AMELIORATION OF ADVERSE EFFECTS OF SALT STRESS ON MAIZE (ZEA MAYS L.) CULTIVARS BY EXOGENOUS APPLICATION OF SULFUR AT SEEDLING STAGE

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Abstract

Sulfur is an important plant nutrient involved in seed germination and seedling establishment. It also plays an important role in response of plants to tolerate abiotic stresses such as salinity. A study was conducted to assess the role of sulfur on salinity tolerance of maize (Zea mays L.) at seed germination stage. Six varieties (Sadaf, MMRI, Pearl Basic, Agaitti 2003, Saiwal 2002 and Pak Afgoi 2003) and two hybrids (Yusaflwa Hybrid and Hybrid 1898) of maize were used to assess the modulation of salt stress by exogenously applied sulfur. Three NaCl (25, 50 and 75 mM) and five potassium sulfate (20, 40, 60, 80 and 100 mM) levels were applied to plants as sand amendment at sowing time along with a control. The experiment was laid down in a Completely Randomized Design (CRD) with three replicates. The data for various germination attributes were recorded. The results revealed that sulfur application significantly modulated all germination parameters i.e., germination percentage, germination index, coefficient of velocity of emergence, mean emergence time, vigour index, germination energy, germination speed, mean daily germination and germination value and thus reduced the toxic effect of salinity. It was found that sulfur at 60 and 80 mM had more pronounced effect in enhancing seed germination. Application of sulfur at 60 to 80 mM improved all germination parameters and reduced time needed for 50% seed to germinate. The phylogenetic tree constructed by NTSysPC clearly clustered all genotypes into two distinct clusters. The tolerant cluster mainly contained 4 varieties (Sadaf, MMRI, Pearl Basic and Agaitti 2003) while the sensitive cluster included two varieties (Saiwal 2002, Pak Afgoi 2003) and two hybrids (Hybrid 1898 and Yusaflwa hybrid). Based on the distance matrixes generated by software, Agaitti 2003 proved to be the most tolerant genotype. In comparison, a maize variety (Pak Afgoi-2003) and a Hybrid-1898 showed the least improvement by exogenously applied sulfur. These results proved that sulfur enhanced salinity tolerance of maize by significantly improving seed germination. Additionally, a highly variable intra-specific response was observed where the salinity tolerance of maize varieties was much higher than hybrids screened in this study.

Key words: Screening, Sulfur, Salinity, Seed germination, Interspecies variation.

Introduction

Salinity is an important factor affecting agricultural productivity throughout the world (Meloni et al., 2003; Rengasamy, 2006). It has been estimated that salt stress has affected approximately 6% of the land area of world that is above 800 million hectares of dry land (Anonymous, 2008). Salinity has affected approximately 45 million hectares out of 230 million hectares of total irrigated land worldwide (Ashraf, 2010). A number of salts are present in the salt affected soil, such as NaCl, Na2SO4, Na2CO3, MgCl2, CaSO4, KC1 and MgSO4. Each of which has differential contribution towards salt stress, but the most important of these is NaCl (Rengasamy, 2002; Munns & Tester, 2008; Tavakkoli et al., 2010; Shahzad et al., 2012; Abbasi et al., 2014).

Sulfur has an important contribution in regulating cell metabolism and hormone signaling pathway. It is a key biochemical regulating seed germination (Lauchli & Epstein, 1990; Johnson et al., 1992). Sulfur is involved in the synthesis of proteins, vitamins, chlorophyll and glutathione involved in tolerance to various stresses (Rausch and Wachter, 2003; Spadaro et al., 2010). Many amino acids have a considerable proportion of sulfur compounds. Thus, the amino acid composition is likely to change by application of sulfur (Singh, 2003). Addition of sulfur to salinity grown plants is likely to improve the plant growth by improved cellular functions (Taiz & Zeiger, 2006).

Maize (Zea mays L.) is very valuable crop. Globally, it is ranked third stable food crop after wheat and rice. Maize is a leading commercial crop of high agro-economic importance due to its use in agro-industries. Worldwide, total annual production of maize is 3.341 million tons. In Pakistan the production area of maize is 9.39 million hectare with an average yield of maize 3.56 tons per hectare (Anonymous, 2011). Thus, maize has achieved key importance in providing food, feed and fodder in Pakistan (Khaliq et al., 2010).

Maize is very sensitive to salinity (Maas, 1986) though it has been reported that maize has interspecies inconsistency of salinity resistance (Maas et al., 1983). Even though substantial efforts have been done in the last decades for the development of salt tolerant crops but only a little success has been achieved. Since, resistance is a multigenic trait, it is very hard to attain success through conventional or molecular breeding (Flowers & Yeo, 1995; Gorham & Jones, 2002). Screening for salinity tolerance at germination and seedling stage has many advantages such as being less laborious, quick, and inexpensive as compared to investigations at mature growth stages (Dasgan et al., 2002). The physiological information generated in screening experiments can be used to improve the salt tolerance crops plants (Romea & Flowers, 2008).

A variety of strategies for counteracting the adverse effects of salt stress on plants are currently in practice. Of these strategies, exogenous application of osmoprotectants, and inorganic salts is contemplated to be an economical and shot-gun approach to alleviate the
harmful effects of salinity on plant growth (Ashraf et al., 2008). From the work of Tlig et al. (2008) and Guan et al. (2009a) it can be concluded that germination test is a useful tool for screening plants under stressed environments. Keeping in view all these details, this study was carried out to establish the role of sulfur in improving salt tolerance of maize based various seed germinability attributes. In addition, the classification of maize cultivars according to their salt tolerance was another objective of this study.

**Materials and Methods**

**Seed sowing:** The seeds of six varieties (Sadaf, MMRI, Pearl Basic, Agaitti 2003, Saiwal 2002 and Pak Afgoi 2003) and two hybrids (Yusafwala hybrid and Hybrid 1898) of maize were acquired from Maize and Millet Institute, Yousefwa, Sahiwal. For sowing, grading was done and healthy and uniform seeds were selected. The sand used in this experiment was thoroughly washed and dried before sowing seeds.

**Treatment application and experimental design:** Three levels of salinity (25, 50, 75 mM NaCl) and six levels of sulfur (20, 40, 60, 80, 100 mg L\(^{-1}\) K\(_2\)SO\(_4\)) were applied as sand amendment at the time of sowing along with control. The experimental design was a three factor factorial experiment set in a Completely Randomized Design (CRD) with three replicates. The Hogland’s nutrient solution was used throughout the experiment for irrigation.

**Estimation of seed germination:** The germination was recorded daily from the day of sowing to completion of germination.

**Percent germination:** The germination percentage was calculated by using the following formula:

\[
\text{% Germination} = \frac{\text{Total seed emerged}}{\text{Total seeds sown}} \times 100
\]

**50% germination:** Time taken to 50% emergence of seedlings (E\(_{50}\)) was calculated according to the formulae of Coolbear et al. (1984) as modified by Farooq et al. (2005).

\[
E_{50} = t_i + \frac{(N-n_t)}{n_t-n_i} (t_j-t_i)
\]

where \(N\) is the final number of emerged seeds, and \(n_i\) and \(n_j\) are the cumulative number of seeds emerged by adjacent counts at times \(t_i\) and \(t_j\), respectively, when \(n_i < N/2 < n_j\).

**Mean emergence time:** Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981) as under:

\[
\text{MET} = \frac{\sum n_t}{n}
\]

where \(n\) is the number of seeds, which were emerged on day \(D\), and \(D\) is the number of days counted from the beginning of emergence.

**Coefficient of uniformity of emergence:** Coefficient of uniformity of emergence (CUE) was calculated using the following formulae of Bewley and Black (1985).

\[
\text{CUE} = \frac{\sum \frac{n_t}{N} - \frac{n_t}{N}}{\left(\frac{n_t}{N}\right)^2}
\]

where \(t\) is the time in days, starting from day 0, the day of sowing, and \(n\) is the number of seeds completing emergence on day \(t\), and \(t\) is equal to MET.

**Emergence index:** Emergence index (EI) was calculated as described in the Association of Official Seed Analysis (Anonymous, 1983) as the following formula:

\[
\text{EI} = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}
\]

**Coefficient of velocity of germination:** Coefficient of velocity of germination is an index for germination speed (Maguire, 1962).

\[
\text{CVG} = \frac{G}{(X_n-Y_n)+(X_n-Y_n)+\cdots+(X_n-Y_n)} \quad \text{(Seed day}^{-1}\text{)}
\]

where, \(G\) is number of germinated seeds.

**Vigour index:** Vigour Index= Root length + Shoot length x % germination

**Germination energy:** Germination Energy was calculated by formula proposed by Maguire (1962).

\[
\text{GE} = \frac{X_n}{Y_n} + \frac{(X_n-X_n)}{Y_n} + \cdots + \frac{(X_n-X_n)}{Y_n}
\]

where \(X_n\) is the number of germinants on the nth counting date and \(Y_n\) is the number of days from sowing to the nth count.

**Speed of germination:** The speed of germination (%) was calculated using the following formula (Krishnaswamy and Seshu, 1990).

\[
\text{Speed of germination (\%)} = \frac{\text{Number of seed germinated at 72h}}{\text{Number of seeds germinated at 168h}}
\]

**Mean daily germination (MDG):** This is an index of daily germination speed and calculated by (Abbasian and Moemeni, 2013).

\[
\text{MDG} = \frac{\text{P}}{\text{d}}
\]

FGP: final germination percent \(d\): test period.
Daily germination speed (DGS): This index is converse of mean daily germination and calculated by (Askkan and Jalal, 2013).

Statistical analysis: Data were statistically analyzed by using computer software Microsoft Excel to explore possible treatment variations. The Analysis of Variance (ANOVA) was performed by using Co-Stat software. The salt tolerance of various genotypes at germination stage was established by cluster analysis using NTSysPC software (v2.10m). All germination attributes were used to construct phylogenetic tree based on their distance coefficient using SAHN (Sequential Agglomerative Hierarchic and Non-over lapping) method. The genotypes having higher distance are likely to have differential salt tolerance potential.

Results

Statistical analysis showed that salt stress significantly reduced the germination of all studied maize cultivars. These findings were confirmed from statistically significant V x Sa interactive effect for various germination attributes such as 50 % germination, emergence index, mean emergence time, coefficient of uniformity of emergence, vigour index, coefficient of velocity of germination, germination energy, germination speed and germination value (Table 1). The maximum reduction in germination attributes was found at 75 mM treatment level. The application of sulfur (20, 40, 60, 80, 100 mM) substantially improved all germination parameters at all salt levels (25, 50, 75 mM). However its 60, 80 mM level proved to be the most effective in enhancing seed germination of maize plants. The highest sulfur level (100 mM) caused a significant reduction in seed germination as compared to other lower sulfur levels 60, 80 mM in all studied maize cultivars (Figs. 1-11).

Sulfur application also reduced the toxic effects of salinity on maize seedlings by improving seed germination at all salt levels (25, 50, 75 mM). A statistically significant Sa x S interaction was observed for 50 % germination, percent germination, coefficient of uniformity of emergence, vigour index, mean daily germination and germination value (Table 1). The interaction between V x Sa x S showed a variable response. For example, 50 % germination, emergence index, mean emergence time, coefficient of uniformity of emergence, vigour index, germination energy and germination speed were highly significant while percent germination, coefficient of velocity of germination, mean daily germination and germination value showed non-significant interactive effect (Table 1). Overall the optimal levels of sulfur i.e. 60 mM and 80 mM affected all seed germination parameters positively while high concentration of sulfur (100 mM) lowered all germination parameters studied (Figs. 1-11).

### Table 1. Mean squares from analysis of variance (ANOVA) of the data for germination parameters of maize subjected to salt stress and sulfur application.

<table>
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<tr>
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<th>df</th>
<th>E50</th>
<th>PG</th>
<th>EI</th>
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<tr>
<td><strong>GV</strong></td>
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<td></td>
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<td></td>
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<td>5.43</td>
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<td>V x Sa x S</td>
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<td>0.015</td>
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<tr>
<td>Error</td>
<td>384</td>
<td>3.13</td>
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**Abbreviations:** e = Exponent; E50 = time to 50% germination; PG = Percent germination; EI = Emergence Index; MET = Mean Emergence Time; CUE = Coefficient of Uniformity of Emergence; VI = Vigor index; CVG = Coefficient of Velocity of Germination; GE = Germination energy; GS = Germination speed; MDE = Mean daily germination; GV = Germination value
Fig. 1. Effect of different levels of exogenously applied sulfur (S) on days to achieve 50% germination in maize (Zea mays L.) cultivars under saline conditions.

Fig. 2. Effect of different levels of exogenously applied sulfur (S) on percentage germination in maize (Zea mays L.) cultivars under saline conditions.
Fig. 3. Effect of different levels of exogenously applied sulfur (S) on emergence index in maize (Zea mays L.) cultivars under saline conditions.

Fig. 4. Effect of different levels of exogenously applied sulfur (S) on mean emergence time in maize (Zea mays L.) cultivars under saline conditions.
Fig. 5. Effect of different levels of exogenously applied sulfur (S) on coefficient of uniformity of emergence (CUE) in maize (*Zea mays* L.) cultivars under saline conditions.

Fig. 6. Effect of different levels of exogenously applied sulfur (S) on vigor index in maize (*Zea mays* L.) cultivars under saline conditions.
SCREENING OF SALT STRESSED MAIZE CULTIVARS UNDER EXOGENOUS APPLICATION OF SULFUR

Fig. 7. Effect of different levels of exogenously applied sulfur (S) on coefficient of velocity of germination (CVG) in maize (Zea mays L.) cultivars under saline conditions.

Fig. 8. Effect of different levels of exogenously applied sulfur (S) on germination energy in maize (Zea mays L.) cultivars under saline conditions.
Fig. 9. Effect of different levels of exogenously applied sulfur (S) on germination speed in maize (*Zea mays* L.) cultivars under saline conditions.

Fig. 10. Effect of different levels of exogenously applied sulfur (S) on mean daily germination in maize (*Zea mays* L.) cultivars under saline conditions.
Fig. 11. Effect of different levels of exogenously applied sulfur (S) on germination value in maize (*Zea mays* L.) cultivars under saline conditions.

Fig. 12. The clustering based on distance matrixes generated for germination, growth and ionic contents of maize (*Zea mays* L.) cultivars as modulated by exogenously applied sulfur under saline conditions. The genotypes were clustered into (a) salt tolerant and (b) salt sensitive groups based on phylogenetic distance generated by NTS Sys PC.
Among all studied maize cultivars, Agaitti 2003 had high % germination, vigour index, speed of germination, emergence index, coefficient of velocity of germination, germination energy, mean daily germination, coefficient of uniformity of germination, mean emergence time and germination value and took less time to achieve 50 % germination. So Agaitti 2003 was proved salt tolerant, among all the varieties and hybrids used in this study. Similarly, Pak Afgoi 2003 and Hybrid 1898 exhibiting the least seed germination potential were classified as the most sensitive genotypes.

The germination data was fed to NTSysPC software (v2.10m) and used to construct the phylogenetic trees based on SADHN method. Since the tree was constructed on the basis of distance coefficients (DC), a higher distance between two groups reflected least similarity or higher differences in their salt tolerance potential and vice versa. All genotypes were clustered in two distinct groups (DC = 237.30) reflecting higher variation in salt tolerance potential of both groups. The tolerant group included four varieties (Agati 2003, Sadaf, MMRI and Peral Basic). In comparison, the sensitive group included two varieties (Sahiwal and Pak Afgoi 2003) and two hybrids (Yusafwala Hybrid and Hybrid 1898) (Fig. 12).

Among the tolerant group, maize variety Agati 2003 branched at the most distance (DC = 119.12) reflecting to be the most salt tolerant variety. In comparison, Sadaf branched at lesser distance (DC = 80.33) and was ranked as moderately salt tolerant. The other two varieties in this group i.e. MMRI and Pearl Basic branched at the least but equal distance coefficients (DC = 46.36) and thus were classified as the least tolerant maize varieties (Table 2).

The sensitive group contained two varieties and two hybrids. In this group, maize variety Sahiwal branched at the most distance (DC = 121.88) indicating this variety to be the least sensitive one. Yusafwala Hybrid being branched at lesser distance (DC = 54.36) was identified to be as moderately sensitive. One of the maize varieties (Pak Afgoi 2003) and Hybrid 1898 having the least distance coefficients (DC = 46.29) were ranked as the highly sensitive maize cultivars.

### Table 2. The clustering based on seed germinability attributes of maize (Zea mays L.) cultivars as modulated by exogenously applied sulfur under saline conditions. The tree was based on distance coefficients. A higher distance between the two cultivars within a cluster reflected greater variation in their salt tolerance while the least distance between two cultivars within a cluster reflected more similarity in their degree of salt tolerance.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Cultivars</th>
<th>Distance coefficient</th>
<th>Salt tolerance</th>
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<tr>
<td></td>
<td>Agaitti 2003</td>
<td>237.30</td>
<td>Tolerant group</td>
</tr>
<tr>
<td></td>
<td>Sadaf</td>
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<tr>
<td></td>
<td>MMRI</td>
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<td>Least tolerant</td>
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<td>Pak Afgoi 2003</td>
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<td>Highly sensitive</td>
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<tr>
<td></td>
<td>Hybrid 1898</td>
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Discussion

In this study, the application of salinity showed a marked reduction in germination attributes. While exogenously applied sulfur showed a significant improvement in germination parameters. Seed germination is amongst the most important physiological processes which is indicator of a crop to tolerate environmental stress (Peralta et al., 2001). The reduction in seed germination by the application of salt stress is attributed to lowering the water potential of the growth media, thereby causing reduction in water uptake. Salinity causes dehydration of proteins and disturbance in many enzymatic activities involved in the process of seed germination. It also causes seed shrinkage and seed become non-viable soon after changes in metabolic activities due to imposition of salt stress, thereby reducing the process of seed germination (Kramer, 1983; Dubey & Rani, 1990; Garg et al., 1993). The results of present investigation showed that salt stress reduced the vigor index of maize seedlings. These findings are supported by previous studies as reported by Janmohammadi et al. (2008) and Djanaguiraman et al. (2003). They reported a significant positive correlation in vigor index and salt stress. Salinity showed a marked reduction in germination percentage (Song et al., 2008; Tlig et al., 2008; Guan et al., 2009b). It might be due to the deposition of Na+ and Cl- ions in the seed that affect the mobilization of various minerals and organic reserves with resumption of respiration (Mahdid et al., 2014; Rasheed et al., 2015).

The results of current study revealed that salt stress caused increased mean germination time. Kaya et al. (2009) reported that salinity caused increase in mean germination time. Similarly, Redondo-Gomez et al. (2008) showed that mean germination time was increased in Limonium emarginatum by increasing the salt stress. It might be due to the reason that salt stress caused reduction in osmotic potential of the rooting zone that ultimately lowers the absorption and expansion of embryo causing the reduction in seed germination time (Al-Niem et al., 1992; Goodman et al., 2001; Patel & Pandey, 2007; Patel et al., 2009). Salt stress also lowered the germination index. These findings were supported by previous reports by Carpici et al. (2009) who observed reduction in germination index by imposition of salinity in maize (Zea mays L.) plants. In a similar way, Khan et al. (1997) reported a marked reduction in germination index by salt application.

Among various methods to improve stress tolerance, the application of various inorganic salts is cheap and easy strategy to counteract the toxic effect of salinity on plants (Ashraf et al., 2008). Inorganic salt like sulfur is well known for its ameliorating effects to the damages caused by salt stress. In this study, sulfur was found very effective in increasing germination of maize seeds sown in saline medium. Sulfur application significantly increased the coefficient of uniformity of emergence and vigor index and decreased a delay to achieve 50% germination of maize seeds. Higher germination rate and percentage under sulfur treatment might be due to accumulation of various secondary metabolites of sulfur such as methionine. It plays an important role in a variety of metabolic processes and can alleviate the impact of salt stress.
of metabolic processes for instance synthesis of protein, S
adenosylmethionine, ethylene and polyamine, all of which
are crucial for germination of seeds as well as seedlings
growth. Methionine synthase and S adenosylmethionine
synthetase are basic components during switching from a
dormant to a highly active metabolic state in the seed
germination (Gallardo et al., 2002). Similarly glutathione
(GSH) is also a reduced compound of sulfur and highly
active under stress conditions and it has the ability to
suppress the inhibition of germination caused by ABA
produced during abiotic stress. In the presence of both
GSH and ABA, germination rate of the seeds of
Arabidopsis thaliana was higher over the seeds which
have alone ABA (Chen et al., 2012).

In this study, the tree was generated on distance
coefficients by SAHN method. All genotypes were clearly
divided into two groups and their tolerance was
established from individual germination data. Since the
tree was generated on the basis of cumulative germination
data, the clustering pattern clearly indicated strong
relationships in their salt tolerance potential under
exogenously applied sulfur. Such clustering has already
been used to screen a wide variety of screening
experiments on different crops (Bayuelo-Jimenez et al.,
2002; Saboora et al., 2006). The clustering results of
current study indicated that this method is very effective
in screening the available germplasm for salinity
tolerance under exogenously applied nutrients.

Conclusion and Recommendations

Although, salinity stress decreased germination and
growth attributes of maize under saline condition, the
exogenous application of sulfur proved very beneficial in
the alleviating the adverse effect of salinity stress. The
lower levels of S showed some improvement in salt
tolerance potential of maize hybrids as compared to the
control plants. S from 60 mM and 80 mM was the most
effective levels in ameliorating the adverse effects of
salinity stress. The higher level i.e. 100 mM reduced the
seed germinability attributes. Thus it is recommended that
for improving maize germination and seedling
establishment grown in low to moderately saline media
the sulfur can be applied at 60 mM concentration at
ermination stage.

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