JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

# GLUTAMATE SUPPLEMENTATION ALLEVIATES THE OXIDATIVE EFFECTS OF SALINITY ON GROWTH AND CHLOROPHYLL SYNTHESIS IN *VIGNA RADIATA* BY UP-REGULATING TOLERANCE MECHANISMS

*Mohammed O. Alshaharni*

***Address(es):***
Biology Department, College of Science, King Khalid University, Abha 61321, Saudi Arabia.

*Corresponding author: maasalim@kku.edu.sa

https://doi.org/10.55251/jmbfs.12705

ARTICLE INFO

Received 20. 4. 2025
Revised 29. 10. 2025
Accepted 17. 12. 2025
Published 1. 2. 2026

Regular article

OPEN ACCESS

**ABSTRACT**

Influence of the exogenously applied glutamate (0, 0.5, 1, 2 and 5 mM) was investigated on growth, chlorophyll synthesis and some tolerance mechanisms in *Vigna radiata* grown on salt stress. Salt stress (100 mM NaCl) reduced height, fresh and dry weight of shoot while as glutamate supplementation mitigated the decline. Applied glutamate affectively increased glutamate 1-semialdehyde, δ-amino levulinic acid and the activity of δ-amino levulinic acid dehydratase, and in addition assuaged the decline caused by salt stress. Treatment of glutamate alleviated the decline in total chlorophyll, carotenoids and the activity of rubisco with highest increase imparted due to 1 and 2 mM glutamate. Activity of chlorophyllase increased due to salt stress and glutamate treated plants exhibited a decline in its activity. The activity of nitrate reductase and glutamate synthase was reduced due to salt stress and glutamate supplementation mitigated the decline and also increased their activities in unstressed conditions. Content of proline and sugars increased due to the supplementation of glutamate in both unstressed and salt stressed plants. The activities of antioxidant enzymes (SOD, POD, APX and CAT) and the content of reduced glutathione and tocopherol increased due to glutamate treatments. Salinity triggered enhancement in hydrogen peroxide and the lipid peroxidation was mitigated by the exogenous treatment of glutamate. Nitrogen and potassium content was reduced while as sodium and chloride accumulation increased due to salt stress, however treatment of glutamate mitigated the decline in nitrogen and potassium and also reduced the accumulation of sodium and chloride. Hence, glutamate treatment can be exploited to prevent the adverse effects of salinity on *Vigna radiata* growth and metabolism.

**Keywords:** *Vigna radiata*, glutamate, chlorophyll synthesis, rubisco, antioxidants, osmolytes

## INTRODUCTION

Salinity is the global problem that influences growth and yield of plants significantly. Salinity reduces germination, chlorophyll synthesis and influences the enzyme functioning therefore causing considerable harmful effects on the plant metabolism (**Abid *et al.*, 2020; Soliman *et al.*, 2020**). Salt stress reduces root and shoot growth, photosynthesis, mineral and water uptake, protein synthesis, alters the redox homeostasis etc thereby imparting severe decline in the overall growth of plants (**Fatma *et al.*, 2016; Qin *et al.*, 2020, 2021**). Environmental stresses reduce growth by influencing the chlorophyll synthesis, photosynthesis and the functioning and synthesis of key enzymes like rubisco (**Fatma *et al.*, 2016; Qin *et al.*, 2024**). Chlorophyll synthesis is drastically hampered by stresses through the impact on synthesis of key precursors and the intermediate components of chlorophyll biosynthesis pathway (**Li *et al.*, 2024**). In addition, the stresses cause increased accumulation of the toxic radicals which result in significant decline in the normal plant metabolic functioning (**Iqbal *et al.*, 2015; Fatma *et al.*, 2016; Lee *et al.*, 2021**). The reactive radicals cause oxidation of the proteins, lipids, nucleic acids etc resulting in altered growth by influencing the structural and functional integrity of cellular organelles. Significant increase in nitrogen metabolism can help in improving the synthesis of amino acids (**Ahanger *et al.*, 2017**) which sever as precursors for several key components like chlorophyll. Salt stress leads to osmotic and ionic stresses therefore intensifying the negative effects and hampering the normal plant functioning severely.

To counter the above mentioned growth alterations, plants are equipped with certain mechanisms that assist them to prevent the damage caused by stresses. The major tolerance mechanisms include the antioxidant system and the osmolytes which protect the plants from the ill effects of the stresses. Antioxidant system has both enzymatic and non-enzymatic machinery that together or individually neutralize the toxic reactive species from the cells to prevent their damaging effects on metabolism (**Ahanger *et al.*, 2019**). The efficient activity of antioxidant system also contributes to growth stability and maintenance of metabolism, redox homeostasis etc therefore contributing to better growth and metabolic performance under stressful conditions (**Qin *et al.*, 2024**). It has been reported that increased activity of antioxidant system significantly prevents the damaging impact of salt stress and also protects the growth, photosynthesis, mineral uptake and assimilation (**Fatma *et al.*, 2016; Taie and Rady, 2024**). Besides this the accumulation of osmolytes like sugars, glycine betaine, proline etc is believed to have significnat contribution to prevent or alleviate the negative effects of stresses. These metabolites help to scavenge the toxic radicals, maintain the tissue water potential and redox homeostasis, protect enzyme functioning and also have important role in stress signalling (Chakraborty and Kumari 2024). It has been reported that salt stress increased the buildup of osmolytes (Gharsallah *et al.,* 2016; **Ahanger *et al.*, 2019**). Plants exhibiting greater functioning of antioxidant system and the osmolyte buildup show better adaptation to stresses and can also perform well (Gharsallah *et al.,* 2016; **Guo *et al.*, 2017; El-Metwally *et al.*, 2022**). Strengthening these tolerance mechanisms can prevent the damaging impact of stresses. In this context, to improve the functioning of indigenous existing tolerance mechanisms through the exogenous application of protective molecules like phytohormones, amino acids, mineral nutrients is considered as efficient management strategies. Therefore, in this backdrop the influence of glutamate supplementation was worked out in this study.

Glutamate is an important acidic amino acid occupying a central position in plants linking nitrogen and carbon metabolism, and acts as key signalling molecule (**Qiu *et al.*, 2020; Liao *et al.*, 2022**). Glutamate is generated by glutamate synthase from glutamine and 2-oxoglutarate, and glutamate itself acts as a substrate for glutamine synthesis from ammonia that is catalysed by glutamine synthetase (**Forde and Lea, 2007**). By the activity of aminotransferases, glutamate is involved in synthesis of several amino acids. In addition, it forms a building block of glutathione, folate and tetrapyrroles, and can also conjugates auxin for its oxidative degradation and isochorismate for the synthesis of salicylic acid (Liao et al. 2022). The signalling role of glutamate regulates the growth and development by affecting seed germination, root architecture, pollen germination, pollen tube growth and defense responses to range of stresses (**Forde, 2014; Kan *et al.*, 2017; Qiu *et al.*, 2020; Ramakrishna and Atanu, 2020**). In animals, glutamate is considered as neurotransmitter and its functioning in plants has not been worked exclusively. Exogenous supplementation of glutamate increased the germination and radical length of *Cucumis sativus* under salt stress (**Chang *et al.*, 2010**). Pretreatment of glutamate increased the survival percentage under heat stress (**Li *et al.*, 2019**). It should be mentioned here that the research studies evaluating the role of applied glutamate in mitigating the influence of abiotic stresses have not been worked as much. Therefore, in present study the impact of different concentrations of glutamate on the growth, chlorophyll synthesis and the tolerance mechanisms was evaluated in mungbean (*Vigna radiata*). It is a legume crop of great importance

grown for food and fodder. It is rich in essential proteins and forms an important part of human diet. The increasing salinity levels in soils can adversely affect the growth and productivity of mungbean. Therefore, present study was carried to investigate the hypothesis that exogenous glutamate can protect mung bean growth, nitrogen assimilation and chlorophyll synthesis by regulating the antioxidant system and the osmolyte synthesis mechanisms.

## MATERIAL AND METHODS

### Seed sterilization and experimental treatments

Seeds of mung bean (*Vigna radiata*) were disinfected using 0.001% mercuric chloride for ten minutes. The sterilized seeds were then washed and blot dried using a filter paper. Sowing of seeds was done in earthen pots filed with soil, sand and compost (5:2:1), and were irrigated with Hoagland nutrient solution to full saturation. After the germination of seeds, four healthy growing seedlings were kept in each pot and others were removed. All pots were fully saturated by irrigating them twice a week with nutrient solution. After ten days of normal growth, the pots were divided into two major groups in which one group was supplied with 100 mM NaCl along with nutrient solution while as other group received the normal nutrient solution. Within the both group (normal and NaCl treated) of pots, different concentrations of L-glutamate i.e., 0, 0.5, 1, 2 and 5 mM were also supplied along with nutrient solution. Pots were kept in green house under natural conditions with three replicates for each treatment and were arranged in complete randomized block design. The plants were allowed to grow for another three weeks. Therefore, four week old plants i.e., three weeks after NaCl and glutamate treatment, were analysed for different growth and biochemical parameters discussed below.

### Measurement of height, fresh and dry weight

The height of treated and untreated plants was measured with the help of a tape. Fresh weight was immediately recorded after harvesting the plants while as the same plants were dried in oven set at 70 ºC for 48 hours to record the dry weight.

### Estimation of mineral ions

The content of nitrogen was estimated according to method described by **Jackson (1973**) and the plant samples were digested in sulphuric acid. The sodium and potassium were estimated flame photometrically after the samples were digested in mixture of sulphuric acid, nitric acid and perchloric acid. Dry plant material was digested in acid until clear solution was obtained and the digested samples were diluted using distilled water and filtered. Thereafter the filtered solution was read using flame photometer to determine the content of sodium and potassium. The content of chloride was determined following the titration method with silver nitrate. Briefly, dry plant material was boiled in 100 mL distilled water for half hour and the extract was filtered and titrated against 0.1 N silver nitrate.

### Measurement of hydrogen peroxide and lipid peroxidation

Hydrogen peroxide was extracted by macerating the fresh 100 mg tissue in 0.1% trichloro acetic acid (TCA) and optical density was taken at 390 nm (**Velikova *et al.,* 2000**). Method of **Heath and Packer (1968)** was followed for measuring the lipid peroxidation. Tissue was homogenized in 1% TCA and the supernatant was mixed with thiobarbituric acid (0.5%) and the resultant was incubated in water bath at 95 ºC for one hour. Thereafter the samples were cooled and centrifuged at 5000g. Then the mixture was read at 532 and 600 nm. The lipid peroxidation was measured as the malonaldehyde (MDA) formed after allowing the extract to react with thiobarbituric acid.

### Determination of δ-amino levulinic acid (δ-ALA) and glutamate 1-semialdehyde (GSA)

The δ-ALA content was estimated by following the method of **Harel and Klein (1972**). Briefly, two sets of leaf tissues from each treatment were taken and one set was incubated with 60 mM levulinic acid for four hours under light. However, the other set was extracted instantly in 1 M sodium acetate buffer (pH 4.6). The extract was centrifuged at 15000g for 10 min and the supernatant was mixed with acetylacetone and mixture was incubated in boiling water bath for 10 minutes. Thereafter samples were cooled and Ehrlich's reagent was added. After ten minutes optical density was recorded at 555 nm. The other set of samples that were incubated were also handled in the same way. Content of ALA was calculated by subtracting the ALA synthesized after four hour incubation from the zero hour incubation. Method described by **Kannangara and Schouboe (1985)** was used to measure the GSA. Two separate sets of 200 mg fresh leaf tissue from each treatment were taken and one set was immediately homogenised in 0.1 N HCl while as the other set was incubated in 500 µM gabaculine for 4 hours under light. Centrifugation was done at 15 000g for 10 minutes and supernatant was mixed with HCl and 3-methyl-2-benzothiazolinonehydrazone (MBTH). The mixture was incubated for two minutes in boiling water bath. Thereafter samples were cooled and $FeCl_3$ was added. Absorbance was taken at 620 nm.

### Measurement of δ-amino levulinic acid dehydratase (δ-ALAD) activity

The method described by **Shemin (1962**) was followed for measuring the ALAD activity. Briefly, fresh leaf was macerated in a prechilled pestle and mortar using a chilled buffer containing 100 mM Tris buffer (pH 7.6) supplemented with 10 mM mercaptoethanol. Homogenate was centrifuged for 10 minutes at 12000g at 4 ºC and supernatant was collected and used as enzyme source. Assay mixture contained 60 mM Tris buffer, $MgCl_2$ (15 mM), ALA (0.2 mM), EDTA, 200 mM sucrose, BSA (0.5%) and enzyme extract. The assay mixture was incubated at 28 ºC for 1 hour followed by addition of trichloroacetic acid to stop the reaction. Thereafter the amount of porphobilinogen formed was measured by Ehrlich reagent and was read at 555 nm. Absorption coefficient of $6.2 \times 10^4$ $M^{-1}$ $cm^{-1}$ was used for calculation.

### Measurement of chlorophyllase activity

Fresh leaf was extracted in 0.1 M potassium phosphate buffer (pH 7.0) having 50 mM potassium chloride, 1 mM diethylenetriaminepentaacetic acid (DPTA), 5 mM sodium diethyldithiocarbamate (DECA), triton X and polyvinyl pyrrolidone. After centrifugation for 15 minutes at 15000g, the supernatant was taken. Briefly, the constituents of the assay were phosphate buffer (pH 7.0), 0.24% triton X, 0.22 µ mol Chl a dissolved in acetone and enzyme. Samples were incubated in dark at 40 ºC for 15 minutes and after that 500 µL of sample was taken and mixed with separation mixture that contained acetone, hexane and 10 mM KOH (2:3:0.2). Mixture was vortexed and centrifuged at 12000 g for 5 minutes. Chlide *a* was determined in acetone phase by measuring the optical density at 667 nm (**Gupta *et al.,* 2012**).

### Measurement of chlorophylls, carotenoids and the activity of Rubisco

Pigments were determined by the method of **Arnon (1949**). Rubisco activity was assayed by the method described by Sharwood et al. (2016) and the absorbance was read at 340 nm. **Lowry et al. (1951**) method was used for protein determination.

### Activity of nitrate reductase and glutamate synthase activity

The activity of nitrate reductase (E.C. 1.6.6.1.) was assayed following **Jaworski (1971**). Fresh 300 mg leaf was incubated in 100 mM potassium phosphate buffer (pH 7.5) supplemented with $KNO_3$ and 0.5% n-propanol (v/v) for 3 h in dark at 30 °C. One 1 mL aliquot was mixed with 1% sulphanilamide and 0.2% 1-nephthylethylene diamine dihydrochloride. After 20 minutes the absorbance was taken at 540 nm. Activity of NADH-glutamate synthase was assayed in accordance to Lea et al. (1990) and the absorbance change was recorded at 340 nm. Fresh 100 mg leaf tissue was homogenized in chilled 100 mM Tris-HCl buffer (pH 8.0) containing magnesium chloride, cysteine, EDTA and 1% PVP. After centrifuging the extract for 20 minutes at 13,000g at 4 ºC, supernatant was collected as used as enzyme source. The absorbance of mixture containing 100 mM potassium phosphate buffer (pH 7.5), enzyme, 100 µM NADH, 10 mM glutamine and 2-oxoglutarate was taken at 340 nm.

### Estimation of proline and sugar

Proline was estimated according to the method of **Bates et al. (1973**). Plant tissue was extracted in 3% sulphosalicylic acid and extract was centrifuged at 3000g for 20 minutes. Supernatant was mixed with acid ninhydrin and glacial acetic acid, and the mixture was incubated at 100 ºC for one hour. Thereafter the samples were cooled on ice and proline was separated using toluene. Optical density was taken at 520 nm. For the estimation of the sugar content, tissue was extracted in 80% ethanol. After centrifugation at 5000g, the anthrone method was used according to **Shields and Burnet (1960**). The optical density was taken at 585 nm.

### Measurement of the reduced glutathione and tocopherol

The content of reduced glutathione was estimated as per **Ellman (1959).** Briefly, fresh 100 mg fresh tissue was extracted in phosphate buffer (pH8.0) and extract was centrifuged at 1000g for 10 minutes. Thereafter, 500 µL supernatant was reacted with 5, 5-dithiobis-2-nitrobenzoic acid and left for ten minutes. Absorbance of the mixture was taken at 412 nm and the concentration of GSH was calculated using standard curve of GSH. Tocopherol was measured following Backer et al. (1980) and the sample was macerated in mixture of ethanol and petroleum ether prepared at the ratio of 1.6:2. Supernatant was mixed with 2, 2-dipyridyl (2%) and absorbance taken at 520 nm.

**Assay of the antioxidant enzyme activities**

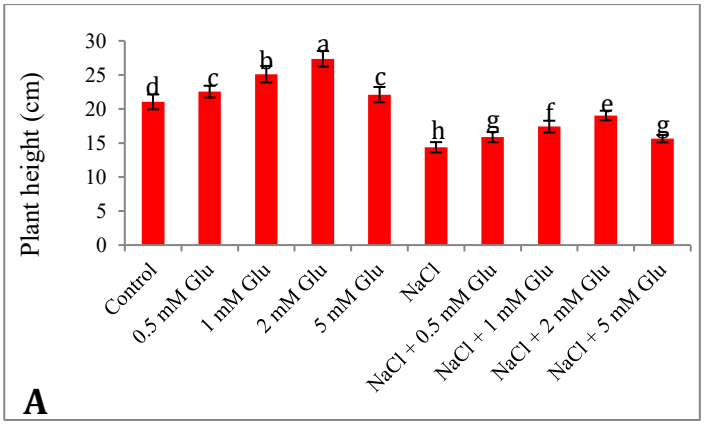
500 mg fresh leaf tissue was extracted in chilled extraction buffer that has 0.1 M phosphate buffer (pH 7.8) containing1% polyvinyl pyrolidine, 0.1 mM of each EDTA and PMSF. The extract was centrifuged for ten minutes at 12,000g and the supernatant was used as enzyme for measuring the activity of enzymes.
Superoxide dismutase activity was measured by following the method described by **van Rossum *et al.*, (1997**). Assay mixture having phosphate buffer, methionine, riboflavin, sodium carbonate, nitroblue tetrazolium, EDTA and enzyme was incubated under light for fifteen minutes. Thereafter the optical density was taken at 560 nm. Catalase activity was measured according to Aebi (1984) and the reduction in the absorbance was monitored at 240 nm for 2 minutes. The method described by **Zhou and Leul, (1998)** was adapted for assaying the activity of guaiacol peroxidase. Change in absorbance at 470 nm was recorded for two minutes in the assay mixture containing potassium phosphate buffer, enzyme extract, $H_2O_2$ and guaiacol. Activity of the ascorbate peroxidase was determined following **Nakano and Asada, (1981).** Assay mixture has phosphate buffer, EDTA, ascorbate, $H_2O_2$and enzyme. Change in optical density was taken for two minutes at 290 nm.

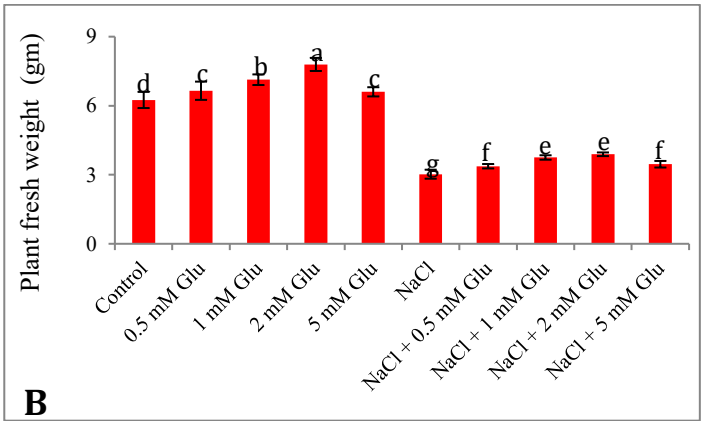
**Statistical analysis**

For every treatment data is the mean (±SE) of three replicates. Data were statistically analysed using (ANOVA) analysis of variance with the help of Statistix 8.1 software and the treatment means were analysed by using LSD test at $P < 0.05$.

**RESULTS**

Figure 1 shows the results of plant height, fresh weight and dry weight of shoot as influenced by the treatment of glutamate and NaCl. Different concentrations of glutamate resulted in increase in these growth parameters in a concentration dependent manner and the highest increase was observed in 2 mM. Compared to the control, at 1 mM glutamate height, fresh weight and dry weight increased by 19.35%, 14.08% and 23.92% respectively while as 2 mM glutamate resulted in an increase of 30.09%, 24.64% and 41.14% respectively. Salt stress declined height by 31.71%, fresh weight by 51.68% and the dry weight by 43.06% contrary to control. When glutamate was supplied to NaCl treated plants it caused increase in these parameters reflecting in the mitigation of the decline. Highest mitigation of the decline was exhibited by the plants treated with 2 mM glutamate. Compared to NaCl stressed plants, the height, fresh weight and dry weight showed an increase of 32.52%, 28.80% and 34.45% respectively in plants receiving NaCl + 2 mM glutamate (Figure 1).



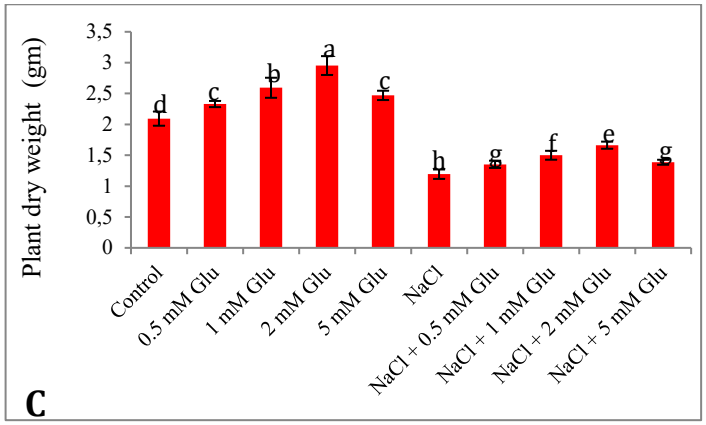
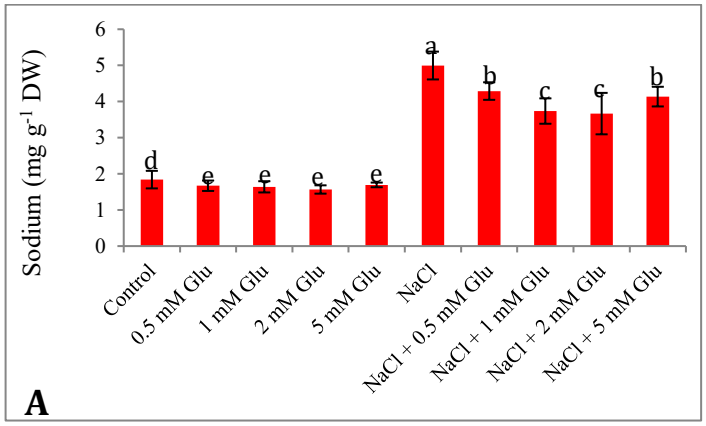
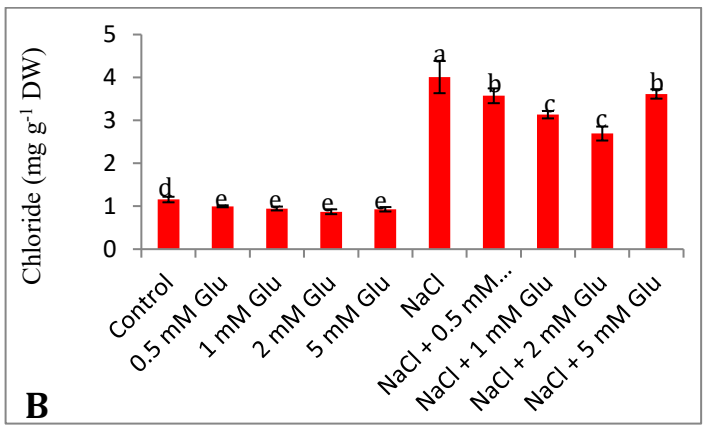




**Figure 1** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) plant height, (B) plant fresh weight and (C) plant dry weight of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

The content of sodium and chloride increased by 171.73% and 159.48% in NaCl stressed plants over the unstressed control plants however, treatment of glutamate to NaCl stressed plants reduced the accumulation of sodium and chloride. When compared with NaCl stressed plants, percent decline in the sodium and chloride was 14.20% and 7.30% in NaCl + 0.5 mM glutamate, 25.20% and 22.59% in NaCl + 1 mM glutamate, 26.60% and 30.56% in NaCl + 2 mM glutamate and 17.20% and 16.61% in NaCl + 5 mM glutamate treated counterparts respectively (Figure 2A and B). Nitrogen and potassium increased by the exogenous treatment of glutamate as compared to control and also mitigated the reduction caused by NaCl stress. Compared to control, both nitrogen and potassium increased in concentration dependent manner exhibiting the highest increase of 33.47% and % 38.42by the application of 2 mM glutamate. Salt stress reduced nitrogen by 45.63% and potassium by 41.52% contrary to the control however, when glutamate was supplied to NaCl stressed plants mitigation of the decline was observed. As compared to NaCl stressed counterparts, nitrogen and potassium exhibited an increase of 18.63% and 15.00% in NaCl + 0.5 mM glutamate, 38.42% and 40.30% in NaCl + 1 mM glutamate, 50.04% and 41.00% in NaCl + 2 mM glutamate and 40.19% and 22.70% in NaCl + 5 mM glutamate respectively (Figure 2C and D).



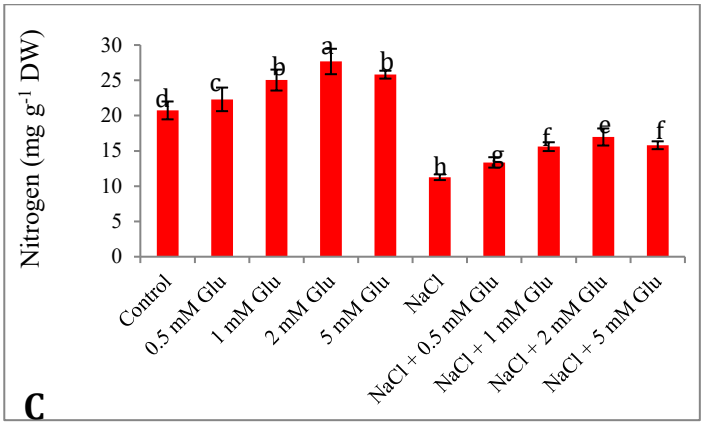
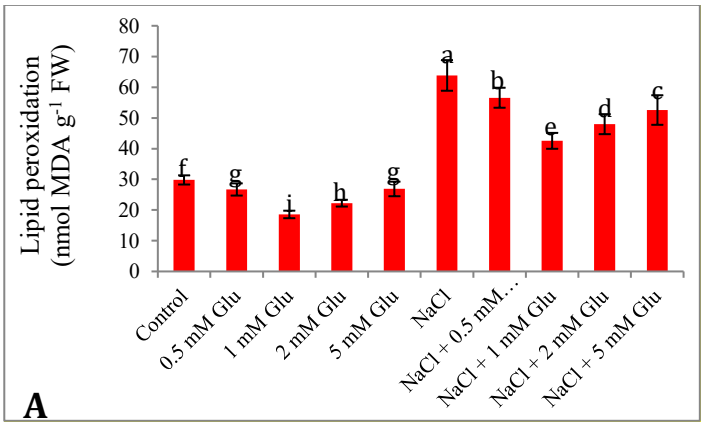



**Figure 2** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) sodium, (B) chloride, (C) nitrogen and (D) potassium of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

Salt stress increased hydrogen peroxide by 74.57% and the lipid peroxidation by 114.19% over the control. Supplementation of glutamate resulted in reduced hydrogen peroxide and lipid peroxidation at all concentrations, though 1 and 2 mM glutamate imparted highest decline under unstressed and the stressed conditions. When compared to NaCl treated plants, hydrogen peroxide and lipid peroxidation exhibited a decline of 24.28% and 33.36% in NaCl + 1 mM glutamate and 26.77% and 24.84% in NaCl + 5 mM glutamate. However, in normal grown plants the decline in hydrogen peroxide and lipid peroxidation was 5.63% and 10.40% by 0.5 mM glutamate, 34.05% and 37.68% by 1 mM glutamate, 29.71% and 25.40% by 2 mM glutamate and 17.20% and 9.83% by 5 mM glutamate treatment contrary to control (Figure 3A and B).



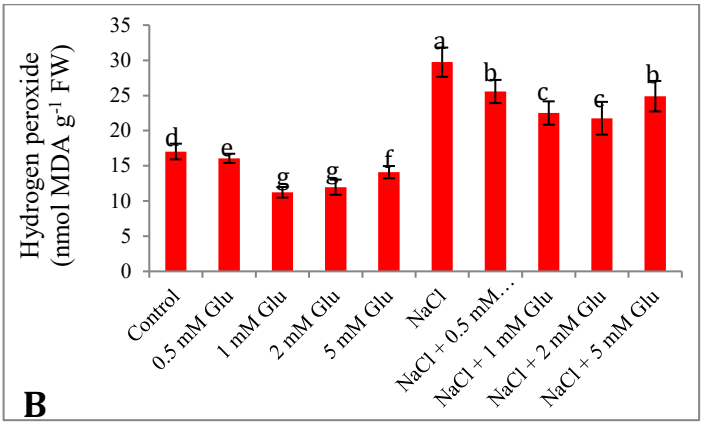
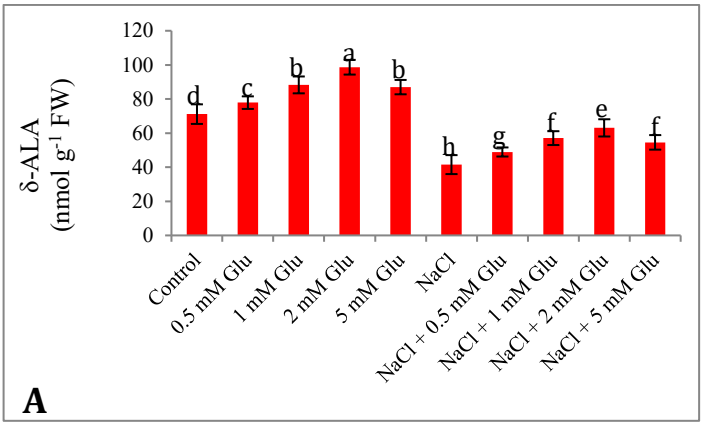




**Figure 3** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) lipid peroxidation and (B) hydrogen peroxide of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

The δ-amino levulinic acid (δ-ALA), glutamate 1-semialdehyde (GSA), total chlorophylls and carotenoids decreased significantly by the salt stress. In comparison to control, a decline of 41.61%, 43.43%, 43.73% and 34.81% was exhibited in δ-ALA, GSA, total chlorophylls and carotenoids respectively in NaCl stressed plants. The treatment of glutamate to NaCl stressed plants caused enhancement in these parameters. Relative to NaCl stressed plants, δ-ALA, GSA, total chlorophylls and carotenoids increased by17.81%, 17.84%, 5.88% and 15.53% due to NaCl + 0.5 mM glutamate, by 37.41%, 32.80%, 22.48% and 32.00% due to NaCl + 1 mM glutamate, by 51.86%, 53.36%, 35.76% and 36.02% due to NaCl + 2 mM glutamate and by 31.39%, 34.64%,10.92% and 7.24% due to NaCl + 5 mM glutamate. In unstressed conditions, glutamate increased these parameters in concentration dependent manner causing greater increase of 38.66%, 40.85%, 26.50% and 20.56% in δ-ALA, GSA, total chlorophylls and carotenoids at 2 mM concentration as contrary to control (Figure 4 and 5).



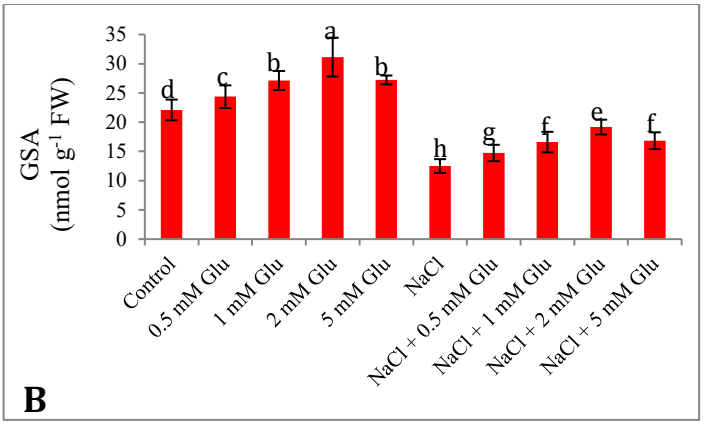
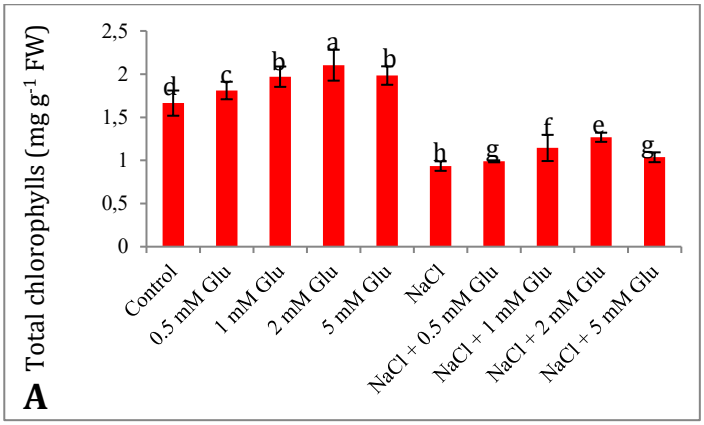




**Figure 4** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) δ-amino levulinic acid (δ-ALA) and (B) glutamate 1-semialdehyde (GSA) of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.



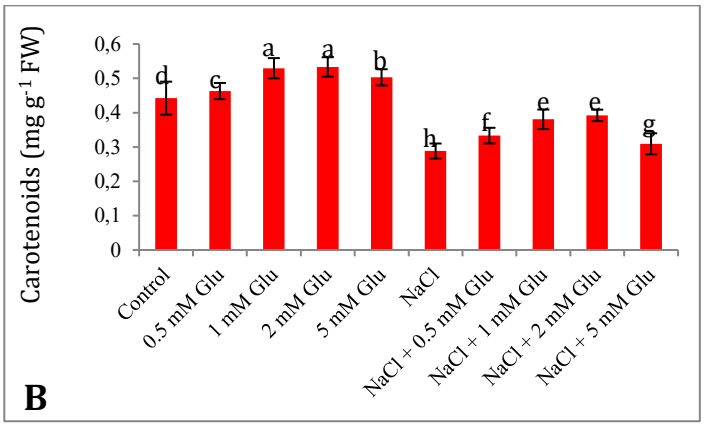
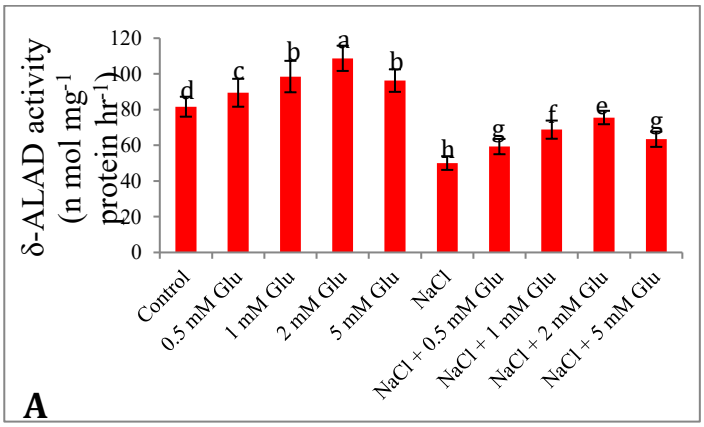




**Figure 5** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) total chlorophylls and (B) carotenoids of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

The treatment of glutamate was affective in increasing the activities of δ-amino levulinic acid dehydratase (δ-ALAD) and Rubisco while as reduced the chlorophyllase activity (Figure 6 and 7). In normal grown plants the exogenous application of 2 mM glutamate resulted in highest increase of 33.19% and 58.29% in the activity of δ-ALAD and Rubisco, even though the effect of other concentrations was also obvious. Salt stress caused a reduction of 38.66% and 44.50% in the activities of δ-ALAD and Rubisco as contrary to control (Figure 6). The treatment of glutamate resulted in mitigation of the decline in their activities at all concentration. Compared to the NaCl stressed plants, the activities of δ-ALAD and Rubisco increased by 18.43% and 7.50% in NaCl + 0.5 mM glutamate, by 37.34% and 31.65% in NaCl + 1 mM glutamate, by 50.92% and 66.47% in NaCl + 2 mM glutamate and by 26.62% and 41.03% in NaCl + 5 mM glutamate (Figure 6). Activity of chlorophyllase decreased by 8.39%, 29.92%, 28.26% and 9.31% in plants treated with 0.5, 1, 2and 5 mM glutamate as contrary to control however, NaCl treated plants exhibited an increase of 86.20% in its activity. The application of glutamate to salt stressed plants declined the activity of chlorophyllase and in comparison to NaCl treatment the decline in activity was 11.74% in NaCl + 0.5 mM glutamate, 24.24% in NaCl + 1 mM glutamate, 33.79% in NaCl + 2 mM glutamate and 13.70% in NaCl + 5 mM glutamate (Figure 7).



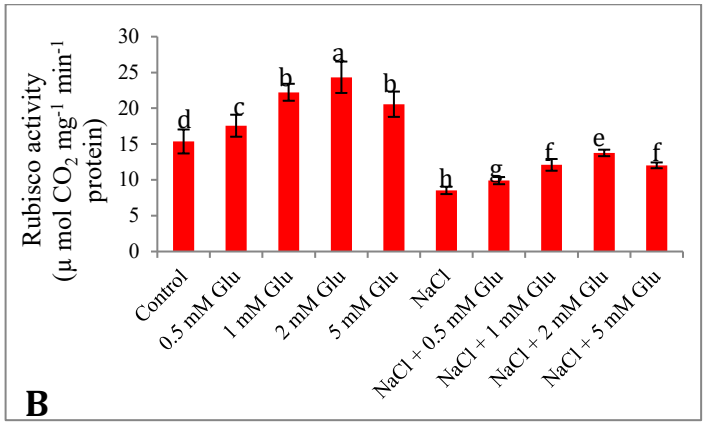




**Figure 6** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on the activity of (A) δ-amino levulinic acid dehydratase (δ-ALA) and (B) Rubisco of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

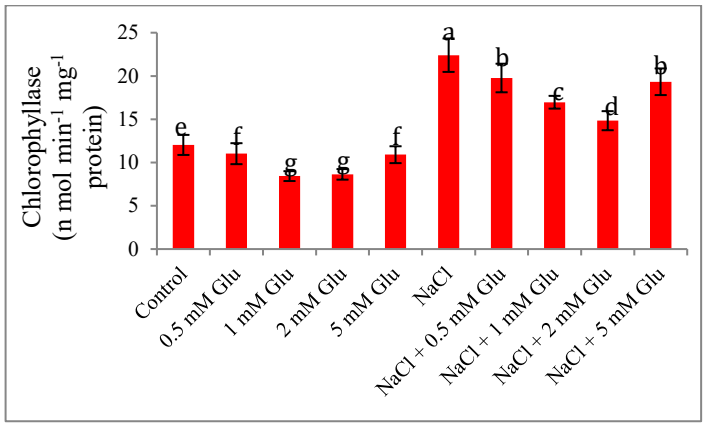
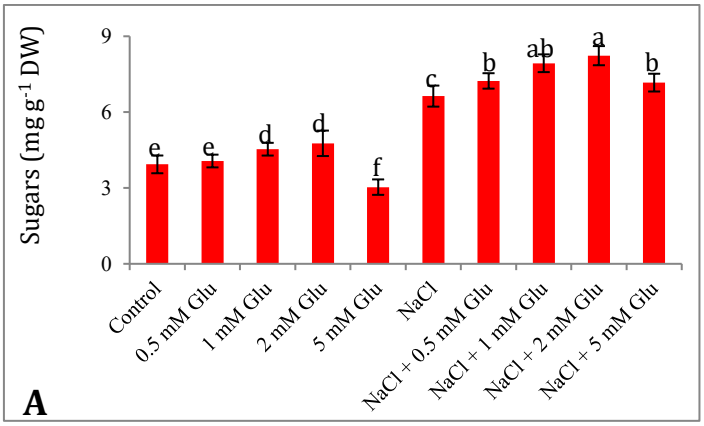



**Figure 7** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on the activity of chlorophyllase of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

The content of sugars and the proline exhibited an increase with the supplementation of glutamate in both unstressed and stressed conditions. Salt stress resulted in 68.64% and 44.90% increase in the sugar and proline content over the control plants. The treatment of glutamate to NaCl stressed plants resulted in further increase in sugar and proline accumulation. In comparison to NaCl stressed plants, the sugars and proline increased by 9.04% and 13.45% in NaCl + 0.5 mM glutamate, by 19.60% and 35.01% in NaCl + 1 mM glutamate, by 23.87% and 50.87% in NaCl + 2 mM glutamate and by 8.40% and 24.43% in NaCl + 5 mM glutamate respectively (Figure 8A and B). In normal plants the content of sugars showed highest increase of 21.11% by the 2 mM glutamate treatment while as proline exhibited highest increase of 101.42% by the 5 mM glutamate as contrary to control (Figure 8A and B).



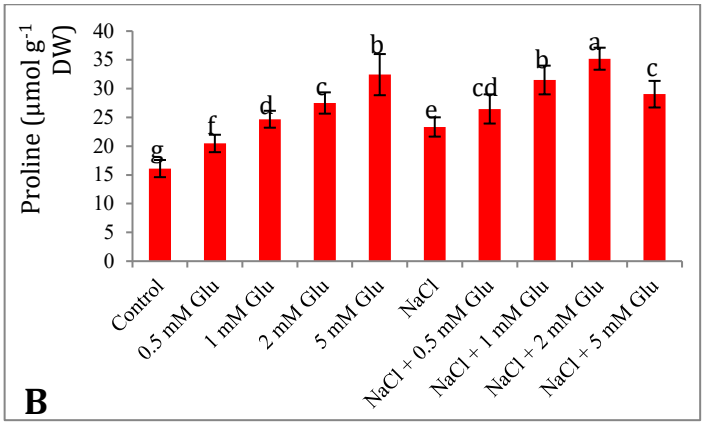



**Figure 8** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) sugars and (B) proline of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

The activity of nitrate reductase and glutamate synthase decreased by 43.24% and 34.73% respectively in NaCl conditions over the control. Supplementation of glutamate at all concentrations increased their activities and also mitigated the reduction caused by NaCl. Compared to NaCl treatment, an increase of 17.30% and 16.73% was observed in NaCl + 0.5 mM glutamate, 30.84% and 33.07% in NaCl + 1 mM glutamate, 49.58% and 50.95% in NaCl + 2 mM glutamate and 27.15% and 22.81% in NaCl + 5 mM glutamate in the activity of nitrate reductase and glutamate synthase respectively. In unstressed plants, glutamate supplementation increased their activities at all concentrations however 1 and 2 mM imparted much evident effects. Highest increase in activities of nitrate reductase and glutamate synthase was 31.84% and 32.25% in 2 mM glutamate contrary to the control (Figure 9A and B).

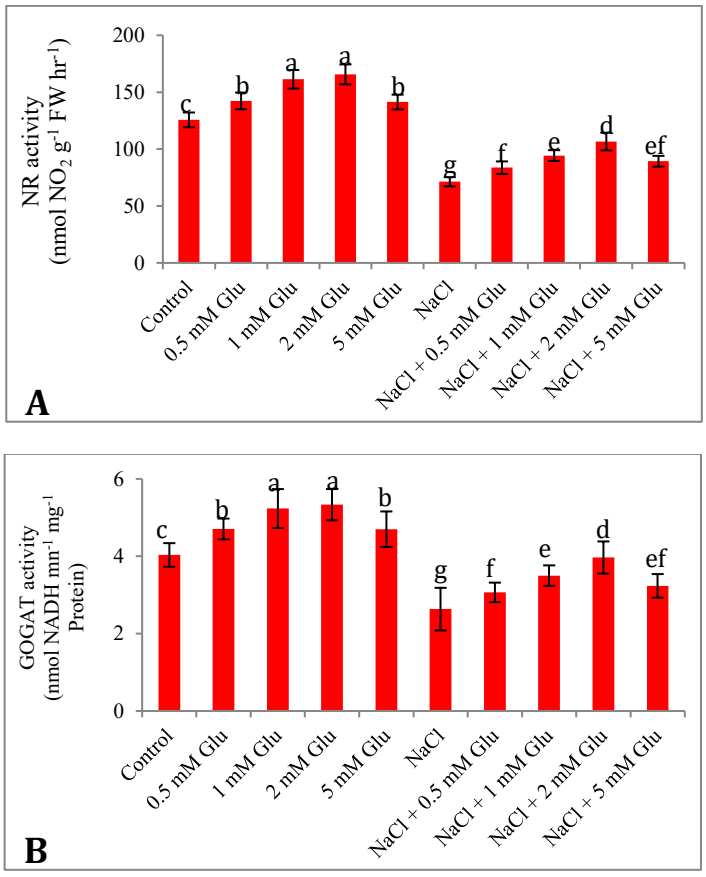
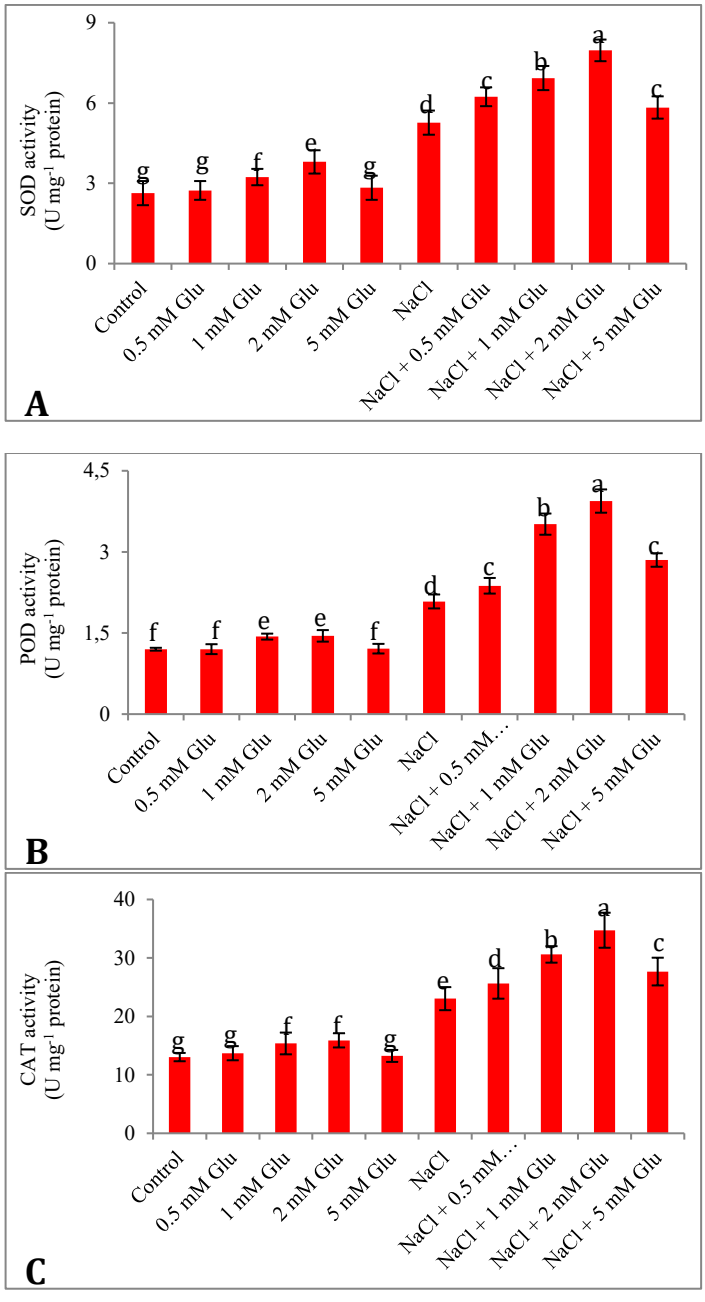



**Figure 9** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on the activity of (A) nitrate reductase and (B) glutamate synthase of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

Besides, the activities of antioxidant enzymes showed an increase due to the supplementation of glutamate in both stressed and unstressed plants. Salinity stressed plants showed 100.03%, 73.33%, 76.74% and 88.03% increase in the activities of SOD, POD, CAT and APX contrary to normal. The application of glutamate to NaCl treated plants further increased the activities exhibiting highest increase of 202.66%, 228.33%, 166.53% and 139.66% in the activities of SOD, POD, CAT and APX over the control in NaCl + 2 mM glutamate. In normal grown plants the supplementation of glutamate also increased the activities of these enzymes at all concentrations (Figure 10A-D).In addition the reduced glutathione and tocopherol contents increased by 44.33% and 46.93% in NaCl stressed plants. Application of glutamate increased the accumulation of reduced glutathione and tocopherol. Compared to NaCl treated plants, the content of reduced glutathione and tocopherol further increased by 6.38% and 18.67% in NaCl + 0.5 mM glutamate, by 19.47% and 46.68% in NaCl + 1 mM glutamate, by 28.09% and 49.14% in NaCl + 2 mM glutamate and by 12.85% and 19.65% in NaCl + 5 mM glutamate. In non stress conditions the exogenous glutamate supplementation increased reduced glutathione and tocopherol at all concentrations and the highest increase was 12.34% and 32.49% respectively in 2 mM glutamate treatment (Figure 11A and B).



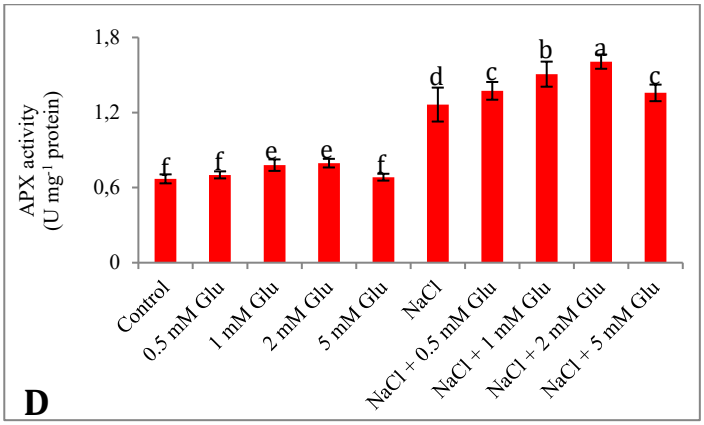
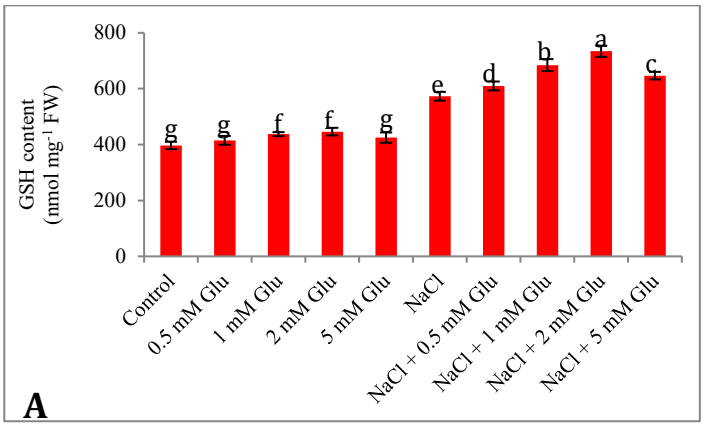



**Figure 10** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on the activity of (A) superoxide dismutase, (B) guaiacol peroxidase, (C) catalase and (D) ascorbate peroxidase of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.



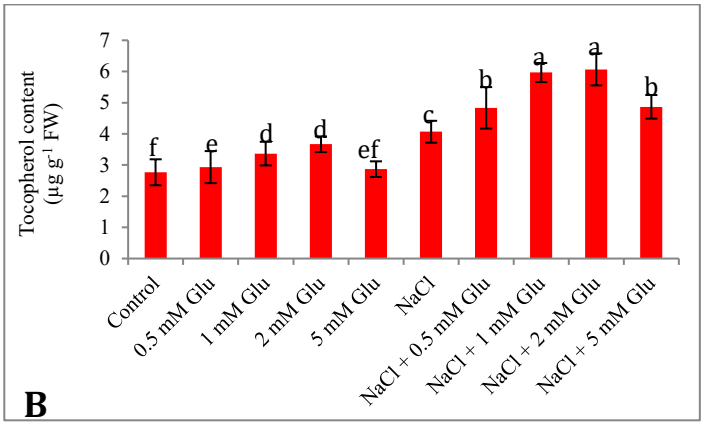




**Figure 11** Effect of supplementation of glutamate (0, 0.5 1, 2 and 5 mM) on (A) reduced glutathione and (B) tocopherol of *Vigna radiata* under unstressed and salt (100 mM NaCl) stress. The data presented in mean (±SE) of three replicates and the different letters on bars show significant difference at P<0.05.

## DISCUSSION

In the contemporary era the intensity of abiotic stresses is increasing day by day causing a significant reduction in the productive agricultural land. To protect the crop plants from the damaging effects of salinity various strategies have been tested and implemented from last decades. In present study the beneficial impact glutamate was investigated in reducing the adverse effects of salinity stress in one of the important crop plant, *Vigna radiata*. Different concentrations of glutamate tested showed a significant effect on the growth and morphological parameters. Supplying glutamate showed a beneficial effect on the growth parameters in unstressed plants and also helped in alleviating the decline resulting due to NaCl treatment. The reports showing that salt stress decreases germination, root and shoot growth, fresh and dry weight of plants are available (**Qados 2011; Ahanger and Agarwal 2017; Soliman et al. 2020; Ahanger *et al.*, 2019; Abid et al. 2020**). Salinity decreases the access and uptake of water, reduces the enzyme functioning, photosynthesis, mineral uptake and affects the cellular division therefore influencing the growth (Ahanger and Agarwal 2017; Abid et al. 2020; Zhao et al. 2021; Lu et al. 2023). However, glutamate supplementation alleviated the decline caused in the growth parameters to some extent with 1 and 2 mM concentration imparting much obvious impact. Exogenous treatment of poly-γ-glutamic mitigated the decline in height and weight of wheat under salt stress (**Guo *et al.*, 2017**). **Alfosea-Simon et al. (2020)** have also observed significant mitigation of the decline in growth parameters in tomato by the supplementation of aminoacids including glutamic acid. Therefore, from the results of present study, it seems that the applied glutamate can help mungbean plants to counter the decline in growth parameters under salt stress. Additional studies will be useful in comprehending the exact role and mechanisms. It was also observed that glutamate treatments reduced the accumulation of excess sodium and chloride ions which was otherwise significantly increased in NaCl treated plants. Under salt stress the sodium and chloride concentrations are increased manifold and to reduce its accumulation either by restricting its uptake from soil or sequester and compartmentalize the accumulated ions to vacuoles or intercellular spaces can reduce their toxic effects on growth (**Deinlein *et al.*, 2014**; **van Zelm *et al.*, 2020**). Increased accumulation of sodium and chloride ion has been reported in mung bean (**Nazar *et al.*, 2011**), wheat (**Ahanger and Agarwal, 2017**), kiwi fruit genotypes (**Abid *et al.*, 2020**) and pumpkin (**Irik and Bikmaz, 2024**), and they have also observed a noteworthy decline in the concentration of essential elements due to salt stress. This increased accumulation of sodium and chloride directly affects the growth and development of plants. For sensing sodium toxicity and subsequently reducing its accumulation to toxic levels within the tissue or organelles plants involve the efficient functioning of salt overly senstive1 (SOS1), Na/H antiporters, AHK1/ATHK1 etc has been considered to be important (**Wu, 2018**). Plants exhibiting the over expression of the transporters mediating sensing and exchange of sodium have been reported to show improved salt tolerance (**Chen *et al.*, 2007; Gouiaa *et al.*, 2012; Yue *et al.*, 2012**). Application of poly glutamic acid alleviated the salinity effects in wheat by mediating increased potassium and reduced sodium accumulation (**Guo *et al.*, 2017**). Glutamate supplementation may have affected these pathways to trigger quick sodium sequestration and reducing its excess accumulation and the damaging effects. Not only did applied glutamate reduce accumulation of sodium and chloride but also resulted in significant improvement in the uptake of nitrogen and potassium. The nitrogen and potassium regulate several plant growth and developmental events leading to increased stress tolerance and better yield productivity (**Ahanger and Agarwal, 2017; Sarraf *et al.*, 2023; Singhal *et al.*, 2023**).

In addition, the supplementation of glutamate caused an increase in the activities of the nitrate reductase and glutamate synthase which were reduced by the salt stress. The decline in the activities of the enzymes mediating the nitrogen assimilation in plants by NaCl has been reported by others also (**Iqbal *et al.*, 2015; Zhang *et al.*, 2023).** Both nitrate reductase and glutamate synthase are key to the efficient nitrogen assimilation in plants and decline in their activities by the salinity stress severely influences the nitrogen uptake, assimilation, remobilization and subsequent synthesis of amino acids thereby hindering growth (**Liu *et al.*, 2022**). Nitrate reductase catalyzes key step in nitrogen assimilation while as glutamate synthase assimilates the ammonium derived from the primary nitrogen uptake and the several internal nitrogen recycling pathways therefore playing a crucial role in remobilization of protein derived nitrogen (**Bernard and Habash, 2009**). Glutamate application increased the activity of both enzymes and also assuaged the reduction caused by the salt stress. Improved functioning of the nitrogen assimilation enzymes by glutamate application can contribute to enhanced amino acid synthesis and also trigger better salinity tolerance through regulation of key pathways as glutamate itself is a precursor for synthesis of many stress protective metabolites.

It was obvious that salt stress reduced the synthesis of chlorophyll and carotenoids however, plants that were grown with exogenous treatment of glutamate exhibited mitigation of the decline. This reduced decline in chlorophyll synthesis in glutamate treated plants was associated with greater synthesis of glutamate 1-semialdehyde and aminolevulinic acid which are the key primary intermediates in chlorophyll biosynthesis pathway. Glutamate is a precursor for chlorophyll synthesis that is converted to glutamate 1-semialdehyde and aminolevulinic acid by enzymatic reactions (**Tripathy and Pattanayak, 2012**). Stresses including drought, salinity, metals etc has been reported to reduce the synthesis of chlorophyll by hindering the generation of glutamate 1-semialdehyde and amino levulinic acid (Dalal and Tripathy, **2012; Turan and Tripathy, 2015; Qin *et al.*, 2024**). Salinity reduces the chlorophyll and carotenoid synthesis in many crop plants (**Ahanger and Agarwal, 2017; Kwon *et al.*, 2019; Lu *et al.*, 2023**). Salinity results in distorted chloroplasts, scattering and deformation of grana, disintegration of the thylakoid and also significantly declines the expression of genes controlling chloroplast development **(Lu *et al.*, 2023**) therefore affecting overall chloroplast functioning and the chlorophyll synthesis. Glutamate supplementation increased glutamate 1-semialdehyde, aminolevulinic acid and chlorophyll content. In addition, glutamate treatment alleviated the decline in these parameters caused by the salt stress. The activity of δ-amino levulinic acid dehydratase (δ-ALAD) was also increased while as the chlorophyllase was reduced by the supplementation of glutamate. Similar results of increased activity of chlorophyll synthesizing enzymes and the declined activity of the degrading enzymes due to glutamate has been reported in bentgrass (**Rossi *et al.*, 2021**). Stresses affect chlorophyll synthesis by hampering the biosynthetic pathway and also hindering the expression

of related genes, declining the uptake key elements like magnesium and nitrogen that form central components of chlorophyll (**Li *et al.*, 2024**). Glutamate supplementation prevented the decline in chlorophyll by regulating the synthesis of intermediate components and also making availability of enough glutamate for chlorophyll synthesis. Treatment of glutamate alleviated the decline in chlorophyll under salt stress in tomato (**Alfosea-Simon *et al.*, 2020**). **Franzoni et al. (2021**) have also confirmed mitigation of decline in chlorophyll and photosynthetic parameters due to application of 1.9 mM glutamic acid in water stressed lettuce. Treatment of glutamic acid increased chlorophyll and carotenoids in peanut causing enhancement in yield (**El-Metwally *et al.*, 2021**).

Besides this, the activity of antioxidant enzymes showed an increase due to treatment of salt stress. Increase in the activities of the antioxidant enzymes in salt stressed plants has been observed by other workers in different crops like *Broussonetia papyrifera* (**Zhang *et al.*, 2013**), wheat (**Ahanger and Agarwal, 2017**), soybean (**Soliman *et al.*, 2020**) and *Moringa oleifera* (**Azeem *et al.*, 2023**). The antioxidant functioning determines the tolerance potential of crop genotype to thrive under extreme growth conditions (**Neto *et al.*, 2006**). Glutamate treatment resulted in further increase in the antioxidant enzymes thereby avoiding the excess buildup of toxic radicals therefore leading to prevention of their deleterious effects on plant cellular structure and functioning. Reports showing increase in the antioxidant enzyme activities due to treatment of glutamic acid conferring tolerance to stresses like salinity (**Guo *et al.*, 2017**), cold (**Lee *et al.*, 2021**) and cadmium (**Saleem *et al.*, 2024**) are available. The exogenous treatment of glutamic acid to *Lens culinaris* resulted in increased activity of antioxidants therefore attenuating the oxidative damage and increasing photosynthetic pigments and growth (**Fardus *et al.*, 2021**). Increased activity of SOD neutralizes superoxide and CAT, POD and APX eliminates hydrogen peroxide therefore protect the cells from their damaging impact. Exogenous glutamic acid increased the expression the antioxidant enzyme coding genes under different stresses to strengthen the indigenous potential to counter their adverse effects (**Lee *et al.*, 2021; Franzoni *et al.*, 2022**). In addition, exogenous treatment of glutamate caused a significant increase in the reduced glutathione and tocopherol which are important redox components and have the antioxidant potential also. Both reduced glutathione and tocopherol scavenge the radicals and also mediate the enzymatic antioxidant reactions especially reduced glutathione causing neutralization of the toxic radicals (**Mehla *et al.*, 2017).** Tocopherols are present in thylakoid membranes and protect the polysaturated fatty acids and the lipoprotein by protecting them from lipoxygenase attack (**Sathishkumar *et al.*, 2010; Waskiewicz *et al.*, 2014**). Salinity stress increased the reduced glutathione content and tocopherol in *Artemisia sphaerocephala* (**Chen *et al.*, 2018**), wheat (Ahanger et al. 2019) and soybean (**Soliman *et al.*, 2020; Alnusairi, 2022**). Tocopherol improves membrane permeability and also maintains Na/K (**Chen *et al.*, 2018**), and can contribute to lessen the oxidative impact of salinity on chlorophyll synthesis, photosynthesis and growth by improving the antioxidant functioning and secondary metabolite accumulation (**Taie and Rady, 2024**).

The exogenously supplied glutamate further contributed to salt tolerance by increasing the accumulation of sugars and proline. Nevertheless, salt stress treatment triggered the accumulation of sugars and proline however, application of glutamate resulted in additional increase in concentration dependent manner. Salt stress increased the sugar and proline content in different plants including tomato (Gharsallah *et al.,* 2016), wheat (**Ahanger *et al.*, 2019; Hussain *et al.*, 2022**) and soybean (**Soliman *et al.*, 2020**). Glutamate acts as precursor for proline synthesis and applied glutamate makes the precursor available in sufficient concentration and may have also affected the biosynthetic pathway which needs to be evaluated. In olive plants, **Saleem et al. (2024**) has also observed increased proline and sugar accumulation due to glutamic acid treatment contributing to increased growth and chlorophyll content under cadmium stress. Exogenously applied glutamic acid increased proline in *Solanum lycopersicum* resulting in alleviation of oxidative effects and increasing the yield under salt stress (**Lee *et al.*, 2021**). Greater accumulation of these compatible osmolytes (proline and sugar) significantly contributes to maintenance of water content and regulating the major cellular functioning including photosynthesis and enzyme functioning. Osmolytes scavenge radicals and mediate signalling events to elicit responses for alleviating the adverse impact of stresses on growth and overall performance of plants (**Varshney *et al.*, 2023; Fu ad Yang, 2023; Chakraborty and Kumari, 2024**). Further studies to evaluate the role of glutamate in gene expression analysis of osmolyte pathways can be very helpful in unraveling the exact mechanisms.

## CONCLUSION

Salinity stress caused a significant decline in growth, chlorophyll synthesis and nitrogen assimilation, and glutamate supplementation was affective in alleviating the negative effects to significant levels. Glutamate treatment increased the chlorophyll synthesis and reduced chlorophyllase activity. Increased functioning of antioxidants and the buildup of osmolytes due to glutamate supplementation contributed to mitigate the deleterious effects of salinity in mungbean. Taken together, glutamate proved beneficial in protecting mungbean from the unpleasant impact of salinity by preventing the oxidative effects on growth, mineral uptake, chlorophyll synthesis, nitrogen assimilation and rubisco activity. Glutamate at 1 and 2 mM proved much affective and future studies to understand the influence of glutamate on gene expression and key signalling molecules under salinity can be very helpful.

**Acknowledgments:** The author extends his appreciation to the Deanship of Scientific Research at King Khalid University, Abha, Saudi Arabia, for funding this work through the General research Project under grant number GRP/145/45.

**Conflict of interest statement:** The author declares that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Author further declares that no animal or human material was used during study which could have lead to the requirement of any former consent.

## REFERENCES

Abid, M., Zhang, Y. J., Li, Z., Bai, D.F., Zhong, Y. P., & Fang, J. B. (2020). Effect of Salt stress on growth, physiological and biochemical characters of four kiwifruit genotypes. *Scientia Horticulturae*, *271*, 109473 https://doi.org/10.1016/j.scienta.2020.109473

Aebi H. 1984. Catalase in vitro. Methods Enzymol.105:121–6.

Ahanger, M. A., & Agarwal R. M. (2017). Salinity Stress Induced Alterations in Antioxidant Metabolism and Nitrogen Assimilation in Wheat (*Triticum aestivum* L) as Influenced by Potassium Supplementation. *Plant Physiology and Biochemistry*, *115*: 449-460 https://doi.org/10.1016/j.plaphy.2017.04.017

Ahanger, M. A., Qin, C., Begum, N., Maodong, Q., Dong, X. X., El-Esawi, M., El-Sheikh, M.A., Alatar, A. A., & Zhang, L. (2019). Nitrogen Availability Prevents Oxidative Effects of Salinity on Wheat Growth and Photosynthesis by Up-Regulating the Antioxidants and Osmolytes Metabolism, and Secondary Metabolite Accumulation. *BMC Plant Biology*, *19*, 479 https://doi.org/10.1186/s12870-019-2085-3

Ahanger, M. A., Tittal, M., Mir, R. A., & Agarwal R. M. (2017). Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. *Protoplasma*, *254(5)*, 1953-1963 https://doi.org/10.1007/s00709-017-1086-z

Alfosea-Simon, M., Zavala-Gonzalez, E. A., Camara-Zapata, J. M., Martinez-Nicolas, J. J., Simon, I., Simon-Grao, S., & Garcia-Sanchez, F. (2020). Effect of Foliar Application of Amino Acids on the Salinity Tolerance of Tomato Plants Cultivated Under Hydroponic System. *Scientia Horticulturae*, *272*, 109509 https://doi.org/10.1016/j.scienta.2020.109509

Alnusairi GSH. 2022. Improved Salt Tolerance by α-Tocopherol in Soybean Involves Up-Regulation of Ascorbate-Glutathione Cycle and Secondary Metabolites. *Journal of Applied Botany and Food Quality*, *95*, 31 - 42, https://doi.org/10.5073/JABFQ.2022.095.005

Arnon, D. I. (1949). Copper Enzymes in Isolated Chloroplast Polyphenol Oxidase in *Beta vulgaris*. *Plant Physiology*, *24,* 1–15. https://doi.org/10.1104/pp.24.1.1

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., & Rahimi, M. (2023). Salinity Stress Improves Antioxidant Potential by Modulating Physio-Biochemical Responses in *Moringa oleifera* Lam. *Scientific Reports, 13*, 2895. https://doi.org/10.1038/s41598-023-29954-6

Backer, H., Frank, O., de Angells, B., & Feingold, S. (1980). Plasma Tocopherol in Man at Various Times after Ingesting Free or Ocetylaned Tocopherol. *Nutr Rep Int*, *21,* 531–6. https://doi.org/10.1093/ajcn/53.3.723

Bates, L. S., Waldre, R. P., & Teare, I. D. (1973). Rapid Determination of Free Proline for Water Stress Studies. *Plant Science*, *39*, 205–207 https://doi.org/10.1007/BF00018060

Bernard, S. M., & Habash, D. Z. (2009). The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. *New Phytologist, 182(3),* 608-620 https://doi.org/10.1111/j.1469-8137.2009.02823.x

Chakraborty, P., & Kumari, A. (2024). Role of Compatible Osmolytes in Plant Stress Tolerance Under the Influence of Phytohormones and Mineral Elements. In: *Improving Stress Resilience in Plants, Physiological and Biochemical Basis and Utilization in Breeding*, 165-201 https://doi.org/10.1016/B978-0-443-18927-2.00011-X

Chang, C., Wang, B., Shi, L., Li, Y., Duo, L., & Zhang, W. (2010). Alleviation of Salt Stress-Induced Inhibition of Seed Germination in Cucumber (*Cucumis sativus* L.) by Ethylene and Glutamate. *Journal of Plant Physiol*ogy, *15*, 167(14):1152-6. https://doi.org/10.1016/j.jplph.2010.03.018

Chen, H., An, R., Tang, J. H., Cui, X. H., Hao, F. S., Chen, J., & Wang, X. C. (2007). Over-expression of a Vacuolar $Na^+/H^+$ Antiporter Gene Improves Salt Tolerance in an Upland Rice. *Molecular Breeding, 19*, 215-225. https://doi.org/10.1007/s11032-006-9048-8

Chen, X., Zhang, L., Miao, X., Hu, X., Nan, S., Wang, J., & Fu, H. (2018). Effect of Salt Stress on Fatty Acid and A-Tocopherol Metabolism in Two Desert Shrub Species. *Planta,* 247, 499–511 https://doi.org/10.1007/s00425-017-2803-8

Dalal, V. K., & Tripathy, B. C. (2012). Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. *Plant Cell and Environment*, *35(9)*, 1685-703. https://doi.org/10.1111/j.1365-3040.2012.02520.x

Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., & Schroeder, J. I. (2014). Plant salt-tolerance mechanisms. *Trends in Plant Sciences*. *19(6*), 371-9. https://doi.org/10.1016/j.tplants.2014.02.001
Ellman, G. L. (1959). Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics*, 82, 70–7. https://doi.org/10.1016/0003-9861(59)90090-6
El-Metwally, I. M., Sadak, M. S., & Saudy, H. S. (2022). Stimulation Effects of Glutamic and 5-Aminolevulinic Acids On Photosynthetic Pigments, Physio-biochemical Constituents, Antioxidant Activity, and Yield of Peanut. *Gesunde Pflanzen,* 74, 915–924. https://doi.org/10.1007/s10343-022-00663-w
Fardus, J., Hossain, M. S., & Fujita, M. (2021). Modulation of the Antioxidant Defense System by Exogenous L-Glutamic Acid Application Enhances Salt Tolerance in Lentil (*Lens culinaris* Medik.). *Biomolecules, 11(4),* 587. https://doi.org/10.3390/biom11040587
Fatma, M., Masood, A., Per, T. S., & Khan, N. A. (2016). Nitric Oxide Alleviates Salt Stress Inhibited Photosynthetic Performance by Interacting with Sulfur Assimilation in Mustard. *Frontiers in Plant Sciences*, *7*, 521. https://doi.org/10.3389/fpls.2016.00521
Forde, B. G. (2014). Glutamate Signaling in Roots. *Journal of Experimental Botany*, 65, 779-787 https://doi.org/10.1093/jxb/ert335
Forde, B. G., & Lea, P. J., (2007). Glutamate in Plants: Metabolism, Regulation, and Signalling. *Journal of Experimental Botany*, *58(9)*, 2339-58. https://doi.org/10.1093/jxb/erm121
Franzoni, G., Cocetta, G., Trivellini, A., Garabello, C., Contartese, V., & Ferrante, A. (2022). Effect of Exogenous Application of Salt Stress and Glutamic Acid on Lettuce (*Lactuca sativa* L.). *Scientia Horticulturae*, *299*, 111027 https://doi.org/10.1016/j.scienta.2022.111027
Franzoni, G., Cocetta, G., & Ferrante, A. (2021). Effect of Glutamic Acid Foliar Applications on Lettuce Under Water Stress. *Physiology and Molecular Biology of Plants, 27*, 1059–1072. https://doi.org/10.1007/s12298-021-00984-6
Fu, H., & Yang, Y. (2023). How Plants Tolerate Salt Stress. *Current Issues Molecular Biology*, *45(7)*, 5914–5934. https://doi.org/10.3390/cimb45070374
Gharsallah, C., Fakhfakh, H., Grubb, D., & Gorsane, F. (2016). Effect of Salt Stress on Ion Concentration, Proline Content, Antioxidant Enzyme Activities and Gene Expression in Tomato Cultivars. *AoB PLANTS, 8*, plw055, https://doi.org/10.1093/aobpla/plw055
Gouiaa, S., Khoudi, H., Leidi, E. O., Pardo, J. M., & Masmoudi, K. (2012). Expression of Wheat $Na^+/H^+$ Antiporter *TNHXS1* and $H^+$-Pyrophosphatase *TVP1* genes in Tobacco from a Bicistronic Transcriptional Unit Improves Salt Tolerance. *Plant Molecular Biology*, *79*, 137-155 https://doi.org/10.1007/s11103-012-9901-6
Guo, Z., Yang, N., Zhu, C., & Gan, L. (2017). Exogenously Applied Poly-γ-Glutamic acid Alleviates Salt Stress in Wheat Seedlings by Modulating Ion Balance and the Antioxidant System. *Environmental Science and Pollution Research, 24*, 6592–6598. https://doi.org/10.1007/s11356-016-8295-4
Gupta, S., Gupta, S. M., Sane, A. P., & Kumar, N. (2012). Chlorophyllase in *Piper betle* L. has a Role in Chlorophyll Homeostasis and Senescence dependent chlorophyll breakdown. *Molecular Biology Reports*, 39, 7133–7142. https://doi.org/10.1007/s11033-012-1545-8
Heath, R. L., & Packer, L. (1968). Photoperoxidation in Isolated Chloroplasts: I. Kinetics and Stoichiometry Of Fatty Acid Peroxidation. *Archives of Biochemistry and Biophysics*, *125,* 189–98. https://doi.org/10.1016/0003-9861(68)90654-1
Hussain, S., Zhang, R., Liu, S., Wang, Y., Ahmad, I., Chen, Y., Hou, H., & Dai, Q. (2022). Salt stress affects the growth and yield of wheat (*Triticum aestivum* L.) by altering the antioxidant machinery and expression of hormones and stress-specific genes. *Phyton-International Journal of Experimental Botany, 92*(3), 2022 861-881 https://doi.org/10.32604/phyton.2023.025487
Iqbal, N., Umar, S., &Khan, N. A. (2015). Nitrogen Availability Regulates Proline and Ethylene Production and Alleviates Salinity Stress in Mustard (*Brassica juncea*). *Journal of Plant Physiology*, *178*, 84-91. https://doi.org/10.1016/j.jplph.2015.02.006
Irik, H. A., & Bikmaz, G. (2024). Effect of Different Salinity on Seed Germination, Growth Parameters and Biochemical Contents of Pumpkin (*Cucurbita pepo L.*) seeds cultivars. *Scientific Reports, 14*, 6929 https://doi.org/10.1038/s41598-024-55325-w
Jackson, M. L., (1973). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd, New Delhi.
Jaworski, E. G. (1971). Nitrate Reductase Assay in Intact Plant Tissue. *Biochemical and Biophysical Research Communication*, *43*, 1274–1279 https://doi.org/10.1016/S0006-291X(71)80010-4
Kan, C. C., Chung, T. Y., Wu, H. Y., Juo, Y. A., & Hsieh, M. H. (2017). Exogenous glutamate rapidly induces the expression of genes involved in metabolism and defense responses in rice roots. *BMC Genomics*, 18, 1-17 https://doi.org/10.1186/s12864-017-3588-7
Kannangara, C. G., & Schouboe, A., (1985). Biosynthesis of d-ALA in Greening Barley Leaves. VII. Glutamate-1-Semialdehyde Accumulation in Gabaculine treated Leaves. *Carlsberg Research Communication*, *50*, 179–191. https://doi.org/10.1007/BF02907144
Kwon, O. K., Mekapogu, M., & Kim, K. S. (2019). Effect of Salinity Stress on Photosynthesis and Related Physiological Responses in Carnation (*Dianthus caryophyllus*). *Horticulture Environment and Biotechnology, 60*, 831–839. https://doi.org/10.1007/s13580-019-00189-7
Lea, P. J., Blackwell, R. D., Chen, F. L., & Hecht, U., (1990). Enzymes of Ammonia Assimilation. In: Lea, P.J. (Ed.), *Methods in plant biochemistry, enzymes of primary metabolism*, 3. Academic Press Limited, London, UK, pp. 257–276.
Lee, H. J., Lee, J. H., Wi, S., Jang, Y., An, S., Choi, C. K., & Jang, S. (2021). Exogenously Applied Glutamic Acid Confers Improved Yield Through Increased Photosynthesis Efficiency and Antioxidant Defense System Under Chilling Stress Condition in *Solanum lycopersicum* L. cv. Dotaerang Dia. *Scientia Horticulturae*, 277, 109817 https://doi.org/10.1016/j.scienta.2020.109817
Li X, Zhang W, Niu D, Liu X. 2024. Effects of abiotic stress on chlorophyll metabolism. *Plant Science, 342*, 112030. https://doi.org/10.1016/j.plantsci.2024.112030
Li, Z. G., Ye, X. Y., & Qiu, X. M. (2019). Glutamate Signaling Enhances the Heat Tolerance of Maize Seedlings by Plant Glutamate Receptor-like channels-mediated calcium signaling. *Protoplasma*, *256(4*), 1165-1169. https://doi.org/10.1007/s00709-019-01351-9
Liao, H. S., Chung, Y. H., & Hsieh, M. H. (2022). Glutamate: A Multifunctional Amino Acid in plants. *Plant Science*, *318*, 2022, 111238 https://doi.org/10.1016/j.plantsci.2022.111238
Liu, X., Hu, B., & Chu, C. (2022). Nitrogen Assimilation in Plants: Current Status and Future Prospects. *Journal of Genetics Genomics*, *49(5*), 394-404. https://doi.org/10.1016/j.jgg.2021.12.006
Lu, C., Li, L., Liu, X., Chen, M., Wan, S., & Li, G. (2023). Salt stress inhibits photosynthesis and destroys chloroplast structure by down regulating chloroplast development–related genes in *Robinia pseudoacacia* seedlings. *Plants, 12(6*), 1283 https://doi.org/10.3390/plants12061283
Mehla, N., Sindhi, V., Josula, D., Bisht, P., & Wani, S.H. (2017). An Introduction to Antioxidants and Their Roles in Plant Stress Tolerance. In: Khan, M., Khan, N. (eds) *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*. Springer, Singapore. https://doi.org/10.1007/978-981-10-5254-5_1
Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*. *22,* 867–80. https://doi.org/10.1093/oxfordjournals.pcp.a076232
Nazar, R., Iqbal, N., Syeed, S., & Khan, N. A. (2011). Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *Journal of Plant Physiology, 168*, 807–815 https://doi.org/10.1016/j.jplph.2010.11.001
Neto, A. D. A., Prisco, J. T., Enéas-Filho, J., de Abreu, C. E. B., & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, *56(1*), 87-94 https://doi.org/10.1016/j.envexpbot.2005.01.008
Qados, A. M. S. A. (2011). Effect of Salt Stress on Plant Growth and Metabolism of Bean Plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*. *10(1*), 7-15 https://doi.org/10.1016/j.jssas.2010.06.002
Qin, C., Ahanger, M. A., Lin, B., Huang, Z., Zhou, J., Ahmed, N., Ai, S., Mustafa, N. S. A., Ashraf, M., & Zhang, L. (2021). Comparative Transcriptomic Analysis Reveals the Regulatory Effects of Acetylcholine on Salt Tolerance of *Nicotiana benthamiana*. *Phytochemistry,* 181, 112582. https://doi.org/10.1016/j.phytochem.2020.112582
Qin, C., Ahanger, M. A., Zhou, J., Ahmed, N., Wei, C., Yuan, S., Ashraf, M., & Zhang, L. (2020). Beneficial role of Acetylcholine in Chlorophyll Metabolism and Photosynthetic gas exchange in *Nicotiana benthamiana* seedlings under salinity stress. *Plant Biology, 22(3)*, 357-365 https://doi.org/10.1111/plb.13079
Qin, C., Lian, H., Alqahtani, F. M., & Ahanger, M. A. (2024). Chromium Mediated Damaging Effects on Growth, Nitrogen Metabolism and Chlorophyll Synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming. *Scientia Horticulturae*, *323*, 112494 https://doi.org/10.1016/j.scienta.2023.112494
Qiu, X. M., Sun, Y. Y., Ye, X. Y., & Li, Z. G. (2020). Signaling Role of Glutamate in Plants. *Frontiers in Plant Science*, *10,* https://doi.org/10.3389/fpls.2019.01743
Ramakrishna, A., & Atanu, B. (2020). Glutamate: Physiological Roles and Its Signaling in Plants. In: Baluška, F., Mukherjee, S., Ramakrishna, A. (eds) Neurotransmitters in Plant Signaling and Communication. Signaling and Communication in Plants. Springer, Cham. https://doi.org/10.1007/978-3-030-54478-2_13
Rossi, S., Chapman, C., Yuan, B., & Huang, B. (2021). Glutamate Acts as a Repressor for Heat-Induced Leaf Senescence Involving Chlorophyll Degradation and Amino Acid Metabolism In Creeping Bentgrass. *Grass Research*, *1*, 4 https://doi.org/10.48130/GR-2021-0004
Saleem, M. H., Zafar, S., Javed, S., Anas, M., Ahmed, T., Ali, S., Mirmazloum, I., & Ahmad, A. (2024_. Modulatory effects of glutamic acid on growth, photosynthetic pigments, and stress responses in olive plants subjected to cadmium stress. *Journal of King Saud University - Science, 36(11)*, 103540 https://doi.org/10.1016/j.jksus.2024.103540

Sarraf, M., Janeeshma, E., Arif, N., Farooqi, M. Q. U., Kumar, V., Ansari, N.A., Ghani, M. I., Ahanger, M. A., & Hasanuzzaman, M. (2023). Understanding the role of Beneficial Elements in Developing Plant Stress Resilience: Signalling and Crosstalk with Phytohormones and Microbes. *Plant stress*, *10*, 100224 https://doi.org/10.1016/j.stress.2023.100224

Sathishkumar, R., Lakshmi, P. T. V., & Annamalai, A. (2010). Comparative Analyses Of Non Enzymatic And Enzymatic Antioxidants Of *Enicostemma littorale* blume. *International Journal of Pharmacology and Bio Science*, *1*, 1–16

Sharwood, R. E., Sonawane, B. V., Ghannoum, O., & Whitney, S. M. (2016). Improved Analysis of $C_4$ and $C_3$ Photosynthesis via Refined in vitro assays of their Carbon Fixation Biochemistry. *Journal of Experimental Botany*, *67*, 3137–3148. https://doi.org/10.1093/jxb/erw154

Shemin, D. (1962). 5-aminolevulinic acid dehydratase from *Rhodo-pseudomonas spheroids*. *Methods in Enzymology* 5, 883–884. https://doi.org/10.1016/S0021-9258(19)56976-9

Shields, R., & Burnett, W. (1960). Determination of Protein-Bound Carbohydrate In Serum By A Modified Anthrone Method. *Annals of Chemistry, 32*, 885–886 https://doi.org/10.1021/ac60163a053

Singhal, R. K., Fahad, S., Kumar, P., Choyal, P., Javed, T., Jinger, D., Singh, P., Saha, D., Prathibha, M. D., Bose, B., Akash, H., Gupta, N. K., Sodani, R., Dev, D., Suthar, D. L., Liu, K., Harrison, M. T., Saud, S., Shah, A. N., & Nawaz, T. (2023). Beneficial elements: new players in Improving Nutrient Use Efficiency And Abiotic Stress Tolerance. *Plant Growth Regulation, 100*, 237–265. https://doi.org/10.1007/s10725-022-00843-8

Soliman, M., Elkelish, A., Souad, T., Alhaithloul, H., & Farooq, M. (2020). Brassinosteroid Seed Priming With Nitrogen Supplementation Improves Salt Tolerance In Soybean. *Physiol Molecular Biology of Plants, 26(3),* 501-511. https://doi.org/10.1007/s12298-020-00765-7

Taie, H. A. A., & Rady, M. M. (2024). α-Tocopherol mediates alleviation of salt stress effects in *Glycine max* through up-regulation of the antioxidant defense system and secondary metabolites. *Acta Physiologia Plantarum, 46*, 39 https://doi.org/10.1007/s11738-024-03654-8

Tripathy, B. C., & Pattanayak, G. K. (2012). Chlorophyll Biosynthesis in Higher Plants. In: Eaton-Rye, J., Tripathy, B., Sharkey, T. (eds) *Photosynthesis. Advances in Photosynthesis and Respiration*, vol 34. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1579-0_3

Turan, S., & Tripathy, B. C. (2015). Salt-stress induced modulation of chlorophyll biosynthesis during de-etiolation of rice seedlings. *Physiologia Plantarum*, *153*(*3*), 477-91. https://doi.org/10.1111/ppl.12250

van Rossum, M. W. P. C., Alberda, M., & van der Plas, L. H. W. (1997). Role of oxidative damage in tulip bulb scale micropropagation. *Plant Science*, *130*, 207–216. https://doi.org/10.1016/S0168-9452(97)00215-X

van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt Tolerance Mechanisms of Plants. Annu Rev Plant Biol. 2020 71:403-433. https://doi.org/10.1146/annurev-arplant-050718-100005

Varshney, V., Singh, J., & Salvi, P. (2023). Sugar Signaling and Their Interplay in Mitigating Abiotic Stresses in Plant: A Molecular Perspective. In: Sharma, D., Singh, S., Sharma, S.K., Singh, R. (eds) *Smart Plant Breeding for Field Crops in Post-genomics Era*. Springer, Singapore. https://doi.org/10.1007/978-981-19-8218-7_12

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain treated bean plants, protective role of exogenous polyamines. *Plant Science*. *151*, 59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Waskiewicz, A., Beszterda, M., & Golinski, P. (2014). Nonenzymatic antioxidants in plants. In: *Oxidative damage to plants, antioxidant networks and signaling*, 201-234 https://doi.org/10.1016/B978-0-12-799963-0.00007-1

Wu, H. (2018). Plant salt tolerance and $Na^+$ sensing and transport. *The Crop Journal*, *6(3),* 215-225 https://doi.org/10.1016/j.cj.2018.01.003

Yue, Y., Zhang, M., Zhang, J., Duan, L., & Li, Z. (2012). SOS1 gene over expression increased salt tolerance in transgenic tobacco by maintaining a higher $K^+/Na^+$ ratio. J. Plant Physiol., 169, 255-261 https://doi.org/10.1016/j.jplph.2011.10.007

Zhang, X., He, P., Guo, R., Huang, K., & Huang, X. (2023). Effects of salt stress on root morphology, carbon and nitrogen metabolism, and yield of Tartary buckwheat. *Scientific Reports*, *13(1),* 12483. https://doi.org/10.1038/s41598-023-39634-0

Zhang, M., Fang, Y., Ji, Y., Jiang, Z., & Wang, L. (2013). Effects of salt stress on ion content, antioxidant enzymes and protein profile in different tissues of *Broussonetia papyrifera. South African Journal of Botany*, 85, 1-9. https://doi.org/10.1016/j.sajb.2012.11.005

Zhao S, Zhang Q, Liu M, Zhou H, Ma, C., & Wang, P. (2021). Regulation of Plant Responses to Salt Stress. Int J Mol Sci. 22(9):4609. https://doi.org/10.3390/ijms22094609

Zhou, W., & Leul, M. (1998). Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. *Plant Growth Regulation, 26(1*), 41–47. https://doi.org/10.1023/A:1006004921265