Effect Of Sulphur On Plant Growth & Defense System Against Salinity Stress

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ABSTRACT:
The given investigation was undertaken to evaluate the performance of black gram (vigna mungo L.) under different salinity levels (i.e. 50mM, 100mM and 150mM NaCl) and to find out the remedial effect of two doses of S (i.e. 2mM and 4mM) against salinity stress on growth of studied crop by providing tolerance against salinity stress. The experiment was carried out in Botany department, Jinnah University for women, Nazimabad, Karachi, in controlled laboratory condition by using plate culture technique followed as completely randomized design with three replication of each treatment. The observation of given research showed that the percentages of shoot and root length and Fresh and dry seedling weights were higher in control treatment. The low (50mM NaCl) level of salinity treatment had no deleterious effects on plant vegetative growth, while at higher concentration of NaCl (100mM), all the growth parameters were drastically reduced. Both application rates of MgSO₄ were found satisfactory to eliminate the negative effect of saline environment inside rhizosphere by promoting plant tolerance against stress thus support treated plants growth and development.

Keywords: Soil salinity; Sulphur; Remedial effect; Tolerance; Rhizosphere.
INTRODUCTION:
Crop productivity is adversely affected by high salt content in soils (Alam et al., 2000). Approximately, 7% of the world’s land area, 20% of the world’s cultivated land, and nearly half of the irrigated land is affected with high salt contents (Szabolcs, 1994; Zhu, 2001). Effects of salinity are more obvious in arid and semiarid regions where limited rainfall, high evapo-transpiration, and high temperature associated with poor water and soil management practices are the major contributing factors (Azevedo Neto et al., 2006). Salinity in soil can be originated from soil parent material; from irrigation water or from fertilizers, manures, composts, or other amendments. Improper irrigation practices and lack of drainage have also aggravated the problem leading to significant reductions in crop productivity (FAO, 2003). Highly saline/alkali water qualities can cause problems for irrigation, depending on the type and amount of salts present, the soil type being irrigated, the specific plant species and growth stage, and the amount of water able to pass through the root zone. Salt stress causes hyper osmotic stress and ion disequilibrium, thereby disabling the vital cellular functions of a plant. Reduced availability of water, increased respiration rate, altered mineral distribution, membrane instability, failure in the maintenance of turgor pressure in tissues. The increased levels of Na+ inside the cells change enzyme activity resulting in cell metabolic alteration; disturbance in K+ uptake and partitioning in the cells and throughout the plant that may even affect stomatal opening, thus diminishing the ability of the plant to grow. The salinity-induced reduction in growth and development of plants is associated with ionic/osmotic effects, nutritional imbalance or oxidative stress. The cause of injury is probably due to the salt load exceeding the ability of the cells to compartmentalize salts in the vacuole. Salts then would rapidly build up in the cytoplasm and inhibit enzyme activity. Alternatively, they might build up in the cell walls and dehydrate the cell (Munns, 2005).

Plants have developed a wide range of adaptive/resistance mechanisms to maintain productivity and ensure plant survival under salt stress. Of the several possible mechanisms to reduce the effects of salinity stress, management of mineral nutrients status of plants can be the efficient defense system. In some plants increased resistance to a biotic stresses has been achieved by exogenous application of various organic and inorganic solutes (Afsheen Aamir and Saima Ibrahim 2013; Hamdia and El-Enany 1998). This approach, which may significantly contribute to increased crop production in stress environments. Sulfur (S) is an important plant nutrient involved in plant growth and development. It is an integral part of several important compounds, such as Sulphur containing amino acids (cysteine and methionine) and many other compounds, e.g. glutathione or ferredoxin, vitamins, co-enzymes, phytohormones and reduced sulfur compounds that decipher growth and vigor of plants under optimal and stress conditions. Externally supplied Sulphur has been shown to ameliorate the adverse effects of salinity on plants, presumably by facilitating higher K+/Na+ selectivity (Hasegawa et al., 2000). Another key role attributed to supplemental Sulphur addition is its help in osmotic adjustment and growth via the enhancement of compatible organic solutes accumulation (Giriya et al., 2002). Sulphur has also been implicated in stress protection by stabilizing membranes and reducing the oxidative damage (Larkindale and Knight, 2002). The present review focuses on improving the salinity effects on physiology and metabolism of plants and the importance of sulfur in improving plant tolerance against salinity.

MATERIALS AND METHOD Laboratory experiment (plate culture technique):
In this experiment, Petri plate method was used to study the effect of different NaCl concentrations (50, 100 and 150mM), alone and along MgSO₄ solution (as S source) on the growth parameters of black gram (vigna mungo L.). Three sets of treatment (T1, T2, T3) were designed on the basis of three concentration of salinity (50, 100 and 150Mm NaCl) along with 2 rates of MgSO₄(2mM and 4Mm), while the control set up (T0) was organized with no salinity basis (0mM) along with single doses of both rate of MgSO₄ separately. Each treatment was replicated thrice and arranged in complete randomized block design. Ten healthy chemically sterilized seed of black gram (vigna

| Experimental Layout | NaCl | MgSO₄ |
|---------------------|------|------|
| T0                  | 0mM  | 0mM S| S |
| T1                  | 50mM | 0mM S| S |
| T2                  | 100mMNaCl | 0mM S | S |
| T3                  | 150mMNaCl | 0mM S | S |

| Treatments | NaCl | MgSO₄ |
|------------|------|------|
| T0         | 0mM  | 0mM S|
| T1         | 50mM | 0mM S|
| T2         | 100mMNaCl | 0mM S|
| T3         | 150mMNaCl | 0mM S|
mungo L.) were placed in each Petri plate with one disc of filter paper under normal laboratory condition with temperature ranging from 21-25°C. Five ml of each treatment were added alternately a day. The growth parameters including shoot length, root length, shoot fresh and dry weight and root fresh and dry weight were recorded after 6th day of germination.

**Inhibitory Percentage (I):** The inhibitory percentage of calculated reading was calculated by Surendra and Pota, (1978) formula. 
\[ I = \frac{100 - T/C}{T/C} \times 100 \]

Where, I is the parentage of inhibition, T is treatment reading, and C is control plant reading.

**Statistical Analysis:** The data was subjected to statistical analysis by the software program of SPSS. Data are displayed as mean ± standard deviation for three replications. Significant difference (P< 0.05) is denoted as asterisk (*) between the control and treatments.

**RESULT:**

The given Research indicated the response of plant under salinity stress & its reclamation through S as potential mineral to reduced salinity inhibitory effect on plant growth. Salt stress affects the plant growth and development thereby affecting the yield quantity and quality of stressed crop, similar finding was also experienced by Cuartero et al., 2006.

**SHOOT LENGTH (cm):** Data regarding shoot length of black gram are presented in Table-1a which showed that, alleviated salinity greatly reduced plant shoot length when compared to untreated control plant. Salinity cause enhancement of osmotic pressure leads reduction of water absorbance and disturbance in metabolic and physiological processes. The excess of Na⁺, Cl⁻ ions modifies the metabolic activities of cell wall, which causes deposition of several materials on cell wall and limits the cell wall elasticity, thus cell walls become rigid and turgor pressure efficiency in cell enlargement is decreased with application of elevated salt treatment. (Babu et al.,2012). The inhibitory percentage increased by the rate of 49.08%, 56.16% and 87.915% at 50, 100 and 150M respectively. This high degree of shoot length inhibition greatly affects the plant growth by lowering physiological functioning of plant. Application of sulfur at low strength provide a significant tolerant toward salinity effect and reduced inhibitory percentage from 49.08% to 20.34% and 11.897%, 56.16% to 43.838% and 28.746%, 87.915% to 59.698% and 57.972% at 2mM and 4mM S respectively.

**ROOT LENGTH (cm):** Table-1a showed that increase in salt concentration significantly inhibited the root length of black gram due to their direct exposure to the saline medium. The treated plant showed 36.16%, 24.47% and 84.99% reduction in RL at salinity elevation from 50mM salt to 150mM NaCl respectively over control. The application of MgSO₄ had positive effect on reducing the inhibitory effect of salinity on RL. The percent inhibition RL noticeably reduces 6.8%, 16.77% and 50.64% when S was applied at high rate (i.e 4mM).

**SHOOT FRESH WEIGHT (gm):** Table-1b showed that, all the salinity levels induced reduction in water uptake that resulted in reduced shoot fresh weight in black gram plant. The highest reduction was found with 150mM salinity level of about 69.17% over control. Application of S significantly overcome the water uptake inhibition and increase SFW. Data revealed that, 38.5%, 30.47% and 69.17% inhibition in SFW were reduced to 12.034%, 42.59% and 59.91% with 2mM S-dose and 3.63%, 36.27% and 44.32% with 4mM S application when compared to untreated / control plant SFW.

**ROOT FRESH WEIGHT (gm):** Table-1b showed that, on increasing salinity. The uptake of water from soil solution greatly inhibited from 43.75% to 68.75% from 50mM to 150mM. These reductions in RFW with salinity significantly overcome with 4mM MgSO₄ application, whereas low S dose also induced inhibition in water uptake thus decrease RFW up to 137.5% to 106.2% respectively with 100mM and 150mM NaCl over to control.

**SHOOT DRY WEIGHT (gm):** Table-1c showed that, treatment with 150mM salinity was found inhibitory for shoot dry weight production and caused 67.02% reduction over control plant. Decrease in dry mass accumulation is proportional to increasing levels of salinity. This result is in agreement with those reported by Majkowska et al. (2008). The cause of reduction in dry matter content could be the reduced development and differentiation of tissues, shrinkage of the cell contents, unbalanced nutrition, damage of membrane and disturbed avoidance mechanism (Akram et al. 2007). Salt...
stress also resulted in a disturbance in photosynthesis, enzyme activities, protein synthesis energy and lipid metabolism which effects the metabolites transportation to the storage tissues and hence reduced dry mass accumulation (Parvaiz & Satyawati, 2008). Reduction in total biomass under different salinity levels was also reported by Al-Ansari, (2003). This high degree of influences caused by applied salt was reduced with the application of S at both levels (i.e. 2mM and 4mM).the percent inhibition reduced from 31.91%, 0.106% and 67.021% to 17.02%, 38.29% and 50% with 2mM S and 5.319%, 31.914% and 37.23% with 4mM S over control.

ROOT DRY WEIGHT (gm): Table-1c showed that, all the salinity levels greatly reduced root dry biomass accumulation @35.29%, 58.82% and 90.58% over control. Application of MgSO4 at low dose (2mM) significantly overcome this inhibition and lowered the inhibitory percentages up to 23.52%, 47.05% and 35.29% respectively.

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### Table 1a: Effect of Sulphur on Plant Length (cm) growing against different Salinity levels

| NaCl (mM) | SHOOT LENGTH (cm) | ROOT LENGTH (cm) |
|-----------|--------------------|-------------------|
|           | MgSO4              | MgSO4             |
|           | 0mM                | 2mM               | 4mM               | 0mM            | 2mM               | 4mM               |
| T0 0mM    | 13.40±0.839 (0)    | 10.60±0.890 (7.738) | 10.27±0.823 (5.198) | 0.73±0.894 (0) | 0.20±1.273 (7.300) | 0.84±1.533 (11.377) |
| T1 50mM   | 7.87±1.746 (-49.68) | 12.32±1.959 (20.341) | 13.62±0.574 (-11.897) | 4.31±1.742 (36.16) | 5.53±0.602 (18.11) | 7.21±0.722 (-6.811) |
| T2 100mM  | 6.78±1.157 (-56.16) | 8.86±1.079 (43.831) | 11.02±1.130 (28.746) | 5.1±0.854 (24.47) | 4.83±1.276 (27.646) | 5.62±1.304 (16.777) |
| T3 150mM  | 1.86±1.406 (-87.313) | 0.23±1.090 (-39.098) | 0.35±0.574 (-57.972) | 1.01±1.229 (-84.999) | 3.2±0.307 (32.013) | 3.33±0.823 (30.044) |

Each value is an average of 3 replications ± Standard deviation. Values in ( ) are % of respective control.

### Table 1b: Effect of Sulphur on Plant Fresh Weight (gm) growing against different Salinity levels

| NaCl (mM) | SHOOT FRESH WEIGHT (gm) | ROOT FRESH WEIGHT (gm) |
|-----------|--------------------------|------------------------|
|           | MgSO4                    | MgSO4                  |
|           | 0mM                      | 2mM                    | 4mM                    | 0mM            | 2mM                    | 4mM                    |
| T0 0mM    | 1.15±0.395 (0)           | 1.22±0.258 (-5.628)    | 1.31±0.231 (-19.339)   | 0.03±0.008 (0) | 0.04±0.003 (-34.37)    | 0.027±0.110 (-15.62)   |
| T1 50mM   | 0.71±0.266 (-38.528)     | 1.01±0.307 (12.034)    | 1.13±0.092 (-3.636)    | 0.04±0.040 (-43.75) | 0.08±0.026 (-15.0) | 0.09±0.051 (-181.2) |
| T2 100mM  | 0.80±0.281 (-30.476)     | 0.66±0.297 (-42.597)   | 0.73±0.105 (-36.277)   | 0.02±0.015 (-18.75) | 0.07±0.055 (-137.5) | 0.02±0.017 (-37.5) |
| T3 150mM  | 0.35±0.151 (-69.177)     | 0.46±0.162 (-59.913)   | 0.64±0.257 (-44.329)   | 0.01±0.001 (-68.75) | 0.06±0.081 (-106.2) | 0.016±0.011 (-50) |

Each value is an average of 3 replications ± Standard deviation. Values in ( ) are % of respective control.
Effect of Sulphur on Black gram defense system against Salinity stress (Plate culture)

| NaCl (mM) | SHOOT DRY WEIGHT (gm) | | | | ROOT DRY WEIGHT (gm) | | |
|-----------|------------------------|---|---|---|------------------------|---|---|
|           | MgSO4 0mM | 2mM | 4mM | MgSO4 0mM | 2mM | 4mM |
| T0        | 0mM | 0.094±0.026 (0) | 0.083±0.021 (-11.702) | 0.086±0.015 (-8.510) | 0.017±0.003 (0) | 0.015±0.005 (-10) | 0.011±0.004 (-35.29) |
| T1        | 50mM | 0.064±0.057 (-31.914) | 0.078±0.031 (-17.021) | 0.089±0.11 (-5.319) | 0.011±0.003 (-35.29) | 0.013±0.004 (-23.52) | 0.015±0.004 (-11.76) |
| T2        | 100mM | 0.084±0.031 (-0.106) | 0.058±0.028 (-38.297) | 0.064±0.015 (-31.914) | 0.007±0.004 (-58.82) | 0.009±0.001 (-47.05) | 0.004±0.002 (-76.47) |
| T3        | 150mM | 0.031±0.011 (-47.021) | 0.047±0.012 (-50) | 0.059±0.025 (-37.234) | 0.0016±0.001 (-90.58) | 0.011±0.010 (-35.20) | 0.003±0.001 (-82.35) |

Each value is an average of 3 replications ± Standard deviation.
Values in ( ) are % of respective control.

Fig. 1(a) Effect of Sulphur on Shoot length (cm) of Black gram (vigna mungo L.) growing under different Salinity levels
Fig. 1(b) Effect of Sulphur on Root length (cm) of Black gram (vigna mungo L.) growing under different Salinity levels

Fig. 1(c) Effect of Sulphur on Shoot Fresh Weight (gm) of Black gram (vigna mungo L.) growing under different Salinity levels

Fig. 1(d) Effect of Sulphur on Root Fresh Weight (gm) of Black gram (vigna mungo L.) growing under different Salinity levels
Fig. 1(e) Effect of Sulphur on Shoot Dry Weight (gm) of Black gram (*vigna mungo* L.) growing under different Salinity levels

Fig. 1(f) Effect of Sulphur on Root Dry Weight (gm) of Black gram (*vigna mungo* L.) growing under different Salinity levels