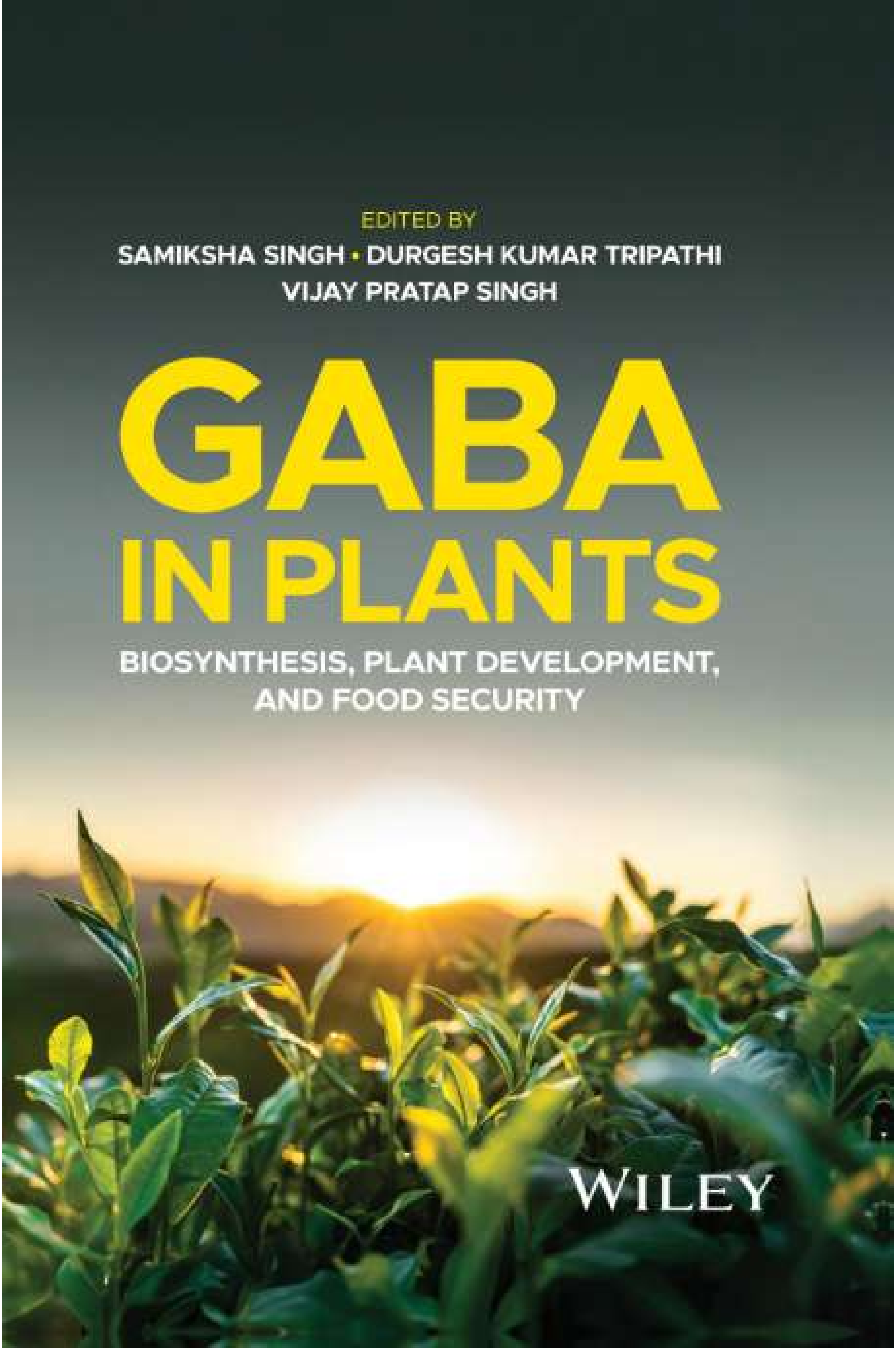

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GABA IN PLANTS

BIOSYNTHESIS, PLANT DEVELOPMENT, AND FOOD SECURITY

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Contents

16

GABA-Mediated Salt Stress Tolerance Through Physiological and Molecular Mechanisms

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Introduction

Salt stress conditions pose a significant challenge, as it is one of the damaging environmental stresses that curtail crop productivity and quality. Increased soil salinity causes cascades of reactions in plants at both the morphophysiological and molecular levels due to ionic toxicity, osmotic stress, and oxidative stress simultaneously (Hasanuzzaman and Fujita 2022). Notably, an excess of salt and chlorine ions can adversely affect plant root systems. Moreover, the presence of excessive sodium and chlorine ions hampers the retention of other essential ions, thereby impeding overall plant development and disrupting metabolic processes in response to salt stress. Additionally, salt stress leads to reduced water absorption capabilities in plants, contributing to dry conditions (Acharya et al. 2022). The detrimental effects of salt stress trigger the production of reactive oxygen species (ROS) within plants, further harming them through oxidative processes. It is crucial to recognize that salt stress adversely impacts all stages of plant development, with seed germination being particularly affected (Kumar et al. 2020).

In recent times, abiotic stressors have prompted researchers to explore newer techniques as a means to enhance global agricultural production. One noteworthy molecule in this context is gamma-aminobutyric acid (GABA), a nonprotein ubiquitous molecule first discovered in plants (potato tuber) and distributed throughout prokaryotic and eukaryotic organisms (Shelp et al. 1999). It is a crucial metabolite/endogenous signaling molecule in various regulatory systems in plants, and it accumulates in plant cells under adverse environmental conditions. Various biotic and abiotic stress conditions, including low temperature, radiation, low pH, hypoxia, darkness, and leaf senescence, trigger a rapid accumulation of GABA in plants (Ansari et al. 2014). Also, GABA plays a critical role in carbon and nitrogen metabolism, essential for plant development (Ansari et al. 2021). Under salt-induced desiccation, GABA functions as an active osmolyte without adverse

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effects and possesses free radical scavenging properties (Carillo 2018). The GABA-shunt pathway also plays a pivotal role in regulating GABA metabolism in cytosol by regulating glutamate dehydrogenase (GAD) through decarboxylating glutamate into GABA (Jalil and Ansari 2020).

Furthermore, the application of GABA has been found to promote plant growth, enhance antioxidant metabolism, and stimulate the production of antioxidant genes in a dose-dependent manner, thereby mitigating oxidative damage caused by stress in plants. GABA application also aids in regulating plant osmoregulation, thereby improving stress resistance. Specifically, exogenous GABA efficiently inhibits free radical production and reduces oxidative damage by modulating the expression of key genes related to free radical production and genes encoding antioxidant enzymes during salt stress (Li et al. 2017). Studies on white clover seeds treated with GABA have shown increased germination rates and reduced salt-related damage during germination (Cheng et al. 2018). While exogenous GABA administration has been shown to enhance abiotic stress tolerance in many plants, there remains a scarcity of information on how GABA regulates salt tolerance. This chapter explores the interconnectedness between osmoregulation, the antioxidant defense system, and important gene expression during salt stress, all of which are influenced by GABA-regulated salt tolerance.

Concept of Salt Stress to Plants

Various biotic and abiotic stresses adversely affect plant growth and development. Salinity is one of the major global problems that negatively affect crop productivity and global food security. The presence of excessive salt concentrations makes soil and water saline. Salt stress is typically brought on by high Na^+ and Cl^- levels. Salt stress lowers water potential, creates ion imbalances, disturbs ion homeostasis, and increases toxicity. Growth inhibition is directly connected to the osmotic potential of soil water or the concentration of soluble salts, as salt stress involves both osmotic and ionic stress. Reduced early development and plant productivity result from this altered water status. According to estimates, more than one billion hectares (ha) of the earth's land are thought to have been salinized. As the world's population grows, the food insecurity problem is made worse by the salinization of the world's shrinking agricultural area (Soltabayeva et al. 2021).

The impacts of stress on morphology can be seen in a variety of ways, including plant height, total biomass (fresh or dry weight), and other morphological markers. Increased salt concentration in the growth environment increases the effects of salinity stress on the development of plants. Germination is a useful indicator of salinity stress because salinity stress influences the pace at which seeds germinate. As one of the most important morphological processes, germination is a better stress indicator because stress can be detected at the early stages of the plant (Soltabayeva et al. 2021). At 100 mM NaCl, the germination rates of tomato and sunflower decreased by 71 and 62%, respectively. While the weight of tomato and sunflower decreased with the application of 50 mM NaCl, the weight of wheat, maize, rice, *Arabidopsis*, and ryegrass decreased at levels of 100–150 mM NaCl. In citrus and acacia, 100 mM NaCl generated biomass modifications during the early stages of growth (Pérez-Tornero et al. 2009; Ghaleb et al. 2010; Abbas et al. 2013).

Reduced photosynthesis due to salinity stress is caused by disruption of the photosynthetic machinery in plants. Increased soil NaCl levels cause osmotic stress, which limits cell growth rate and shrinks stomatal aperture, limiting photosynthetic efficiency. The first noticeable effect of salt stress on plants is the reduction in leaf area. In the initial stages, the smaller leaf surface area caused by the reduced cell extensibility is more significant than the lower photosynthetic rate per unit area (Shabala and Lew 2002). The drop in the regeneration capacity of ribulose bisphosphate (RuBP) and PSII sensitivity are further factors contributing to the reduction in photosynthetic activity. Salt stress interferes with the photosynthetic machinery by affecting the orientation of the chloroplast lamellar system and chloroplast integrity, resulting in reduced photosystem activity (Sharif et al. 2019). The decline in photosynthesis is linked to reduction in chlorophyll content and changes in the chlorophyll's ultrastructure. Chlorophyll content can be considered one of the physiological criteria that is a helpful predictor for choosing salt-tolerant cultivars. With an increase in salinity level, cotton cultivars' chlorophyll contents were found to significantly decrease. Inhibition of enzymes involved in chlorophyll production may be the cause of this decline (Zhang et al. 2014).

The osmotic effects of salt stress on a range of plant metabolic processes result in water deficit, which leads to oxidative stress due to the generation of ROS like superoxides and hydroxyl and peroxy radicals. ROS, which are by-products of ionic and hyperosmotic stressors, lead to membrane dysfunction and cell death. Membrane proteins, lipids, and nucleic acids are oxidatively damaged as a result of this rise in ROS levels in plant tissues (AbdElgawad et al. 2016). Plants create an effective system of enzymatic and nonenzymatic antioxidants to scavenge high ROS levels. Reduced ascorbate (ASC), glutathione (GSH), flavonoids, phenolics, and tocopherols are examples of nonenzymatic antioxidants. Enzymatic antioxidants involved in ROS detoxification include catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), and enzymes of the ascorbate-glutathione cycle, like ASC peroxidase (APX), GSH reductase (GR), monodehydroascorbate dehydrogenase (MDHAR), and dehydroascorbate reductase (DHAR) (Gill and Tuteja 2010).

High salt uptake inhibits the uptake of other nutritional ions, particularly K^+, resulting in a K^+ deficit. Numerous plants respond to increased NaCl treatment by increasing Na^+ and Cl^- levels while decreasing Mg^{2+}, K^+, and Ca^{2+} levels (Isayenkov and Maathuis 2019). A mechanism utilized by plants to balance the osmoticum between the cytosol, vacuole, and external environment is the increase in the content of soluble organic molecules in the cytoplasm of plants exposed to salt stress. These substances also stabilize enzyme structures and protect enzymatic systems. Certain compounds, such as proline, glycine, β-alanine, glycine betaine, and complex sugars like raffinose, that plants frequently produce and/or accumulate in stressful environments can be used to modify the osmotic pressure (Rahneshan et al. 2018).

Salt Stress and Related Metabolic Changes

Different abiotic stressors trigger sensitive and dynamic responses from plant metabolism. Metabolic alterations are a key component of plant stress responses because they can occur quickly in response to stresses. Salinity harms plants at almost every stage of life, including seed germination, growth, and development. These effects are a result of salinity-induced activations of molecular networks that are involved in stress recognition, ionic balance, signal transduction, modulation of the expression of stress-related genes, protein, and consequently, metabolisms (Balasubramaniam et al. 2023). Metabolic pathways that produce metabolites are varied and complex in plants. Both primary and secondary metabolites aid in plant adaptability and are intermediates or by-products of cellular regulation mechanisms. Due to their ability to supply energy and chemical building blocks, primary metabolites such as amino acids, sugars, and lipids are essential for the growth and development of plants. Flavonoids, terpenoids, phenolics, saponins, alkaloids, cardiac glycosides, proanthocyanidins, and quinones are just a few of the specialized molecules known as secondary metabolites that have antioxidant activity (Arif et al. 2020). Stress-related increases in the production of secondary metabolites shield cellular structures from the oxidative damage and are essential for interacting with the environment by supplying resistance to biotic and abiotic stressors.

When plants sense salinity stress, they undergo metabolic reprogramming involving modifications in the production of primary and secondary metabolites for regulating osmotic homeostasis and signaling pathways. The accumulation of compatible solutes such as proline, hydroxyproline, sugars, polyamines, glycine betaine, and proteins from the late embryogenesis abundant (LEA) superfamily is an important strategy utilized by plants for maintaining a low intracellular osmotic potential during high salinity. Proline, one of these metabolites, dominates in osmotic adjustment during salt stress. Stimulation of the proline biosynthesis route and the inhibition of the proline catabolic route can result in proline accumulation under osmotic stress (Zhao et al. 2020).

Salinity causes disturbances in secondary metabolites with osmoregulatory functions that are frequently engaged in plant defense and stress adaptation. These substances also play a number of physiological roles, such as signal modulation, ROS scavenging, enzyme activation, and photoprotection. In general, the accumulation of phenolics and flavonoids was significantly influenced by the degree of salt stress (Arif et al. 2020). NaCl stress substantially raised the content of phenolics, as well as the transcription of phenylalanine ammonia-lyase (PAL) genes and their activity in *Lonicera japonica* (Yan et al. 2016). NaCl stress in *Salicornia brachiata* caused a clear and consistent upregulation of numerous flavonoids. There was a noticeable buildup of quercetin, kaempferol, and anthocyanin derivatives such as cyanidin, delphinidin, and hesperidin in the shoots and roots. Additionally, with higher NaCl dosages, *S. brachiata* had significantly higher amounts of flavones and peonidin. In salt-treated *S. brachiata* and *Sesuvium portulacastrum* plants, phenylpropanoids and lignans were upregulated. 1-*O*-feruloyl-ᴅ-glucose, 1-*O*-caffeoyl-ᴅ-glucose, and curcumin diglucoside were among the phenylpropanoid compounds that increased at the higher salt level. Lignans like secoisolariciresinol, sesamolin, pinoresinol, and matairesinol were also accumulated at maximum NaCl dosage (Benjamin et al. 2019).

GABA and Salinity Stress Tolerance

Under circumstances of abiotic stresses such as salinity stress, GABA tends to build up within the plant cells (Mekonnen et al. 2016; Ma et al. 2018). Earlier studies have suggested that the utilization of GABA enhances plants' ability to withstand salinity stress by promoting the activity of antioxidant enzymes and mitigating the accumulation of ROS within the cells (Jalil and Ansari 2020), regulating the C/N ratio, Na^+ and K^+ absorption, enhancing phenolic bioactive compounds, amino acids, and proline, and controlling cell osmosis (Cheng et al. 2018; Xu et al. 2019; Khan et al. 2021). In a study by Wang et al. (2017), GABA application under salinity treatment led to an increase in sugar content, proline accumulation, and seedling growth in maize, when compared to untreated plantlets (Wang et al. 2017). GABA metabolism within *Arabidopsis thaliana* was commonly upregulated in response to NaCl stress (Renault et al. 2010). Recent work on GABA administration in plants under salinity stress is represented in Table 16.1. The NaCl stress initiates the signal transduction pathway, resulting in an increase in the cytosolic calmodulin-dependent activity of the L-glutamate decarboxylase enzyme. Nonetheless, it appears that GABA is essential for plant development. Exogenous GABA has been shown to increase plants' adaptation toward stressed environmental circumstances by enhancing overall growth, photosynthetic efficiency, antioxidative defense mechanisms, and nitrogen metabolism (Vijayakumari et al. 2016; Ma et al. 2018; Salah et al. 2019). The functions of GABA vary across different species, depending on the specific cellular compartment where it is active. A schematic model representing the positive effect of GABA under salinity stress is depicted in Figure 16.1.

GABA Improves Photosynthesis and Chlorophyll Fluorescence Parameters Under Salt Stress

Photosynthetic apparatus and photosynthesis are the targets that are greatly damaged by salt stress. Salt stress inhibits photosynthetic capacity by decreasing CO_2 fixation, stomatal conductance, and transpiration in plants. GABA, a non-proteinogenic amino acid in plant cells, probably plays a dual role as both a signaling molecule and a metabolite when exposed to various biotic and abiotic stresses. GABA application has beneficial effects on various photosynthesis-associated functions in plants. Salinity stress negatively affects plant photosynthetic capacity, and GABA effectively improves salt stress tolerance. Recently, exogenous GABA reduced salinity-associated stress damages, resulting in improved growth and biomass by decreasing ROS concentration but enhanced photosynthetic pigments of mungbean plants (Ullah et al. 2023a).

Exogenous GABA application improved photosynthetic capacity, net photosynthetic rate, transpiration rate, and stomatal conductance under salt stress in maize seedlings, which ultimately resulted in enhancement in CO_2 utilization in maize seedling leaves (Wang et al. 2017). Jin et al. (2019) studied the interactions between cellular redox signaling, chlorophyll biosynthesis, and PSII photochemical efficiency involved in GABA-induced salinity stress tolerance in *Cucumis melon* and found that pretreatment with exogenous GABA mitigated salinity stress by excessive accumulation of Chl and its precursors, thus avoiding photooxidation injury. Furthermore, the genetic, physiological, and molecular mechanisms of GABA-mediated effects and the regulation of stress signaling by GABA in plants under high-salt environmental conditions have been described recently (Dabravolski and Isayenkov 2023). Chen et al. (2018) reported that exogenous GABA increases the tolerance of *Cucumis melon* L. seedlings under salinity-alkalinity stress via effects on the chloroplast antioxidant system.

Table 16.1 Recent works related to the effects of GABA in plants under salinity stress.

Plant species	Salt conc.	GABA conc.	Effect of salinity on plants	Effect of GABA	Reference
Cucumis melon	50 mM alkalic salt (NaCl/Na_2SO_4/ $NaHCO_3$/Na_2CO_3 1 : 9 : 9 : 1 M ratio) solution	50 mM	MDA level, relative electrical conductivity (REC), and the activities of ascorbate peroxidase (APX), ascorbate reductase (DHAR), and SOD all increased during salt stress. Reduced shoot fresh and dry weight and leaf area, reduced ascorbate and glutathione (AsA and GSH) contents, and reduced monodehydroascorbate reductase (MDAR) activities and glutathione reductase (GR) were observed.	Pretreatment with GABA significantly inhibited stress-induced changes in H_2O_2, AsA, and GSH content.	Jin et al. (2019)
Cyperus esculentus	100 and 200 mM NaCl	2 mM	Substantial increase in H_2O_2, MDA, soluble sugars, proteins, and superoxide dismutase. Considerable decrease in growth, activities of peroxidase (POD) and catalase (CAT), glutamine oxoglutarate aminotransferase (GOGAT), NO_3^- and NH_4^+ concentrations, and nitrate reductase (NR).	Exogenous GABA application lowers chlorophyll degradation and improves the photosynthetic efficiency and growth under salinity stress. Compared to untreated GABA peers, the treated GABA peers had considerably lower levels of Na^+ and Cl^- ions, MDA and H_2O_2, as well as higher levels of osmolytes, chlorophyll pigments, antioxidant enzymes, Mg^{2+}, and K^+, and N metabolism.	Ullah et al. (2023b)
Lactuca sativa	40 and 80 mM NaCl	25 µM	Salinity caused a significant decline in the germination percentage and maximum quantum yield of PS II (F_v/F_m). Elevated electrolyte leakage and proline levels were also noted in plants treated with higher concentrations of salt.	GABA considerably shortened the mean germination time in lettuce seeds exposed to salt, improved F_v/F_m, reduced the specific energy fluxes per reaction center, and increased the electron transport flux in the photosynthetic system of lettuce. Furthermore, GABA treatment helped to recover the decrease in non-photochemical quenching (NPQ) and quenching coefficients (qN, qP, qL) caused by salt stress and decreased proline content. Improvement in L-ascorbate peroxidase, superoxide dismutase, and catalase activities were also shown by GABA + NaCl supplemented plants.	Kalhor et al. (2018)

Lolium perenne	175 mM NaCl	1 mM	Under 175 mM NaCl, germination percentage, shoot weight, shoot carbon, and nitrogen levels fell in comparison to the non-stressed control. Salinity stress resulted in increased activity of shoot SOD and decreased CAT and APX activities.	GABA improved seed germination, growth of seedlings, and C and N levels and altered carbohydrate metabolism and antioxidation machinery to improve salinity tolerance.	Tang et al. (2022)
Oryza sativa	150 mM NaCl	0.5 mM	Considerable increase in proline, glutathione reductase (GR), and MDA.	GABA priming significantly improved photosynthetic system and water relations, increased GABA, glutathione reductase levels, and reduced Na^+, proline, free radical, and MDA concentrations. Furthermore, it improved K^+ concentration, antioxidant enzyme activities, phenolic metabolism, and their transcription levels in seedlings under stress.	Sheteiwy et al. (2019)
Triticum aestivum	100 and 200 mM NaCl	0.5 mM	Decline of pigment content, SOD and CAT activities, and increase in MDA and electrolytic activity demonstrated by increased membrane damage.	GABA + NaCl groups were found to have higher net photosynthetic rates, intercellular CO_2 concentrations, PS II efficiency, and NPQ than NaCl groups. GABA + NaCl treatment significantly outperformed the NaCl groups in terms of SOD and CAT activity.	Li et al. (2016)
Triticum aestivum	150 mM NaCl	50 mg/l	Salt stress decreased dry weight, relative water content, and altered the ionic balance in the stressed seedlings.	Exogenous GABA has the power to prevent salt ion transfer to leaves and maintain the overall wellness of leaves. Under salt-stressed circumstances, pretreatment with GABA boosted seedling biomass, K^+ content in leaves, but lowered Na^+ concentration in leaves and roots via enhancing Na^+ exclusion and K^+ retention.	Wang et al. (2019)
Triticum aestivum	100 mM NaCl	2.0 mM	Increased glucose content, nitric oxide (NO) production, and oxidative stress, together with decreased growth traits and photosynthetic efficiency of plants.	GABA application enhanced nitrogen and sulfur assimilation, growth, photosynthesis, and ion homeostasis under salt stress. Oxidative stress mitigation was attained through changes in the ascorbate-glutathione cycle and proline metabolism.	Khanna et al. (2021)

(*Continued*)

Table 16.1 (Continued)

Plant species	Salt conc.	GABA conc.	Effect of salinity on plants	Effect of GABA	Reference
Vigna radiata	50 and 100 mM NaCl : Na_2SO_4 solution (1 : 1 ratio)	1.5 mM	Mungbean plants treated with salinity promote antioxidant enzymes to eliminate ROS at the cost of decreased growth and biomass, since more energy is allocated to antioxidant defense mechanisms instead of organ development.	Exogenous GABA promoted growth and physiological metabolism, greatly reducing the harms brought on by salt stress. Increased K^+/Na^+ ratio, improved production of photosynthetic pigments, and decreased H_2O_2 and MDA levels boosted osmolyte accumulations, and increased N concentration and N-metabolizing enzyme activity were observed.	Ullah et al. (2023a)
Zea mays	150 mM NaCl	0.5 mM	Excessive levels of Na^+ change the amount of potassium and calcium levels, enzyme activity, and metabolic processes, leading to the production of ROS and inducing oxidative stress in maize.	In general, GABA activated enzymes involved in nitrogen metabolism and restored the essential TCA cycle substrates through the modulation of GABA-shunt metabolism, enhancing the balance of carbon and nitrogen metabolism in maize and increasing salt tolerance.	Wang et al. (2023)

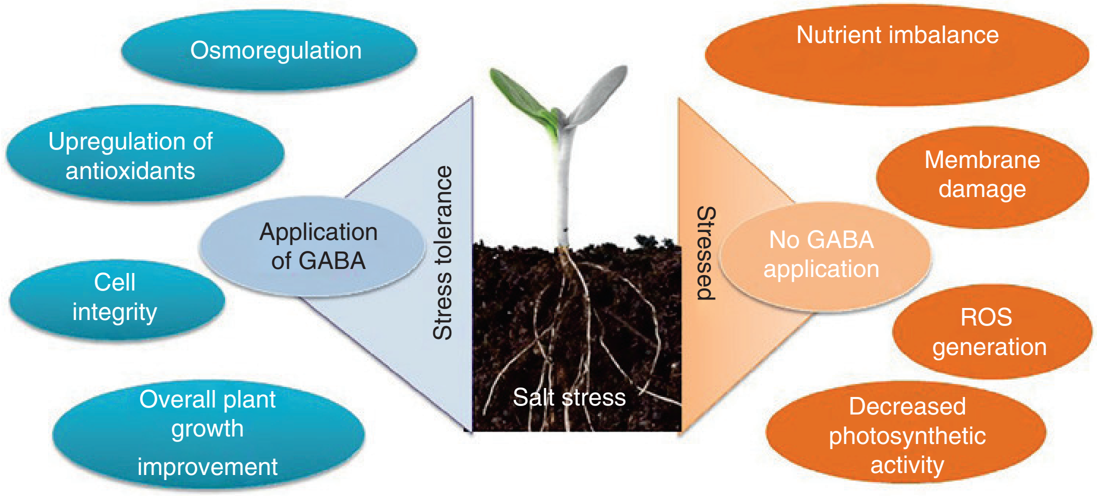

Figure 16.1 Schematic model representing the positive effect of GABA under salinity stress.

PSII is considered to be the primary site of the photosynthetic apparatus injury during salinity stress, due to the decreased excitation energy reaching PSII reaction centers (RCs) and changes in the pigment–protein complexes of thylakoid membranes. GABA protects PSII from salt stress damage by increasing the absorption of light energy and electron transfer in PSII and improves chlorophyll fluorescence parameters in plants. Based on the interpretation of the JIP-test and chlorophyll *a* fluorescence parameter, Xiang et al. (2016) concluded that exogenous GABA alleviated stress-related damage on the acceptor side of PSII in muskmelon seedlings. It also restored energy distribution, the reaction center status, and enhanced the ability of PSII to repair reaction centers in stressed seedlings. Thus, GABA may play a crucial role in protecting the chloroplast structure and function of PSII against the deleterious effects of salinity stress.

Moreover, chlorophyll fluorescence and photosynthesis improved under salinity stress in maize seedlings also (Wang et al. 2017). Additionally, GABA in combination with melatonin (*N*-acetyl-5-methoxytryptamine) effectively improved the salt stress tolerance of broad beans (*Vicia faba* L.) and increased the maximum quantum yield of PS II and minimized the negative effect of stress on the non-photochemical quenching and the energy fluxes of light absorption (Shomali et al. 2021). Even though salinity caused a significant decline in the maximum quantum yield of PSII (F_v/F_m) of lettuce plants, GABA application improved F_v/F_m. GABA decreased specific energy fluxes per reaction center (RC) for energy absorption and dissipation, while enhancing electron transport flux in the photosynthetic apparatus of lettuce plants. Moreover, the decline in non-photochemical quenching (NPQ) and quenching coefficients (qP, qL, qN) by salt stress were recovered by GABA application (Kalhor et al. 2018).

GABA Alleviates Oxidative Injury Induced by Salt Stress via Accumulation of the Osmolytes in Plants

Excessive ROS frequently accumulate under salinity stress, and the cellular ion homeostasis is an important adaptive trait of plants under salt stress. Salt stress decreases the stability and integrity of the biological membranes due to ROS accumulation, and exogenous application of GABA can reduce the inhibitory effects of ROS accumulation in plants. Li et al. (2016) investigated that GABA appeared to impart partial protection to the wheat plants against oxidative disturbance induced by salt. Exogenous GABA regulated the sodium ion flux in tomato plants by reducing the absorption of sodium ions. Due to the reduction of absorption, the sodium ions transported to the leaves are significantly lower than that under salt stress. Thus, Na^+ accumulation in the leaves and roots of tomato was reduced by preventing Na^+ influx in roots and transportation to leaves. Moreover, exogenous GABA under salt stress enhanced the reduction of both superoxide anion and hydrogen peroxide radicals in cells and subsequently decomposed into water and oxygen in plants (Wu et al. 2020; Aljuaid and Ashour 2022).

Electrolyte leakage and Malondialdehyde (MDA) content are often used to assess stress-induced damage in plants. GABA can trap the reactive intermediates during lipid peroxidation, and GABA reacts readily with MDA to form different products (Deng et al. 2010). The electrolyte conductivity and MDA as indexes of membrane injury were lowered by GABA treatments than in the plants under NaCl alone in wheat (Li et al. 2016). MDA content is regarded as an important indicator of oxidative stress in plants, and Wu et al. (2020) observed a significant decrease in MDA content following exogenous GABA treatment under salt stress in tomato plants. Foliar application of 1 mM GABA reduced MDA and H_2O_2 content in maize seedlings (Aljuaid and Ashour 2022).

GABA appears to impart partial protection to various abiotic stresses in most plants by increasing leaf turgor, increasing osmolytes, and reducing oxidative damage by stimulation of antioxidants. Al-Quraan and Al-Omari (2017) investigated the response of two lentil cultivars (*Lens culinaris* Medik.) to salt, osmotic, and oxidative stresses *via* the characterization of seed germination, accumulation of ROS, and GABA level. GABA content in lentil seedlings significantly reduced lipid peroxidation, and the results showed that GABA shunt is a key signaling and metabolic pathway that allows adaptation of lentil seedlings to salt, osmotic, and oxidative stresses. The metabolic and signaling role of GABA in plants against coping with various environmental stresses such as cold, heat, drought, salt, and heavy metal in legumes have been reviewed in detail (Sita and Kumar 2020).

GABA is an important component of the free amino acid pool in plant cells, mainly acts as an osmotic substance and plays an important role in imparting protection of plants to salt stress (Li et al. 2021). Exogenous GABA application in tomato leaves treated with NaCl exhibited increased accumulation of GABA and glutamate. Glutamate can maintain the flow of the GABA shunt, and the increase in glutamate content provides a large amount of raw material for the endogenous synthesis of GABA. This process can reduce the salt-induced damage in plant cells by consuming H^+ and thus reducing the degree of acidification of cells (Wu et al. 2020). They also found that the content of serine, proline, and alanine was increased, and thus the metabolic balance under salt stress with exogenous GABA was maintained in tomato plants. The accumulation of amino acids in leaves may help to reduce MDA content by inducing the formation of strong hydrogen-bonded water around protein and protecting the natural state of cellular protein polymers in plant cells.

GABA can alleviate salinity injury by accumulating osmolytes in plant cells and thus acts as an osmotic substance. Soluble sugars, proline, glycine betaine, and trehalose are among the major osmolytes in plants. Proline is the main nitrogen-containing osmolyte in plants, which can increase the cytoplasm water concentration and protect the metabolic center of the cytoplasm by preventing the vacuoles from absorbing water from the cytoplasm. Moreover, free amino acids are considered to be osmoregulation substances that protect plants from salt stress by reducing membrane permeability. Exogenous application of GABA has been shown to increase proline and soluble sugar content under salt stress conditions (Wang et al. 2017; Aljuaid and Ashour 2022). Exogenous GABA-induced increases in proline and soluble sugar contents might be indirectly associated with its participation in the citric acid cycle. The two intermediate products of the citric acid cycle – succinic acid and α-ketoglutarate – can be generated by the GABA oxidation reaction (Wang et al. 2017). Moreover, the findings of Sheteiwy et al. (2019) recommended that the high tolerance to osmotic and salinity stress in rice is closely associated with the capability of GABA priming to control ROS level by inducing antioxidant enzymes, secondary metabolism, and the expression level of *calcineurin B-like protein-interacting protein kinase* (CIPK) genes. The key physiological and molecular mechanisms associated with GABA under salinity stress are represented in Figure 16.2.

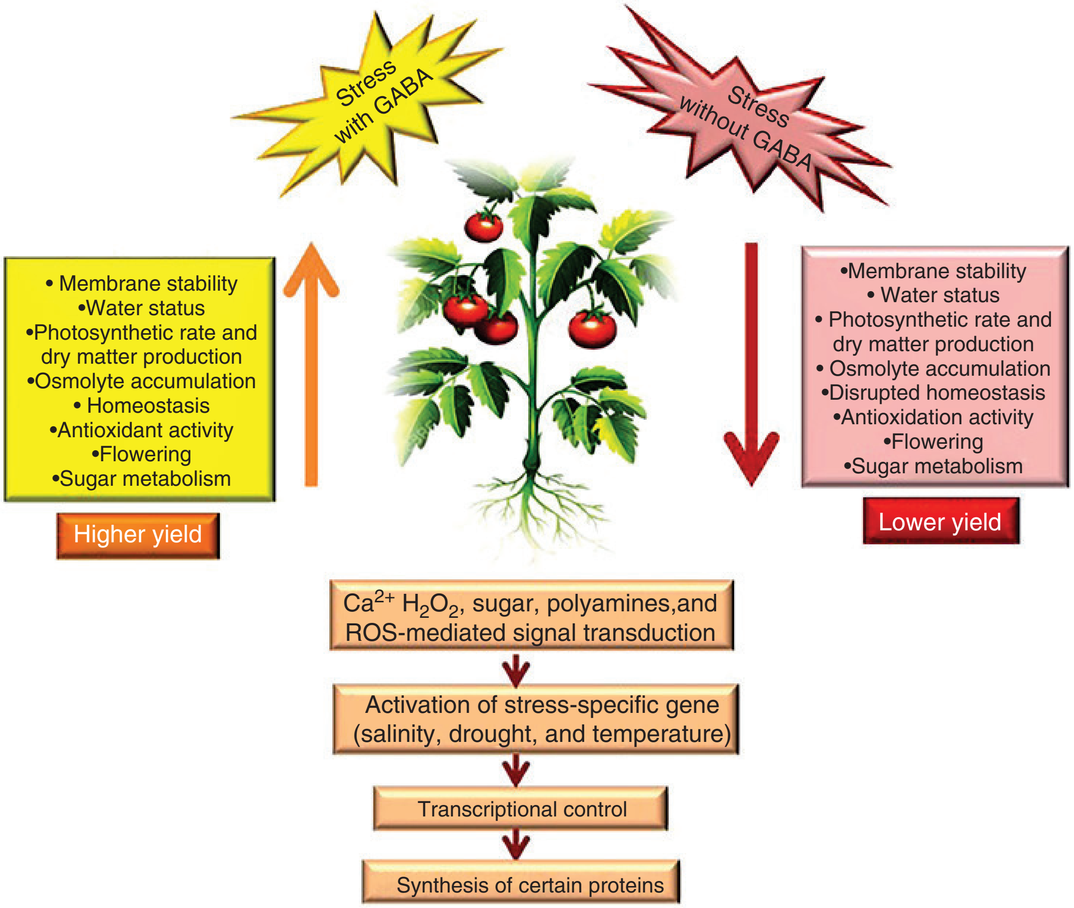

Figure 16.2 Physiological and molecular mechanisms associated with GABA exposure to salinity stress.

Molecular Changes Associated with GABA-Induced Salinity Stress Tolerance

GABA is a potential stress-responsive molecule that can alter certain stress-responsive genes to impart stress tolerance in plants. In the case of salinity stress, GABA plays a major role in maintaining seed viability. During salinity stress, glutamate decarboxylase (*GAD*) genes, which are responsible for the conversion of glutamate (Glu) to GABA, get activated, leading to the accumulation of GABA-shunt metabolites (GABA, glutamate, and alanine). The enhanced levels of GABA aid in respiratory mechanisms, maintaining nitrogen–carbon metabolism and synthesis of osmolytes for enhanced stress tolerance (Al-Quraan et al. 2019). *GAD* genes can also be activated by the exogenous application of GABA, which results in the endogenous accumulation of GABA by *GAD* gene-mediated conversion. It was observed that tomato plants subjected to salinity stress exhibit enhanced stress tolerance (Wu et al. 2020). Apart from this, exogenous application of GABA in muskmelon plants under salinity-alkalinity stress significantly upregulates the expression of *spermidine synthase (SPDS), spermine synthase (SPMS), arginine decarboxylase (ADC), and carbamoyl putrescine amido hydrolase (CPA)* genes, resulting in increased levels of polyamines such as putrescine, spermidine, and spermine, which aid in photosynthesis by maintaining chlorophyll synthesis and also inhibit ROS accumulation by altering redox homeostasis (Xu et al. 2019). Polyamine contributes to stress tolerance with the help of their oxidative product GABA, and this degradation is facilitated by the enzyme diamine oxidase (DAO). When *Glycine max* plants are subjected to salinity stress, *DAO* genes get upregulated, leading to a higher accumulation of GABA (Xing et al. 2007). So, apart from GAD-mediated conversion, GABA is produced endogenously in plants by degradation of polyamines during salinity stress.

Exogenous application and endogenous production of GABA will lead to the differential expression of various stress-responsive genes. The differential expression of *Salt Overly Sensitive 1* (*ZmSOS1*) and intracellular Na^+/H^+ antiporters (*ZmNHX1*) genes, along with the high-affinity potassium transporter 1 (*ZmHKT1) gene*, helps plants survive under saline stress by excluding Na from the cytosol and protecting enzymes. Exogenous GABA application in maize plants leads to overexpression of *ZmHKT1* and downexpression of *ZmSOS1* and *ZmNHX1,* leading to a reduction in Na/K ratio selectivity (Aljuaid and Ashour 2022). Few other stress-responsive genes (*CIPKs*), such as *OsCIPK01*, *OsCIPK03*, *OsCIPK08,* and *OsCIPK15*, were upregulated in GABA-primed rice plants under salinity stress, which activates a number of downstream signaling pathways, including the accumulation of proline and soluble sugars (Sheteiwy et al. 2019). They also observed the overexpression of genes associated with secondary metabolites such as phenylalanine ammonia-lyase (*PAL1*), polyphenol oxidase (*PPO*), Shikimate dehydrogenase (*SKDH*), and Cinnamyl alcohol dehydrogenase (*1bCAD1*) which helps in ROS scavenging and inhibiting the radicle chain reaction (Sheteiwy et al. 2019). Li et al. (2020) revealed that enhanced salt stress tolerance by GABA has a great correlation with lipid, saccharide, and amino acid metabolism. And it is proved by the differential expression of genes such as magnesium-chelatase (*Mg-CHT*), aldehyde oxidase (*AO*), cytochrome P450 (*CYP450*), 3-ketoacyl-CoA synthase 6 (*KCS6*), acetyl-CoA carboxylase 1 (*ACC1*), zinc transporter 29 (*ZTP29*), and alpha-amylase 3 (*AMY3*), which are mainly involved in starch degradation, zinc homeostasis, and biosynthesis of fatty acid, abscisic acid, wax, and chlorophyll in creeping bentgrass subjected to salinity stress. From the above-mentioned reports, it was proven that GABA is an important biocompound for imparting salt stress tolerance in plants.

Conclusion

Salt stress limits crop development and yield, which poses a serious concern for agronomists. GABA is a nonprotein amino acid that plays an important role in plant stress tolerance. It acts in several plant functions, including metabolic processes and signaling under stressful circumstances. It acts as a signaling molecule synthesized in the chloroplasts of plant cells and is transported to the cytoplasm, where it interacts with GABA receptors present on the plasma membrane of plant cells, regulating the expression of stress-related genes. Under salinity stress, GABA acts as an osmoprotectant that helps maintain the osmotic balance of the cell. It can also reduce the growth restriction and oxidative damage caused by salt stress, and it supports the accretion of endogenous GABA and other osmolytes. In a dose-dependent way, the administration of GABA promotes plant growth, antioxidant metabolism, and the expression of antioxidant genes. The effects of salt stress on plants are lessened by GABA, which increases crop productivity. Overall, GABA plays an important role in plant stress tolerance, especially under salinity stress.

Declaration of Interest: None

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