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## **Involvement of Salicylic Acid in Sulfur Induced Salinity Tolerance: A Role of Glutathione**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author RN carried out the experimental work and searched literatures for the work on which this article is based and wrote the first draft of the manuscript. Authors SU and NAK supervised the work and helped in the presentation of the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Plants are severely affected by salinity due to its high magnitude of adverse impacts and worldwide distribution. Among various strategies, nutrient management may be an important aspect in alleviating stress under varied environmental conditions. The uptake and assimilation of sulfur plays an inevitable and imperative role in determining plant growth and development and is a fundamental part of several important compounds, such as glutathione, co-enzymes, vitamins, phytohormones and reduced sulfur compounds that decipher growth and vigour of plants under optimal and stressful environments. Assimilatory sulfate reduction may induce salt tolerance by coordinating various physiological processes and molecular mechanisms which are likely to be induced by phytohormones. Among phytohormones salicylic acid (SA) could provide tolerance against salinity stress by enhancing physiological processes and can improve salinity tolerance by its effect on sulfur assimilation pathway as biochemically or molecular mechanisms. The present review critically evaluates the recent studies on the modulation of sulfur assimilation and gives an insight into the role of glutathione to maintain redox state and their cumulative significance in plants' tolerance to salinity by SA in inducing various physiological responses in plants under salinity stress.

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## 1. INTRODUCTION

Salinity is a major abiotic stress which adversely affects plant distribution, survival and productivity mainly through changes at physiological, biochemical and molecular level [1-4]. Salinization is rapidly increasing on a global scale and currently effects more than 10% of arable land resulting in greater than 50% decline in the average yields of major crops [5]. The overall reduced growth and development of plants under salt stress is a result of cumulative effect on disruption in ion homeostasis, water balance and reduction in photosynthetic capacity of plants [6-8]. It affects almost every aspect of plant physiology at both whole plant and at cellular level through osmotic stress in an early phase and ionic stress at later stage of plant growth [2]. Salinity stress induces oxidative stress through increased production of reactive oxygen species (ROS) that causes lipid peroxidation, damages DNA, photosynthetic inhibition [9-12]. Any adaptation that regulates ROS generation in plants will provide efficient defense mechanism for tolerance against stress.

The availability of mineral nutrients also plays a major role in the regulation of photosynthesis [13,14]. Sulfur (S) deficiency is of wide occurrence throughout the world [15,16] causing alterations in plant metabolism [17]. Khan et al. [18] reported that sulfur-containing compounds have shown increased tolerance to salt-stressed plants by modulating physiological processes. Plants have a well-developed potential mechanism to combat with such adverse environmental salinity stress problems. Manipulation of the antioxidant machinery is an efficient tool for combating salinity induced oxidative stress. Among antioxidants, reduced glutathione (GSH) plays a key role in salt tolerance and its availability is influenced both by S-assimilation and phytohormones action [11]. Glutathione is considered as a marker of oxidative stress in plants and plays multifaceted part in metabolism [19,20,21]. Glutathione biosynthesis, concentration, and redox status were recently identified as the link between SA accumulation and Npr1-dependent induction of pathogen defence genes and, SA were found to be involved in the regulation of genes coding for components of the antioxidant defence systems [22-25]. However, the overall extent of redox regulation of stress perception, signaling, and defence is just emerging [26].

Phytohormones have also been shown to influence salinity tolerance through modulating several physiological processes and biochemical mechanisms [27]. Their role in salinity stress is critical in modulating physiological responses that lead to adaptation of plants to an unfavorable environment. Among them salicylic acid(SA) is a naturally occurring plant hormone, influences various physiological and biochemical functions in plants, acts as an important signaling molecule and has diverse effects on tolerance to biotic and abiotic stress [28,29]. Studies on agricultural plants demonstrated that SA-treatment strongly induces the synthesis of antioxidants (ascorbate and glutathione), antioxidant enzymes (glutathione transferase, glutathione peroxidase, ascorbate peroxidase), and provides increased tolerance against biotic and abiotic stress factors [30,31]. Examples of regulation of plant development by S and phytohormones are provided under optimal and abiotic stress [10,11]. SA plays an essential role in alleviating salt stress by regulating plant growth and development [32]. The roles of glutathione and GSH/thiol-disulphide status in signal transduction cascade and modulation of phytohormones signaling pathways have been shown [33]. It shows that the interaction of phytohormones and S assimilation is crucial in stress tolerance. It is therefore, important to identify to which extent sulfur assimilation is induced by salicylic acid to counteract the deleterious effects of salt stress. The present review focuses on the modulation of the role of glutathione metabolism and its redox state

that could enhance tolerance to salinity stress by SA in inducing various physiological responses in plants under salinity stress.

## 2. CHANGES IN PLANT PHYSIOLOGY AND METABOLISM UNDER SALINITY

Salinity is a major environmental constraint that renders fields unproductive and limits plant growth and productivity [34,35]. The adverse effects of salinity on plant growth are the result of changes in plant physiology which include ion toxicity, osmotic stress, nutrient deficiency and mineral homeostasis, especially  $\text{Ca}^{2+}$  and  $\text{K}^+$  homeostasis [2,36-38]. However, Roshandel and Flowers [39] through molecular studies have shown that the changes induced by salinity are ionic rather than osmotic. They reported that the changes in the expression of genes encoding proline rich proteins, senescence associated proteins and heat-shock proteins were responsive to the ionic rather than osmotic effects of salt in rice.

The toxic effect of salinity is through oxidative stress caused by enhanced production of ROS [40,41]. The increased production of ROS occurs under all kinds of stress, although their identity and compartment of origin may differ [42]. High concentration of ROS can damage photosynthetic pigments, proteins, lipids and nucleic acids by oxidation [43]. Controlling ROS production and scavenging in the chloroplast is shown to be essential for tolerance to salinity in cabbage transgenic plants and in salinity tolerant cultivars [44]. In addition to the destructive role of ROS, they also serve as a stress signal molecule and activate acclimation and defense mechanisms that will in turn counteract stress-associated oxidative stress [45,46,36]. Recently, it has been shown that  $\text{H}_2\text{O}_2$  produced by apoplastic polyamine oxidase influences the salinity stress signaling in tobacco and plays a role in balancing the plant response between stress tolerance and cell death [47]. Thus, ROS act both as the damaging toxic molecule and as the beneficial signal transduction molecule and there is need to control the steady-state level of ROS in cells during normal metabolism as well as in response to different stresses [46]. Elucidation of mechanisms that control ROS signaling in cells during salt stress can provide a powerful strategy to enhance the tolerance of crops to these environmental stress conditions.

Stress tolerance can be increased by over-expressing ROS-responsive regulatory genes that regulate a large set of genes involved in acclimation mechanisms, including ROS-scavenging enzymes. Over-expression of transcription factors such as *Zat10*, *Zat12* or *JERF3* enhanced the expression of ROS-scavenging genes and tolerance to salt, drought or osmotic stresses [48,49]. Furthermore, over-expression of mitogen-activated kinase kinase 1 (MKK1) in *Arabidopsis* enhances the activity of mitogen-activated protein kinase (MAPK) cascade, which is also activated by ROS [50], reduced stress-associated ROS levels and increased tolerance to salinity stress [51]. Many transgenic plants have been produced with high salinity tolerance by engineering MAPK cascade. There are also calcium dependent protein kinases (CDPKs), which are involved in salt stress response. Asano et al. [52] have characterized one CDPK gene *OsCDPK21* from rice whose overexpression results in an enhanced salinity tolerance in transformants. One of the best-characterized signaling pathways specific to salinity involves sensing calcium by the calcineurin B-like protein (CBL) CBL4/SOS3 and its interacting protein kinase CIPK24/SOS2 [2,40,53]. NaCl stress-induced SOS2/SOS3 complex is targeted to the plasma membrane enabling the phosphorylation and activation of the membrane-bound  $\text{Na}^+/\text{H}^+$  antiporter, salt overly sensitive 1 (SOS1) [2]. In *Arabidopsis*, the over-expression of SOS1 improves salinity tolerance [54]. SOS gene is involved in the regulation of  $\text{Na}^+$  and  $\text{K}^+$  ion homeostasis through the SOS pathway. SOS2 kinase is an especially important regulatory compound because of its interaction with other signaling molecules. SOS2 is found to interact with

nucleoside diphosphate kinase 2 (NDPK2) that is involved in ROS signaling [55]. NDPK2 is induced in response to oxidative stress and is shown to be important for the H<sub>2</sub>O<sub>2</sub>-induced activation of MPK3 and MPK6 [56].

Therefore, in order to reduce salinity stress, plants speed up their rate of ROS production that sends signal to activate antioxidants for ROS scavenging. Plants containing high levels of antioxidants can scavenge/detoxify ROS thereby contributing to increased salt tolerance [57,58]. The increased expression of antioxidant compounds can be used as prospective selection criteria for breeding for salt tolerance in different crops [59]. Among the antioxidants involved in ROS scavenging and maintaining steady-state ROS level reduced GSH plays an important role under salinity stress. GSH is a tripeptide found abundantly in all cell compartments in its reduced form [26]. The ratio of GSH to GSSG plays an important role in maintaining redox equilibrium in the cell during H<sub>2</sub>O<sub>2</sub> degradation [60,61]. Maintaining a high ratio of GSH/GSSG that functions as a redox couple is shown to play an important role in salt and drought tolerance as observed in *Lycopersicon esculentum*, *Myrothamnus flabellifolia*, and *Triticum aestivum* [62,63].

The increasing knowledge of genomics, molecular markers helped in understanding the mechanism of salt stress [64,65]. Manipulation of small RNA guided gene regulation represents a novel and feasible approach to improve plant stress tolerance [66]. Türkan and Demiral [34] have studied the involvement of numerous small RNAs in salinity tolerance. High salt stress causes accumulation of H<sub>2</sub>O<sub>2</sub>, and both salt and H<sub>2</sub>O<sub>2</sub> induce the expression of SRO5 protein. Work on the founding member of nat-siRNAs, which is derived from a cis-NAT gene pair of SRO5 and P5CDH genes, demonstrated an important role of nat-siRNAs in osmoprotection and oxidative stress management under salt stress in *Arabidopsis* [67].

### 3. ROLE OF SULFUR METABOLISM IN PLANTS UNDER SALINITY

Sulfur is a critical nutrient for plant growth [68] and regulates photosynthesis by affecting electron transport system [69]. Sulfur is present in the amino acids, methionine (Met; 21%) and cysteine (Cys; 27%), synthesis of protein, chlorophyll and oil in the oilseed crops [70]. The process of S acquisition and assimilation play an integral role with plant metabolism, and its deficiency leads to reduced chlorophyll content, photosystem II (PS II) efficiency and ribulose 1,5-bisphosphate carboxylase (Rubisco) content [69]. Excess sulfate transported into leaf cells accumulates mainly in the vacuoles and constitutes a large internal S reserve [71]. A good part of S incorporated into organic molecules in plants is located in thiol (-SH) groups in proteins (Cys-residues) or non-protein thiols, reduced GSH [72,73]. These bonds are important for the stabilization of protein structure. The sensing of S nutrition state occurs through elaborate systems that modulate flux involving different component pathways [74]. Adequate S nutrition is also required for plant health and resistance to pathogens [75]. S regulates photosynthesis and plant growth through improving nitrogen (N) acquisition [76].

Sulfur assimilation is highly regulated in a demand-driven manner [10,77,78]. The pathway is induced when there is a high demand for growth and development [79]. A surplus of reduced S compounds represses the pathway [80]. It has been reported in barley that the uptake capacity reached a maximum after 4 d of S deprivation and even decreased after this; however, sulfate transporter mRNA abundance continued to increase [81]. In a similar experiment with potato, the sulfate transporter mRNA abundance increased over an 8 d period; however, the measured increased uptake capacity showed only a transient rise [82]. It was proposed that a repression mechanism operated in which some downstream reduced S compounds acted to repress uptake, probably acting on the transcription of the genes for

the uptake transporters [83]. When S supply becomes limiting, the levels of these compounds fall and the repression is relieved. Indirect evidence using inhibitors supported a rapid turnover of the sulfate transporter proteins and the importance of transcriptional regulation [83,84]. Higher expression of ATPS activity has been shown necessary for the maintenance of optimal GSH levels required for the proper functioning of Ascorbate (AsA)-GSH cycle in plants. Nazar et al. [11] reported that S nutrition may provide a novel strategy to reduce the adverse effect of salinity because of S-induced increased N utilization and synthesis of reduced S compounds such as Cys and GSH. Adaptation of sulfate uptake and assimilation is assumed to be a crucial determinant for plant survival in a wide range of adverse environmental conditions since different sulfur containing compounds are involved in plant responses to both biotic and abiotic stresses [75].

Sulfate withdrawal from the growth medium decreases the levels of sulfate, Cys and GSH in plant tissues leading to the induction of sulfate transporter systems and key enzymes along the assimilatory pathway [77]. The increase in steady-state levels of mRNAs for high-affinity sulfate transporters, ATP-sulfurylase (ATPS), and adenosine 5-phosphosulfate reductase (APR) upon S starvation has been detected by Northern analysis [85,86] or cDNA arrays [87,88]. It reveals that the de-repression is regulated at the level of transcription. This de-repression correlates with the time of exposure to S-deficiency; and the activity of APR and ATPS quickly returns to the normal levels when plants are supplied with sulfate again [77]. O-acetylserine accumulates during S starvation and may thus serve as a signal of the S status [89]. O-acetylserine acts most probably as a transcriptional regulator since its addition strongly increases mRNA levels of all the three APR isoforms and also those of sulfite reductase (SiR), chloroplastic O-acetyl serine (thiol) lyase (OAS-TL), and cytosolic serine acetyl transferase (SAT) [90]. O-acetylserine plays a regulatory role in the synthesis of Cys by controlling the oligomerization of the Cys synthase complex, thus coordinating between serine as the nitrogen (N) source and sulfide as the sulfate assimilation intermediate [91].

#### 4. PHYSIOLOGICAL RESPONSE OF GLUTATHIONE IN SALINITY TOLERANCE

Studies have shown the up-regulation of Cysteine synthesis in plants in response to salinity, suggesting a definite possible role of thiol in salt stress tolerance. In order to cope with the salt-induced adverse effects, plants develop defense mechanisms that include the up-regulation of synthesis of GSH which has essential roles within the plant metabolism in reducing the adverse effects of salinity stress [11]. GSH is a low-molecular weight S metabolite (thiol), non-enzymatic antioxidant found in most of the cells. Synthesis of GSH is well regulated by S in a demand driven manner. It has been observed that increases in GSH synthesis are associated with an up-regulation of the Cys synthesis. Similarly, S assimilation pathway enzymes have been found to be involved in the regulation of GSH synthesis. Oxidation state of GSH homeostasis (GSH/GSSG) can maintain cellular redox status of GSH in plant cells [92]. The capacity of GSH to participate in the redox regulation in plant cells is, to a great extent, dependent on its absolute concentration and the ratio of GSH/GSSG under salinity stress [11]. Wild-type *Brassica napus* accumulated glutathione and cysteine upon salt stress, whereas transgenic plants with a high capacity to sequester sodium into the vacuoles did not show these antioxidative responses [93]. Khan et al. [13] reported that in *Brassica juncea* L. cultivar Pusa Jai Kisan high ATP-sulfurylase activity was responsible for a greater tolerance to salinity stress because of efficient antioxidant system and an enhanced glutathione content which led to increased photosynthesis. Enhanced concentrations of glutathione would confer better antioxidative protection and would be considered an acclimation. Recently, Nazar et al. [11] have shown that *Vignaradiata* cultivars differing in salt tolerance have different rates of GSH biosynthesis; the GSH content was

higher in salt tolerant than salt sensitive cultivar. The salt sensitive cultivar showed a greater oxidative damage than the salt tolerant cultivar. Similar reports are also available in *Lycopersicon esculentum* [94], *Oryza sativa* [95] and *Arachis hypogaea* [96]. Cysteine, a final product of S assimilation pathway, is supposed to be the rate-limiting factor for GSH biosynthesis [97,75]. Therefore, GSH biosynthesis can be tightly linked to S assimilation pathway [75,98]. GSH have the ability to directly scavenge the metal induced ROS such as  $O_2^{\cdot-}$ ,  $H_2O_2$  and  $OH^{\cdot}$ . The physiological functions of GSH have been mainly attributed to its reduced form (GSH) in plants; however, it gets oxidized to GSSG. Therefore, it is important to keep high proportion of GSH in the reduced state by the activity of GR with the oxidation of NADPH.

A number of reports are available on the response of GSH to salt stress, but its mode of action in coping salt stress needs elaborative studies. The integrated knowledge on physiological, biochemical and molecular approaches may provide deeper insights towards the precise roles and mechanism of GSH involved in salt stress tolerance. Therefore, novel approaches may be required to monitor the redox potential in different cellular compartments.

## 5. SALICYLIC ACID IN SALINITY TOLERANCE

Plants are frequently exposed to diverse biotic and abiotic stresses throughout their life cycle. In stress-induced growth processes, the initial stimulus seems to be translated into a hormone response that changes the hormonal regime in specific tissues or even in the whole plant. The increase in one hormone level can decrease the response to another. The importance of salicylic acid, jasmonic acid, abscisic acid as primary signals in regulation of plant responses is well established [99]. The outcome of defensive responses depends on the composition and kinetics of the hormones produced [100,101]. It is assumed that the repressive effect of salinity on seed germination and plant growth could be related to a decline in endogenous levels of phytohormones [102]. Besides the role of these phytohormones in salinity tolerance, the role of salicylic acid in stress tolerance is gaining an increasing concern. SA has been known to be present in some plant tissues for quite some time but has recently been recognized as a potential plant growth regulator.

Salicylic acid (SA) is well known as a key signaling molecule in induction of plant defense mechanism [103,104] and reduces symptoms of environmental stress [105]. However, in a concentration-dependent manner, this indicates that SA-ameliorating effects are closely related to the applied concentration [105]. Signaling downstream of SA is regulated by NON-EXPRESSOR OF PR1 (NPR1)-dependent and independent processes. Briefly, the accumulated endogenous SA or applied exogenous SA can induce changes in the cellular redox state, leading to the monomerization of NPR1. Then NPR1 proteins are translocated from the cytosol to the nucleus, where they interact with other transcription factors and activate the expression of defense genes, thus conferring tolerance to the infected pathogens [106].

Increasing evidence in recent years shows that SA elicits defense mechanisms in response to abiotic stresses such as excessive levels of heavy metals [107], ozone and UV radiation [108], salts [10,32,109]. It is a promising compound for reducing susceptibility of crops to stress [105]. SA also influences physiological processes such as seed germination, seedling growth, fruit ripening, flowering, ion uptake and transport, photosynthesis rate, stomatal conductance and chloroplast biogenesis [110,111]. Thus, SA fulfills an important role in the regulation of unspecific plant resistance due to an induction under the influence of a broad

spectrum of protective reactions, which allow considering this growth regulator as a plant stress hormone [111].

## 6. REGULATION OF GLUTATHIONE SYNTHESIS BY SALICYLIC ACID

Phytohormones play a protective role in regulating developmental processes and signaling networks involved in plant responses to a wide range of biotic and abiotic stresses. Information on plant phytohormones and salinity tolerance is scarce, particularly on salicylic acid. Kutz et al. [112] reported that phytohormones control gene expression related to sulfur metabolism. The stress-related hormones, jasmonate, abscisic acid, and salicylic acid, as sulfur-containing compounds have an important function in plant stress defense [75]. Indeed, jasmonate coordinately induces multiple genes of sulfate assimilation [113,114]. Conversely, genes involved in jasmonate synthesis are upregulated in sulfur-starved plants [87,88] and jasmonate levels are affected in plants with disturbed PAPS metabolism [115]. Abscisic acid specifically induces cytosolic OAS-TL [116] but reduces activity of APR by an unknown posttranscriptional mechanism [117]. The interaction of GSH synthesis with stress defence is further corroborated by the finding that GSH contents increased in plants treated with abscisic acid [118] and salicylic acid [119]. It is assumed that modulation of S metabolism in plants would help in alleviating adverse effect of salt stress through the involvement of phytohormones. Among these phytohormones, the role of salicylic acid enhances S-assimilation and N assimilation which is linked to antioxidant system in plants under salinity stresses (Fig. 1).

The higher synthesis of GSH in turn affects the level of antioxidant enzymes. SA-treated mung bean cultivars exhibited changes in the physiological processes to maximize the use efficiency of N and S through higher activity of nitrate reductase (NR) and ATP sulfurylase (ATPS) and synthesis of GSH [11]. Yoshida et al. [120] observed that SA affects S uptake and metabolism by controlling GSH biosynthesis in ozone exposed *Arabidopsis*. Table 1 summarizes studies on the influence of salicylic acid on S-assimilation-related genes under abiotic stress conditions. The table indicates the relationship of SA in modulating s-assimilation. However, little is known about the interaction of salicylic acid with sulfur. High salicylate levels have also been implicated in tolerance to nickel, via induction of SAT activity and an increase in GSH [121]. Both jasmonate and salicylate induce mRNA accumulation and activity of APR [118].

**Table 1. Mediation of salicylic acid in genes involved in sulfur responses**

Gene/s	Sulfur response	Plant	Reference
SAT, OAS,Cys, GSH	activates serine acetyl transferase (SAT) activity which in turn increases reduced GSH	<i>Thlaspi goesingense</i>	[121]
$\gamma$ ECS, GSHS	regulates expression of $\gamma$ ECS, GSHS to increase the level of GSH synthesis	<i>Arabidopsis</i>	[80]
APR1,APR2,APR3	increases mRNA levels of all APR1, APR 2, APR 3	<i>Arabidopsis</i>	[118]
GSH2	regulates total GSH levels	<i>Arabidopsis</i>	[120]
ATPS	increases ATPS activity	<i>Vignaradiata</i>	[11]
GSH	increased the GSH levels	<i>Nicotiana tabaccum</i>	[120]

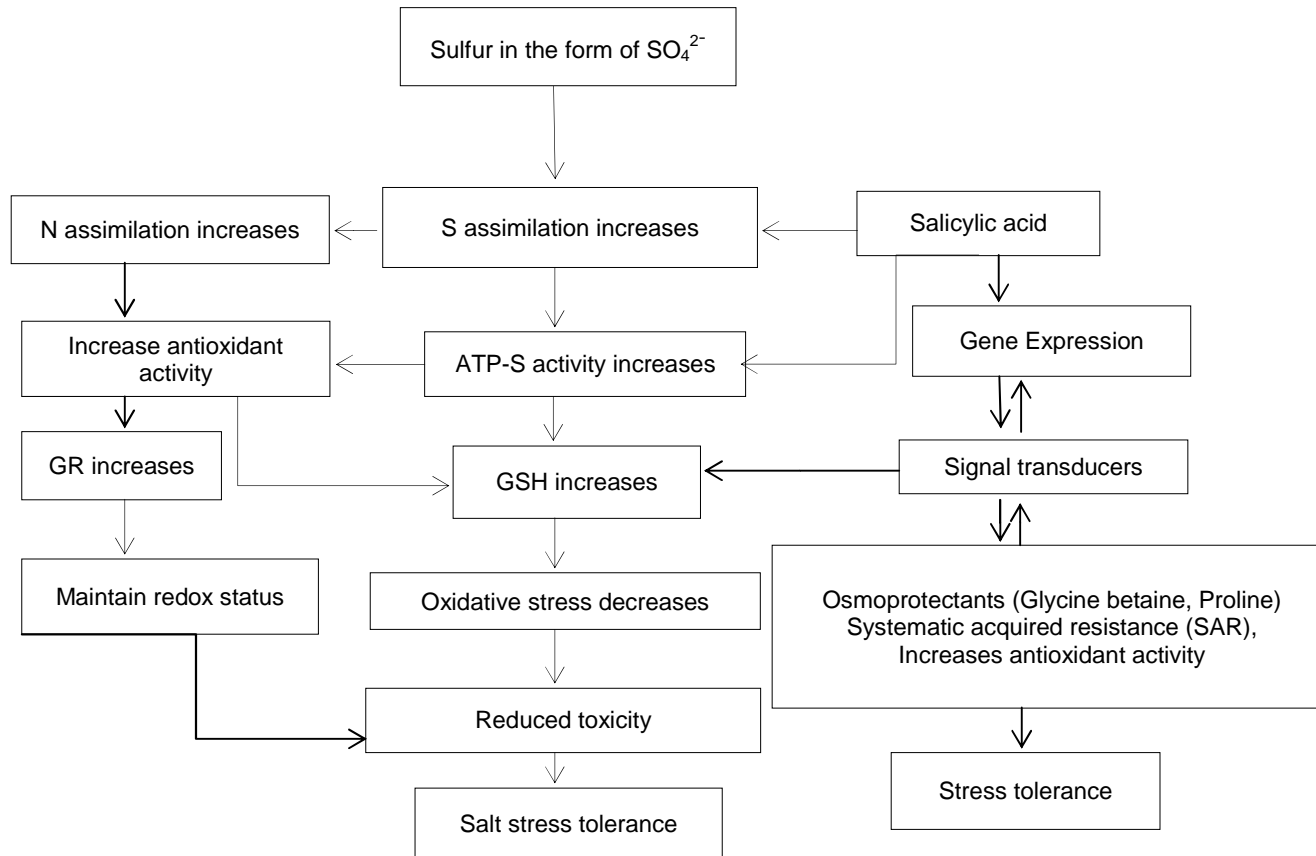


Fig. 1. Schematic representation showing the role of salicylic acid and glutathione under salinity stress to tolerance



SA also accumulates upon pathogen attack, induces expression of pathogenesis-related genes, and is a necessary component of systemic acquired resistance [122]. Treatment of tobacco leaves with SA as well as an infection with tobacco mosaic virus resulted in an increase in GSH content in inoculated, but not in systemic leaves [119]. A treatment with the biologically active SA analogue 2,6-dichloroisonicotinic acid also increased the GSH level leading to a reduction of NPR1, a regulator of systemic acquired resistance, and expression of the PR1 gene for a pathogenesis-related protein [22]. If SA regulates the expression of  $\gamma$ -glutamylcysteine ligase (*GSH1*) and glutathione synthetase (*GSH2*) or utilizes another mechanism to increase GSH synthesis remains to be clarified. Srivastava and Dwivedi [123] reported that in presence of salicylic acid the reduced glutathione, GSH: GSSG was found to be distinctly higher than their respective control. However, the increase of GSH was not proportional to that of salicylic acid in a concentration dependent manner throughout the seedling growth in pea plants. GSH has been reported to be involved in detoxification of  $H_2O_2$  and protection of the cell against various oxidative damages by maintaining redox and thiol status of proteins and other cytoplasmic cellular components in pea [124]. Furthermore, GSH, GSSG and their other derivatives may also be involved in modulating gene expression affected by increased reactive oxygen species [125]. A strong correlation between GSH and Ni accumulation in the Ni hyperaccumulators, including *Thlaspi goesingense*, has been shown. It is thought that elevated GSH is involved in resistance to the oxidative stress caused by Ni [126]. It is important to note that GSH's role in this tolerance is not to bind Ni, since Ni-S coordination was not observed in *Thlaspi goesingense* collected in the field or grown in the laboratory [127]. In *Arabidopsis thaliana* elevated GSH was also observed to lead to increased Ni resistance, and again no significant Ni-S coordination was detected [126]. Increased GSH concentration in *Thlaspi goesingense* is thought to be related to increased levels of SA in the hyperaccumulator [126]. Indeed, SA treatment leads to increased Ni resistance in the nonaccumulator *Arabidopsis*. SA treatment was observed to activate S-assimilation, increasing SAT activity, OAS, Cys, and GSH levels [121]. A number of reports are available on the response of GSH to salt stress, but its mode of action in coping salt stress needs elaborative studies. Apparently, phytohormones play important roles in the regulation of GSH synthesis that have to be investigated in detail in future studies. Such studies will provide insight into molecular mechanisms governing stress tolerance in plants and will also facilitate genetic engineering of plants to tolerate stress.

## 7. CROSS TALK BETWEEN SALICYLIC ACID AND GLUTATHIONE UNDER SALINITY

The overall extent of redox regulation of stress perception, signaling, and defence is just emerging [26]. High levels of SA seem to be tightly linked to high glutathione and  $H_2O_2$  content. Interestingly, low levels of SA in *NahG* and *sid2-2* were accompanied by approximately 50% lower  $H_2O_2$  levels, but only by a slight reduction of the glutathione content in *NahG*. Considering the tight and fast mutual interdependence of SA, glutathione and  $H_2O_2$ , caution is necessary when assigning physiological and signaling functions to one of the three molecules. Low and high SA levels were strictly correlated to a lower and higher foliar  $H_2O_2$  content, respectively. Furthermore high SA was also associated with higher GSH contents, suggesting a tight correlation between SA,  $H_2O_2$  and GSH contents in plants [25] whereas, deficiency in SA results in lower  $H_2O_2$  levels and an inability of the plant to increase the GSH level efficiently when it is exposed to short term high light stress (sHL). Feeding GSH to leaves caused an increase in SA and plants engineered to accumulate glutathione suffered from oxidative stress [128]. Zhou et al. [107] reported in *Medicago sativa* that 0.2mM SA application reduced mercury toxicity by increasing GSH content.

Similarly, SA application was found to increase GSH in ozone stress and helped in stress tolerance. Guo et al. [129] studied on *Poa pratensis* L. that 500 $\mu$ M SA application reduced Cd toxicity by increasing GSH content. GSH increases after treatment with the defense related plant hormone SA, and the redox state of this molecule shifts towards a more reduced state [22,25,100]. Kusumi et al. [130] demonstrated that SA degradation in rice resulted in a decrease of the glutathione pool size, leading to an enhanced sensitivity to oxidative stress this suggest that SA functions in the mechanism that balances reactive oxygen intermediates (ROI) scavenging and ROI production during plant growth. In dicotyledonous plants such as *Arabidopsis* and tobacco, SA has been shown to accumulate in response to various oxidizing stresses, including increased levels of hydrogen peroxide [131], ozone [132], heat [133] and NaCl [67], and this accumulation of SA correlates with glutathione levels [133,134,123]. Li et al. [135] reported that 0.5mM SA increased the content of ASA and GSH under salt stress. SA promotes the accumulation of total glutathione and induces GR [136] to maintain an enhanced concentration of GSH [121]. Over expression of a tomato GSH1 gene in tobacco leads to higher salicylic acid-mediated resistance to *Pseudomonas syringaepv tabaci* infection [137]. Treating mustard seedlings with exogenous SA improved their thermotolerance and heat acclimation that was associated with an increased glutathione pool [133]. Very little information is available regarding the fate of GSH in crop following exogenous SA application. Hao et al. [138] studied the effect of exogenous SA application on *Arabidopsis* wild type ecotype Columbia (WT) and mutant *snc1*, transgenic line *nah G* with low SA signaling and a double mutant *snc1nahG*. He observed that GSH content was higher in *snc1* (mutant) plants than in wild type and *snc1nahG* (double mutant) under salt stress suggesting that SA may promote GSH biosynthesis. SA promoted GSH biosynthesis under salt stress through increased activity of ATPS and NR [11]. Agami and Mohamed [139] reported that under Cd stress SA might act directly as an antioxidant to scavenge ROS or indirectly modulate redox balance through activation of antioxidant response in wheat seedlings.

SA could contribute to maintaining cellular redox homeostasis through the regulation of antioxidant enzymes activity [140-142]. Studies regarding on the influence of SA on GSH particularly under salt stress is very little however, the SA-mediated changes on GSH content under salinity stress are shown in (Table 2). Furthermore, detailed study is required to modulate GSH content through SA under salinity stress.

**Table 2. Response of reduced glutathione (GSH) to salicylic acid under salinity stress**

Salicylic acid	Response	Plant	Reference
100, 200 $\mu$ M/L	+	<i>Pisum sativum</i>	[123]
0.5mM	+	<i>Arabidopsis thaliana</i>	[25]
1 mM	+	<i>Lycopersicum esculatum</i>	[143]
0.1, 0.5mM	+	<i>Brassica juncea</i>	[35]
5, 10 $\mu$ g/ml-1	+	<i>Lycopersicum esculatum</i>	[144]
100 mM	+	<i>Picea abies</i>	[145]
0.1, 0.5mM	+	<i>Vigna radiata</i>	[11]
25, 50, 100 $\mu$ M	+	<i>Pisum sativum</i>	[146]
50 $\mu$ M	+	<i>Lotus japonicus</i>	[147]
0.5mM	+	<i>Triticum aestivum</i>	[136]

(+) Sign indicates up-regulation/increase

## 8. CONCLUSION AND FUTURE PROSPECTS

Salt tolerance is a complex phenomenon in plants, and various research methodologies and genetic approaches are used to characterize the diverse biochemical events that occur in response to salt stress. Based on the knowledge of an enhanced expression of a number of functionally related genes and activities of antioxidant enzymes, plants can be protected from oxidative stress damages. Sulfur assimilates not only play key roles in the primary metabolism of plants and provide structural components of essential cellular molecules, but also act as signaling molecules for cellular communication with the environment. The uptake of sulfate by the roots is one of the primary regulatory steps of sulfur assimilations. Additionally, an improved knowledge on the underlying mechanisms of action of exogenously applied salicylic acid is useful to return metabolic activities to their normal levels under salt stress conditions. The present work reveals that the GSH mechanism and its regulatory action which is involved in the protection of plants against salt stress are induced by the application of salicylic acid. Salicylic acid regulates complex signaling network involved in developmental processes and plant responses to environmental stresses including salinity. To understand how plants coordinate multiple hormonal components in response to various developmental and environmental cues is a major challenge for the future. To our knowledge the effect of salicylic acid on glutathione metabolism has not yet been studied. The application of salicylic acid will enhance the production of glutathione and alleviate stress which will facilitate understanding of the effect of SA on ATP-sulfurylase activity and GSH biosynthesis in relation to stress tolerance.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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