

Role of Exogenous Application of Proline and Glycine Betaine in the Salinity Tolerance of Solanaceae Family: A Review

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Abstract

Salinity stress is a major abiotic constraint affecting plant productivity and crop yield throughout the world. Solanaceae family comprising important food, ornamentals and medicinal species is an important family of cultivated crops in which salinity stress is acute. Solanaceae family in general is made of glycophytes and relies on salt-resistant mechanisms. Usage of potent osmoprotectants that are compatible plays an important role in counteracting the effect of osmotic stress. Exogenous application is a shortcut approach to induce protective osmolytes in plant species as compared to conventional breeding and transgenic approaches. Proline and Glycine betaine are widely acknowledged for their reduction in the harmful effects of salinity through osmoregulation. They also act as a scavenger of reactive oxygen species (ROS), stabilize sub-cellular structures, and act as a signal molecule interacting with other metabolic pathways during salt stress. A significant improvement in germination, growth, photosynthesis, and yield is observed when these osmolytes are applied exogenously under saline conditions. Despite numerous pieces of literature agreeing on the positive roles of proline and glycine betaine, the effects are not always apparent. Furthermore, sometimes sensitive plants accumulate higher concentrations of proline, and GB or negative effects are associated with plants due to toxicity. This review evaluates the understanding of the effects of exogenous application of proline and glycine betaine on major solanaceous crops and their relation with salt tolerance. This paradigm is also compared to the multiple salt tolerance mechanisms found in extreme halophytes of genus *Lycium* particularly the antioxidant capacity and proline content.

Keywords: Salinity tolerance; Osmolytes; Proline; Glycine Betaine; Solanaceae

Introduction

Plants are exposed to a range of stresses during their development, resulting in the expression of unique and complicated stress responses involving numerous morphological, biochemical, and physiological alterations. Of many factors affecting plants, abiotic stress is the primary cause of a 50% decrease in the average yield of key crops, resulting in losses worth hundreds of millions of dollars every year [1]. The most well-known stress in this respect is high salinity, which reduces crop productivity on irrigated land across the world and results in the degradation of many hectares of agricultural land each year. As the world population has ascended

exponentially in recent years, arable land has become increasingly scarce. Industrialization, climate change, and poor management practices further exacerbated these problems. Hence, presented with challenges to increase the productivity of our fields and overall food production, attention must be given to the proper utilization of saline soils. The soil with an electrical conductivity (EC) of the saturation extract, of 4 dS/m (approximately 40 mM NaCl at 25 °C) and exchangeable sodium of 15% is regarded as saline soil [2]. Salinity is one of the major threats to sustainable agriculture, especially in arid and semi-arid regions that globally decrease plant production by impairing various physiological,

biochemical, and molecular functions. Salinity when combined with heat, drought, flooding, and heavy metal stress can even be more harmful to plant growth and physiological performance [1]. High salt concentrations can cause inhibition of seed germination and seedling growth, reduction in total plant weight, stomatal closing, reduced photosynthesis, production of Reactive Oxygen Species (ROS), and limitation in the water uptake from soil [3].

Most of the cultivated crops have been domesticated or evolved in arable fields away from acute saline soils and hence show sensitivity to the same. Salt accumulation is characterized by some adverse effects like lowering the osmotic potential of soil solution, nutritional imbalance in plant roots, specific ion effect on plant root-soil solution interface, water deficit ultimately leading to stunted plant growth. Glycophytes are plants that cannot tolerate salt stress and are extremely sensitive to salt concentrations, resulting in severe inhibition or death around 100-200 mM salt. They include the most important, economic foodcrops and rely on salt-resistant mechanisms to combat increased salt concentrations. Halophytes can tolerate salt concentration anywhere from 300-1000 mM of salt by effective exclusion, compartmentalization, secretion of salts, and overexpression of genes for proteins, ion transporters, regulatory molecules, and osmolytes [3]. Under salinity stress plants have evolved various biochemical, molecular, and physiological mechanisms to diminish the adverse effects. Osmotic adjustment by the production of osmolytes is one of the principal adaptive mechanisms for the maintenance of turgor pressure and in preventing the harmful effects of salinity stress. Osmolytes or Osmoprotectants are small, electrically neutral, and highly soluble organic compounds at molar concentrations that efficiently maintain osmotic balance and stabilize proteins and membranes under salt, drought, or other stress conditions [4,5]. Accumulation of osmolytes, such as proline, glycine betaine, trehalose, etc., in the cytoplasm of cells, helps plants regulate cell osmotic pressure under salinity stress [6]. Ashraf, *et al.* 2007 [7] draw three methods for induction of compatible solutes for salt tolerance - developing new cultivars through plant genetics and breeding, genetic engineering of plants, and exogenous application of these osmolytes to plants growing under stress conditions. Exogenous application of proline and glycine betaine was found effective in attenuating salt stress in major crops like rice [4], sugarcane [8], Maize [9] etc.

Despite the putative role of these compounds, certain authors have challenged these conclusions, arguing that the correlation between proline accumulation and abiotic stress tolerance in plants is not always apparent as in the case of salt and cold-sensitive *Arabidopsis thaliana* showing increased levels of proline [10]. Other authors [11,12] have also pointed out the negative correlation of osmolytes in salinity tolerance of chilly and tomato respectively. The review attempts to consolidate the literature regarding the exogenous application of proline and glycine betaine for salt stress in certain solanaceous crops viz Tomato, Potato, Brinjal, Chilli (Sweet pepper), Tobacco, and a brief evaluation of extreme halophyte of Solanaceae family *Lycium humile*.

Overview of Solanaceae family

Solanaceae is one of the major plant families, occupying a wide range of terrestrial habitats which is highly diverse in terms of genetic diversity, having some of the important crops cultivated throughout the world [13]. Certain members are pivotal to human civilization like (Tomato, Potato, Chilly, Brinjal, Pepino, Naranjilla, Tamarillo (tree tomato)) as food sources (Petunia, Datura) as ornamentals and (Tobacco, *Atropa*, *Hyoscyamus*) for drugs and medicinal properties in addition to being used as model plants in molecular genetic studies. In 2020 for instance, 644 million tonnes of Solanaceous crops (Chilli, Eggplant, Potato, Tobacco, Tomato) were produced from a harvest area of 29 million hectares of land [14]. While around 15 genera are utilized for food purposes, only four genera *Solanum*, *Capsicum*, *Physalis*, and *Lycium* have economic significance in the present scenario [13]. Solanaceous crops are highly vulnerable to drought and salt stress due to their body mass, succulence, and high-water requirement during the reproductive stages i.e., flowering, fruiting, and seed development [15]. The survival and perpetuation of a plant depend critically on the ability of its seeds to germinate at a high concentration of salt. In general most of the cultivated species of Solanaceae fall under glycophytes with very few exceptions like *Lycium humile* being able to establish themselves in saline soil. Although species from families Solanaceae are not particularly halophytes, their seeds germinated well at a moderate concentration of NaCl (200 mM) (200 mM) [16].

Proline and glycine betaine

Any perturbations in the cytosol or the cell metabolism could have undesirable effects and are of prime concern in sensitive plants subjected to stress conditions. Compatible osmolytes are nontoxic

molecules that in low concentrations act as osmoprotectants maintaining cellular machinery and provide enough protection to the tertiary structure of proteins and enzymes like proline, glycine betaine, trehalose, ectoine, etc [4]. Proline (Pro) is a proteinogenic organic acid containing a secondary amine group having a rigid-ring-like structure made up of three carbon R-group fused to the α -nitrogen group and is considered crucial for primary metabolism [10]. Accumulation of proline is seen in different organisms like bacteria, fungi, marine invertebrates, and plants under different stresses like drought, high salinity, high light, and UV irradiation, heavy metals, oxidative stress, and in response to biotic stresses [6,17]. The past decade has seen the rapid development of studies on proline and has well established the functions of proline. Hayat, *et al.* 2012 [5] highlighted the effect of exogenous Proline on leaf water potential and Photosynthesis; where strained plants with impaired water uptake, stomatal functioning, and retardation of chlorophyll biosynthesis triggered accumulation of the osmolyte. A strong association is found between proline accumulation and plant osmotic and salt stress tolerance in different plant species. Sorghum varieties studied under salt stress conditions revealed that tolerant varieties accumulated more proline and were correlated to their capacity to tolerate and adapt to salinity conditions [18]. Following the same vein, salt-tolerant rice lines accumulated more Pro than the sensitive ones under salt stress [4]. A study in 2014 [8] concluded that salt-tolerant sugarcane cultivar SP 81-3250 when treated to varied concentrations of saline solution exhibited increased proline content in leaves. Exogenously applied Pro reduced Na^+ and Cl^- and activated antioxidant systems by increasing activities of catalase, ascorbate peroxidase, and superoxide dismutase and levels of reduced ascorbate and glutathione in Maize seedlings grown in saline condition [9]. Collectively, these studies outline a critical role of proline in scavenging Reactive Oxygen Species (ROS), protecting enzymes, acting as a metal chelator, and in the signaling mechanism of plants in addition to its function as a beneficial osmolyte.

Glycine betaine (GB) or the fully N-methyl-substituted derivative of glycine is a zwitterionic, non-toxic, water-soluble cellular osmolyte compound that is electrically neutral over a wide range of pH values able to interact with hydrophilic and hydrophobic molecules like proteins and enzymes. A number of studies [6,19] have postulated a convergence between synthesis of Glycine betaine at enhanced rates in the cytosol to the ability

of plant species to tolerate biotic and abiotic stress. Similar to proline, glycine betaine is synthesized by several plant species as a compatible osmoprotectant to balance the osmotic potential of intracellular ions under salt stress. [7] present an account of GB alleviating salt stress in common bean by regulating the nutrient uptake and activating the antioxidant defense system suppressing Na^+ and encouraging K^+ , N, and P. GB administered topically to Brinjal plants under salt stress marginally increased the yield with improvements in the photosynthesis and aerial shoot growth [20]. Glycine betaine increased the emergence and germination rates of sweet pepper seedlings when given at the recommended amount of 10mM [21].

Evaluation of Tomato (*Solanum lycopersicum. L*)

Tomato (*Solanum lycopersicum. L*) is a high-value vegetable crop grown extensively throughout the world. In terms of salinity, tomato is moderately resistant to salt concentrations, able to tolerate ECs up to 6 dS m^{-1} , making it cultivable in regions displaying certain levels of salinization [22]. Salt stress raised total phenolics and flavonoid contents while decreasing carotenoid levels in the leaves of tomato plants [22]. Proline content in tomato plants exposed to salt concentrations increased dramatically as well as Proline metabolism under the influence of oxidative stress, regulated the synthesis and degradation of various enzymes; conferring salinity tolerance to salt-affected tomatoes [23]. Accumulation of proline was linked to the better performance exhibited by the tolerant genotype of tomato grown under salt stress. On the flip side, the same study [23] noted that GB synthesis produced H_2O_2 which was antithetical to its nature as a compatible osmolyte and indirectly altered coenzyme ATP, NAD(P)H turnover. However, Almeida, *et al.* 2014 [24] study could not find any correlation between the synthesis of proline with the growth of tomato species. Exogenous application of proline on cultivars irrigated with saline water greatly improved the growth parameters and productivity of tomato cultivars [25]. The study extrapolating on the author's earlier paper proposed an optimum spray of 10 mg L^{-1} of proline against salinity for commercial purposes. Other researchers [26], who have looked at the role of Osmolytes like Proline and GB, have found a negative correlation between exogenous application and salt tolerance in tomato seedlings. This study also reported a lowering of K^+ efflux and increased Na^+ efflux when GB is applied exogenously. To determine the effects of salt and water stress on

cherry tomato plants [22], studied the synthesis of osmolytes and found that GB was formed as a secondary osmolyte; providing resistance in the affected plants. Conversely, De la Torre-González, *et al.* 2018 [23] argued that GB did not account for salt tolerance in tomatoes and could rather be a stress indicator. The beneficial influence of GB varied according to different salinity levels with an inability to mitigate the effect on parameters like the number of clusters, fruit set, and weight. Also, the application of GB slowed fruit ripening and enhanced root mass.

Evaluation of Brinjal (*Solanum melongena*.L)

Brinjal also known as Eggplant or Aubergine is a tropical Old World plant from Solanaceae mainly cultivated for its leaves and dark purple fruits. Cultivated brinjal species include widely used *Solanum melongena*. L and minor ones like *Solanum macrocarpon*. L, *Solanum aethiopicum*. L with local importance for food and medicinal purposes. Alkhatib, *et al.* [27] found that Na⁺ ion concentrations above 50 mM and 75 mM reduced leaf area and leaf total sugar content along with the photosynthetic rate respectively in *Solanum melongena*. L. Stomatal conductance, internal CO₂ concentration, chlorophyll content, and transpiration were seriously affected in Brinjal plants exposed to salt stress with more dry matter distribution concentrating more in the roots [28]. Salt and salt-induced oxidative stress raised the levels of Proline, free radicals, and Malondialdehyde, phenolic, and flavonoid contents in the plant, with increased proline concentration showing the least reduction of photosynthetic pigments [29]. Proline accumulation was also pronounced in brinjal plants studied under unfavorable conditions and sometimes exceeded forty times the initial value in tolerant cultivar *Solanum torvum*. L [30]. This is suggestive of the positive correlation between proline biosynthesis and higher salt tolerance in Brinjal. Expression pyrroline-5-carboxylate synthetase enzyme, which plays an important role in the biosynthesis pathway of proline, is amplified in wild cultivars of Brinjal as a result of endogenous proline synthesis. In a recent study, it was observed that leaf proline and leaf glycine betaine were at relatively higher levels when brinjal was supplied with NaCl ions above 80 mM concentration. These osmolytes were regulated on the foliar application of IAA, further stipulating that Pro and GB were not contributing to salinity tolerance but rather formed as a result of unfavorable conditions [31] dismissing the subsiding effect attributed to these compounds by many researchers. Exogenously

applied proline offset the adverse effects on shoot fresh weight of eggplant cultivars but remained ineffective against overall damaging effects associated with increasing salinity [32]. Glycine betaine and thiamine treatment on germinating eggplant seedlings improved the rate of germination and the mean germination time under different salt regimes [16]. More than a 35% increase in the yield of brinjal was observed by Abbas, *et al.* 2010 [20] when GB was foliar applied, increasing the shoot biomass, root fresh and dry weight, and the photosynthetic efficiency of plants experiencing salt stress. Thus the role of glycine betaine in the high osmotic activity and the positive relationship between the osmolyte and the tolerance to increased salt concentration is elucidated. This is also confirmed by Mustafa, *et al.* 2017 [34] who reported overexpression of proline and GB content in salt-tolerant genotypes as opposed to the sensitive variety.

Evaluation of Chilli/Sweet Pepper (*Capsicum annuum*)

Sweet pepper is one of the most important commercial and economically traded vegetables, as it is used in practically every cuisine and culture around the world, and its pungent fruits are known for their antioxidant properties. Salt concentration above 12g/l hampered the vegetative growth of *Capsicum annuum* cultivars and affected parameters like root length, number of leaves, leaf area, chlorophyll level, and energy efficiency of photoautotrophy photosynthesis with subsequent increase in the level of proline [34]. Increased salt concentration also inhibited the relative water content of the leaves tied with osmotic regulation and caused perturbations by amplifying malondialdehyde, suppressing superoxide dismutase, and peroxidase to toxic levels [35]. Proline acts as a beneficial osmolyte for attenuating the toxic effects caused by increased salt concentration in sensitive sweet pepper plants by enhancing K⁺ concentration in the cells and activating antioxidant enzymatic mechanisms [17]. A strong correlation was observed between proline and relative water content of leaves in *C.annuum* plants jeopardized by salt and water deficit conditions [36]. Proline treatments caused remarkable improvements in the yield, phenolic content, ascorbic acid levels, total soluble solid contents, and endogenous proline levels in *Capsicum* fruits. Jamil, *et al.* 2018 [37] noted the synergistic action of proline and L-tryptophan at 50 and 0.12 mM respectively raised the tolerance of Chilli in saline environments, enhancing the growth, yield, and physiology of the plants. However, AL, *et al.* 2012 [38] no-

that proline application produced negative effects in plants ie reduction in leaf area and nodal length of *Capsicum annum* plants. Similar findings were obtained by dos Anjos Soares., *et al.* 2018 [11] who observed that Proline was ineffective in counteracting the hazardous effects observed in Chilly plants irrigated with saltwater. The study also mentions that the excess doses reduced the growth and dry matter deposition in stressed plants. Chilli plants, in general, are non-accumulators of Glycine betaine osmolyte and under stressed conditions, it becomes necessary to supply these exogenously to offset the detrimental effects. The exogenous application of GB increased the growth, metabolic activities, nutrient absorption, and use efficiency in plants as Glycine betaine applied at an optimal rate of 10 mM promoted germination and emergence percentage in Sweet pepper seedlings with a significant reduction in Malondialdehyde content [21]. This is in congruence with the findings made by [19]. Khafagy., *et al.* 2009 [39] noted that treatment of Sweet pepper seeds with GB counteracted the ill effects of salinity on morphological parameters such as the thickness of midrib and integrity of leaf mesophyll tissues. In contrast to the view presented, Escalante-Magana., *et al.* 2019 [36] that Glycine betaine was not an osmolyte when Capsicum plants were subjected to oxidative stress as they were not involved in osmotic activities and did not interfere with the endogenous levels of Proline. This compounded with the minimal effects on Relative water content (RWC), highlighted the inefficient nature of GB in Sweet pepper in moderating salt stress, leaving more room to further studies.

Evaluation of Potato (*Solanum tuberosum*)

Potatoes are annual Solanaceous plants grown widely throughout the world for edible starchy tubers rich in nutrients. Considering the fact that potato crop production is shifting to parts of the world where heat and salt stress are expected to be variables affecting productivity, the demand for versatile potato cultivars that can withstand these unfavorable circumstances grows [13]. Even with the significance of potatoes in agriculture, little is known about the variances and processes of salt tolerance in present potato cultivars. Salinity significantly impacts the potato crop, affecting yield, root formation, aerial growth, dry weight, and biomass output. Under salt stress, a variety of physiological and biochemical processes are harmed, including osmotic balance, transpiration, respiration, water use efficiency, leaf area, germination, and antioxidant generation [40]. The genetic modification of proline

metabolism in plants causes proline buildup during tension and under salt stress, the quantity of proline in the plant can often reach 100 times its normal level [41]. Mosavi., *et al.* 2018 [40] noted that in Potato plants, a shift in nitrogen metabolism linked to the synthesis of molecules like proline, which is utilized in osmotic control, can cause a decrease in chlorophyll levels. Glutamate, a precursor of both chlorophyll and proline, is less important in the chlorophyll biosynthesis pathway as proline production rises. Exogenous proline and essential oils, when used alone, increased the sequestration of reactive oxygen species (ROS) and the ion chelation, making them an important aspect of plant cell abiotic stress responses [42]. According to Ezzat., *et al.* 2019 [43] findings acquired under saltwater irrigation, osmoprotectants like glycine betaine, and proline, administered alone or in sequence exhibited favourable benefits including significant changes in potato plant development traits such as chlorophyll content, relative water content, and membrane stability index. Proline accumulation was increased in transgenic potato plants expressing P5CS genes, which significantly reduced the influence of salt stress on tuber output [6]. However, in some cases, GB and Proline substantially increased the poly phenoloxidase activity causing enzymatic browning, whereas total tuber yield metrics and marketable tuber yield were considerably lowered [43].

Evaluation of Tobacco (*Nicotiana tabacum*)

Tobacco (*Nicotiana tabacum*) is grown commercially in many countries, and the plant's stems and leaves are a major source of nicotine used as a stimulant or anxiolytic. Tobacco plants undergoing salt treatments have increased reactive oxygen species (ROS) levels, nuclear degradation, chromatin miniaturization, oxidative degradation of lipids, and accelerated cell death [44]. Szabados., *et al.* [10] showed that the application of Proline and GB enhanced the antioxidant defense system against the injurious effects of rising salt levels in tobacco Bright Yellow-2 suspension-cultured cells and distinguished proline as being more effective in osmoregulation. This finding is different from that of Celik., *et al.* [45] who argued that Proline synthesis and accumulation don't inherently confer salt tolerance in tobacco cultivars. This view is supported by Suekawa., *et al.* [46] and highlighted that exogenous proline application did not elicit either higher endogenous Proline synthesis or inhibition of Polyphenol oxidases (PPOs) causing negative effects on the growth of tobacco seedlings. The coactive action of glycine betaine

and proline in tobacco plants overexpressing the P5CS gene resulted in an improved antioxidant defense mechanism and overall salt tolerance to salt stress [44]. Tobacco is one of the most commonly used plants for the expression of transgenes from a variety of organisms for the deliverance of desired effects. Targeting genes for overexpression through transgenic techniques and molecular technologies are futuristic in outlook holding great promises in the salinity tolerance of tobacco plants. Transgenic *N.tabaccum* plants have generally good germination-establishment rates, higher levels of the defensive antioxidant system, a stable membrane system, and reduced reactive oxygen species. As proposed by Khatonabadi et al. 2019's [47] assessment of the physiological and antioxidant response of tobacco transgenic plants overexpressing P5Cs genes showed an increase in proline content and further improved salinity tolerance in the plants. Proline levels were high because of the unfavorable salt and water conditions, but they didn't seem to affect transgenic tobacco's osmotic potential or activity.

Evaluation of genus Lycium

Lycium is a prominent genus in the Lycieae tribe of Solanoideae subfamily in the Solanaceae family embracing a variety of xerophytic and halophytic plants, with the greatest genetic diversity in South American and African regions. Around 35 species are utilized for food and medicinal properties including superfoods like goji berry (*Lycium barbarum* and *Lycium chinense*) [48]. Not only are saline environments important contributors to biodiversity, but halophytic plants also act as a powerful tool for understanding salt tolerance like possible sources of salt-responsive genes and promoters, development of transgenic plants, and even phytoremediation. Furthermore, many plant families may be able to build on existing physiological and morphological features to gain enhanced resistance to environmental salinity due to the frequent evolution of salt tolerance in halophytes. Many *Lycium* species, such as *L.barbarum*, *L.californicum*, *L.humile*, *L.carolinianum*, *L.chinense*, *L.europaeum*, *L.ferocissimum*, *L.ruthenicum*, *L.shawii*, and *L.tenuispinosum*, are considered halophytes [48]. *Lycium humile* is arguably one of the world's most salt-tolerant Solanaceae species, capable of growing at high NaCl concentrations of up to 750 mM with a reduction in growth and development only at high concentrations of 1000 mM [15]. The accumulation of the plant hormone abscisic acid, the enhancement of antioxidant capacity and proline content, and an efficient osmotic adjustment are among

the various salt tolerance mechanisms reported in this species. The buildup of proline and ions could be linked to *L. humile*'s technique for dealing with excessive salinity as both chemicals were identified in very low concentrations with the contribution in water conservation and osmotic adjustment [15]. *Lycium ruthenicum* responds to moderate salt concentrations of 100 mM and a low water deficit by increasing their net photosynthetic rate, stomatal conductance, and photosynthetic pigment content, as evidenced by increases in net photosynthetic rate, stomatal conductance, and photosynthetic pigment content. Under specific stress conditions, proline buildup can improve salt and drought tolerance of *L.ruthenicum* [49]. The development of *L. barbarum* is aided by a salinity of 100 mM NaCl, which increases the plant's dry organic matter, betaine, ash content, and photosynthetic pigments. Because proline content grows gradually and dramatically, *L. barbarum* is more vulnerable to salt stress than *L. chinense* under the salinity settings examined [50]. The findings suggested that halophytes' significant proline accumulation may aid in their adaption to a saline environment.

Conclusion

Synthesis of osmolytes like Proline and Glycine betaine increased during periods of salt stress in all the major solanaceous crops under review. Despite the putative roles in salinity tolerance played by these compounds, inconsistencies were found in Tomato, Chilli, Potato, and Tobacco. Similar trends were noticed in the exogenous application of osmolytes *in vitro* and *in vivo*. *Lycium* genus having halophytes like *Lycium humile*, *L.barbarum*, *L.chinense* adapted to extreme saline conditions by accumulating Pro and GB among other salt-tolerant mechanisms under their arsenal.

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