang et al. [179] observed a heritable change in the methylation pattern of Pro metabolizing genes, *P5C synthase (P5CS)*. The existence of a demethylated allele of P5CS was proposed even after removal of the stress cues indicating towards the development of stress memory [176]. Drought- and osmopriming-induced trans-generational drought tolerance (stress memory) mediated by GB has also been reported in wheat seedlings [81,82]. Thus, abiding by the current understanding, it would be better to relate GB and Pro as two synchronized arms of osmotic-stress response, which simultaneously cooperate under sub-optimal conditions. However, over-accumulation of Pro has been observed to be detrimental for mitochondrial and chloroplastic ultrastructures. Treatment with 40-50 mM or higher concentration of Pro also promotes unwanted necrosis [180]. Extremely high concentrations of Pro inhibit *P5CS* by feedback regulation, resulting in retarded organogenesis and growth in *Arabidopsis* and *Distichlis spicata* [181]. At such concentration, Pro lowers the DNA melting point, destabilizes the double helix and makes DNA hyper-susceptible to S1 nuclease [181]. However, no such adverse effects of GB have been observed in plants. Hence from this point of view, it can be hypothesized that GB could be a safer osmolyte compared to Pro, though such a proposition will require rigorous experimentations using high concentrations of GB.

## 13 Engineering Plants for Enhanced GB Biosynthesis

Crop loss due to abiotic stress-induced injuries is a severe agro-economic problem. The biotechnological advancements including maneuvering with molecular targets and generation of transgenic plants have paved the way to better understand GB-mediated abiotic stress tolerance.

### 13.1 Molecular Approaches

The field of molecular biology has not been exhaustively investigated in case of GB production. Some recent data have validated the molecular basis of GB accumulation under stress. Proteomic investigations identified BADH as a salt stress marker in *T. monococcum* seedlings [182]. The proteome of the salt-stressed seedlings was analyzed by two-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF-TOF mass spectrometry. The proteins involved in regulatory, stress protection, protein dynamics, and diverse metabolic pathways were identified. Further analysis verified BADH, leucine aminopeptidase 2, Cu/Zn SOD and 2-Cys peroxiredoxin BAS1 as the molecular markers of the salt stress response [182]. Meng et al. [183] also dissected the proteome of the monocot perennial halophyte, *Puccinellia tenuiflora* exposed to chilling stress. Along with altered protein synthesis and turnover in the cytoplasm and chloroplast, the investigators found an accumulation of osmolytes like GB, Pro, and soluble sugars to be responsible for promoting such adaptation [183]. The importance of BADH in abiotic stress tolerance was illustrated by a knockdown experiment using RNA interference (RNAi) technique. The *BADH1*-RNAi repression lines in rice exhibited reduced yield and tolerance to salt, drought, and cold. This was accounted for due to enhanced MDA and H<sub>2</sub>O<sub>2</sub> accumulation within the tissue. Interestingly, the repression lines did not display any change in the endogenous GB content [184]. Hence, the decrease in stress tolerance was possibly due to the reduced dehydrogenation of the aldehydes accumulating from diverse metabolic processes.

### 13.2 Transgenic Approaches

Due to the inability of the major cereals (barley, wheat, maize) to normally accumulate GB, transgenic approaches to overexpress GB biosynthetic genes have been undertaken to enhance plant tolerance against abiotic stresses [185]. The principal staple food crop, rice has one *CMO* and two *BADH* homologues. The removal of the translational initiation codon, loss of functional domains and generation of premature stop codons due to frameshift mutations altogether result in the formation of non-functional *BADH2* homologues. This gene is involved in the production of the aroma compound, 2-acetyl-1-pyrroline in aromatic cultivars of rice [186]. The functional *BADH1* homologue, on the contrary, is involved in the production of GB under osmotic stresses [187]. Engineering GB biosynthesis in GB non- or low-accumulators by genetic engineering has been tested in diverse plant species ranging from *Arabidopsis*, tobacco, *Brassica*, tomato, maize, rice, potato, and wheat. This resulted in increased tolerance against multiple stresses [185].

Transgenic rice plants overexpressing *choline oxidase (cox)* gene from *Arthrobacter pascens* under stress-inducible and constitutive promoters resulted in increased GB accumulation and tolerance towards salt [188]. Similar observations were also put forward for transgenic tomato plants with chloroplast targeted GB accumulation. These exhibited better tolerance towards chilling stress compared to the sets, where GB was produced in the cytosol and also in greater amounts [189]. These observations might be accounted for due to the effective GB-mediated protection of the photosynthetic apparatus, which is the prime machinery to be affected under oxidative stress [190].

Overexpression of the *codA* gene from *Arthrobacter globiformis* resulted in high GB accumulation in the chloroplasts and the cytoplasm of tomato seedlings. This ensured higher photosynthetic rates, antioxidant activities and efficient scavenging of ROS in the transgenics compared to the wild-type plants exposed to salt stress [42]. The transgenic plants also exhibited better regulation of the ion channels like K<sup>+</sup> transporter, Na<sup>+</sup>/H<sup>+</sup> antiporter and H<sup>+</sup>-ATPase under stress, which led to a higher K<sup>+</sup>/Na<sup>+</sup> ratio compared to the stressed seedlings [42]. The relation between ion channel regulation and GB accumulation has also been observed by Ahire et al. [191] where overexpression of *vacuolar proton pyrophosphatase (VPPase)* from *Sorghum bicolor* in *Bacopa monnieri* conferred salt tolerance via enhanced production of GB. The shoots of the transgenic plants also accumulated low MDA and exhibited lesser membrane damage due to GB-mediated recharging of the antioxidant machinery and effective scavenging of toxic ROS [191]. Overexpression of the *S. oleracea* chloroplastidic *BADH* in

sweet potato cv. Sushu-2 increased GB production within the chloroplast [192]. This resulted in improved tolerance to salinity and oxidative stress in the transgenic sweet potato plants due to better photosynthetic efficiency, membrane integrity, and ROS scavenging compared to the stressed plants [193].

Overexpression of genes encoding stress-associated proteins also induced GB synthesis within transgenic plant tissues [185]. Transformation of chilli pepper with tobacco *osmotin* gene resulted in enhanced salt tolerance due to accelerated GB and Pro synthesis. The transgenics exhibited higher activities of SOD, GR, APX and elevated relative water content (RWC) under stress compared to the control plants [194]. The G-protein, RabAc4, involved in membrane trafficking has been reported to cooperate with GB to promote chilling tolerance in tomato seedlings [189]. Tab. 6 presents a concise list of transgenic approaches applied to enhance GB accumulation and promote abiotic stress tolerance.

**Table 6:** Development of GB accumulating transgenic plants for generating abiotic stress tolerance

Stress Tolerance	Gene	Donor species	Transgenic Species	Physiological Effects	References
Salt	<i>cox</i>	<i>Arthrobacter pascens</i>	<i>Oryza sativa</i>	High GB accumulation and activities of antioxidant enzymes	Su et al. [188]
Salt	<i>codA</i>	<i>A. globiformis</i>	<i>Solanum lycopersicum</i>	Increased photosynthetic rate, antioxidant activity, and ROS scavenging	Wei et al. [42]
Salt	<i>BADH</i>	<i>Spinacia oleracea</i>	<i>Ipomoea batatas</i>	Increased GB accumulation in the chloroplasts	Fan et al. [192]
Salt Drought	<i>BADH</i>	<i>S. oleracea</i>	<i>Nicotiana tabacum</i>	Maintenance of RuBisCO activity	Yang et al. [195]
Heat Drought	<i>BADH</i>	<i>Atriplex hortensis</i>	<i>Triticum aestivum</i>	Maintenance of photosynthetic efficiency	Wang et al. [45]
Cold Drought	<i>betA</i> (encoding choline dehydrogenase)	<i>Escherichia coli</i>	<i>Zea mays</i>	Reduced lipid peroxidation of membranes due to efficient ROS scavenging	Quan et al. [196]
Drought	<i>betA</i>	<i>E. coli</i>	<i>Gossypium hirsutum</i>	Reduced lipid peroxidation of membranes due to efficient ROS scavenging	Lv et al. [197]
Salt Drought Oxidative	<i>codA</i>	Bacterial origin	<i>Solanum tuberosum</i>	Photosynthetic protection	Ahmad et al. [198]

## 14 Conclusion and Outlook

Glycinebetaine, the most important organic osmolyte, plays numerous functions in plant growth and development. Under different environmental stresses, many higher plants naturally accumulate GB as a protectant. Besides acting as an osmoprotectant, GB stabilizes cytosolic pH, proteins, enzymes, and membranes, scavenges toxic ROS, and maintains the balance of cell redox for protecting subcellular structures in stressed plants. Moreover, GB maintains the ionic balance of plants under different stress conditions. Extensive work in recent past confirmed how endogenous and exogenous GB confers tolerance to plants at the time of diverse abiotic stresses. New findings recommend the involvement of differentially expressing endogenous genes in GB arbitrated stress tolerance in higher plants by ROS scavenging, maintaining dealings with oxidative stress, gene expression and the increasing accumulation of GB under the condition of abiotic stress. Because of its broad range of function, the genes of the GB biosynthesis pathway are being used to develop transgenic plants. Such plants are very efficient to

accumulate GB and show improved tolerance to a variety of abiotic stresses, including secondary oxidative bursts. Even though scientists are performing different research work with GB, the signaling behavior mediated by GB is still unclear and needs investigation.

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