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

A. RIFFAT[*] AND M.S.A. AHMAD

Department of Botany, University of Agriculture Faisalabad, Pakistan
[*]*Corresponding author's email: aliariffat@hotmail.com*; *sajidakeel@yahoo.com*

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 (Yusafwala 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 the two distinct clusters. The tolerant cluster mainly contained 4 varieties (Sadaf, MMRI, Pearl Basic and Agati 2003) while the sensitive cluster included two varieties (Sahiwal 2002, Pak Afgoi 2003) and two hybrids (Hybrid 1898 and Yusaf wala hybrid). Based on the distance matrixes generated by software, Agati 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, Na_2SO_4, Na_2CO_3, $MgCl_2$, $CaSO_4$, KCl and $MgSO_4$. 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, 2005; 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 may* 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 (Romeza & 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, Yousafwala, 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_2SO_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).

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

where N is the final number of emerged seeds, and ni and nj are the cumulative number of seeds emerged by adjacent counts at times ti and tj, respectively, when ni < N/2 < nj.

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

$$MET = \frac{\sum Dn}{\sum n}$$

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).

$$CUE = \frac{\sum n}{\sum[(\bar{t} - t)^2 \times n]}$$

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 $\bar{t}$ 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:

$$EI = \frac{\text{No. of emergerd seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of emergerd seeds}}{\text{Days of final count}}$$

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

$$CVG = \frac{G_1 + G_2 + \cdots + G_n}{(1 \times G_1) + (2 \times G_2) + \cdots + (n \times G_n)} \quad (\text{Seed day}^{-1})$$

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).

$$GE = \frac{X_1}{Y_1} + \frac{(X_2 - X_1)}{Y_2} + \cdots + \frac{(X_n - X_{n-1})}{Y_n}$$

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).

$$MDG = \frac{FGP}{d}$$

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)**.**

$$DGS = \frac{1}{MDG}$$

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.

SoV	df	E50	PG	EI
Variety (V)	7	21.56 ***	16853 ***	131.74***
Salinity (Sa)	3	9.15 ***	6609 ***	32.91 ***
Sulfur (S)	5	1.20 ***	8529 ***	26.65 ***
V x Sa	21	0.28 ***	142 ns	2.58 ***
V x S	35	0.018 ***	236 ***	1.62 ***
Sa x S	15	0.061 ***	294 ***	0.28 ns
V x Sa x S	105	0.021 ***	76 ns	0.54 ***
Error	384	0.0019	112	0.28
		MET	**CUE**	**VI**
Variety (V)	7	2.43 ***	$3.11e^{-5}$ ***	1326739 ***
Salinity (Sa)	3	0.39 ***	$4.39e^{-6}$ ***	9077713 ***
Sulfur (S)	5	0.48 ***	$1.46e^{-6}$ ***	8019443 ***
V x Sa	21	0.039 ***	$1.05e^{-7}$ ***	94655 ***
V x S	35	0.040 ***	$4.65e^{-9}$ ***	161386 ***
Sa x S	15	0.0060 ns	$8.94e^{-9}$ ***	288682 ***
V x Sa x S	105	0.013 ***	$5.68e^{-9}$ ***	56288 ***
Error	384	0.0073	$2.01e^{-9}$	18959
		CVG	**GE**	**GS**
Variety (V)	7	25535 ***	18.23 ***	1.084 ***
Salinity (Sa)	3	5102 ***	5.41 ***	0.22 ***
Sulfur (S)	5	5738 ***	4.77 ***	0.019 ns
V x Sa	21	154 **	0.51 ***	0.12 ***
V x S	35	239 ***	0.25 ***	0.14 ***
Sa x S	15	39 ns	0.048 ns	0.027 ns
V x Sa x S	105	75 ns	0.074 ***	0.037 ***
Error	384	66.83	0.042	0.018
		MDE	**GV**	
Variety (V)	7	468.16 ***	5.43 ***	
Salinity (Sa)	3	183.61 ***	3.46 ***	
Sulfur (S)	5	236.93 ***	0.46 ***	
V x Sa	21	3.95 ns	0.13 ***	
V x S	35	6.57 ***	0.025 **	
Sa x S	15	8.18 ***	0.023 *	
V x Sa x S	105	2.12 ns	0.015 ns	
Error	384	3.13	0.012	

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant

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

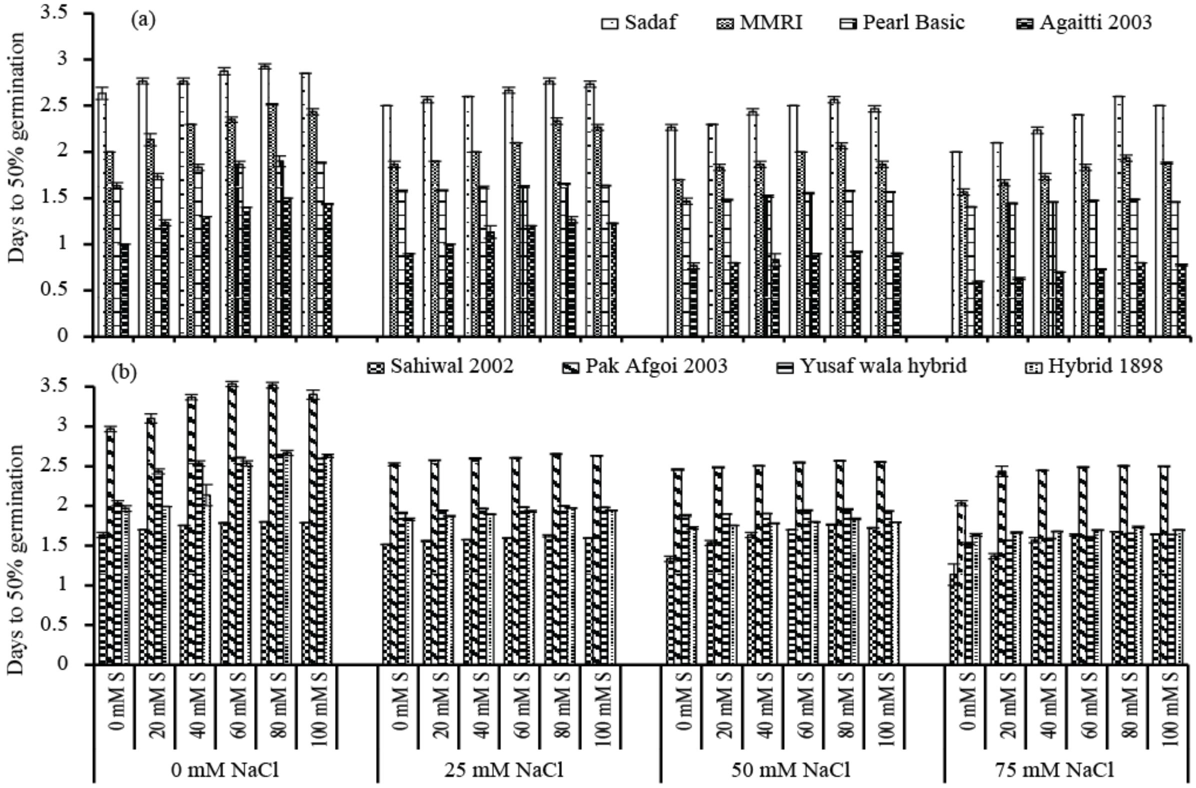

Fig. 1. Effect of different levels of exogenously applied sulfur (S) on days to achieve 50 % germination in maize (*Zea mays* L.) cultivars under saline conditions.

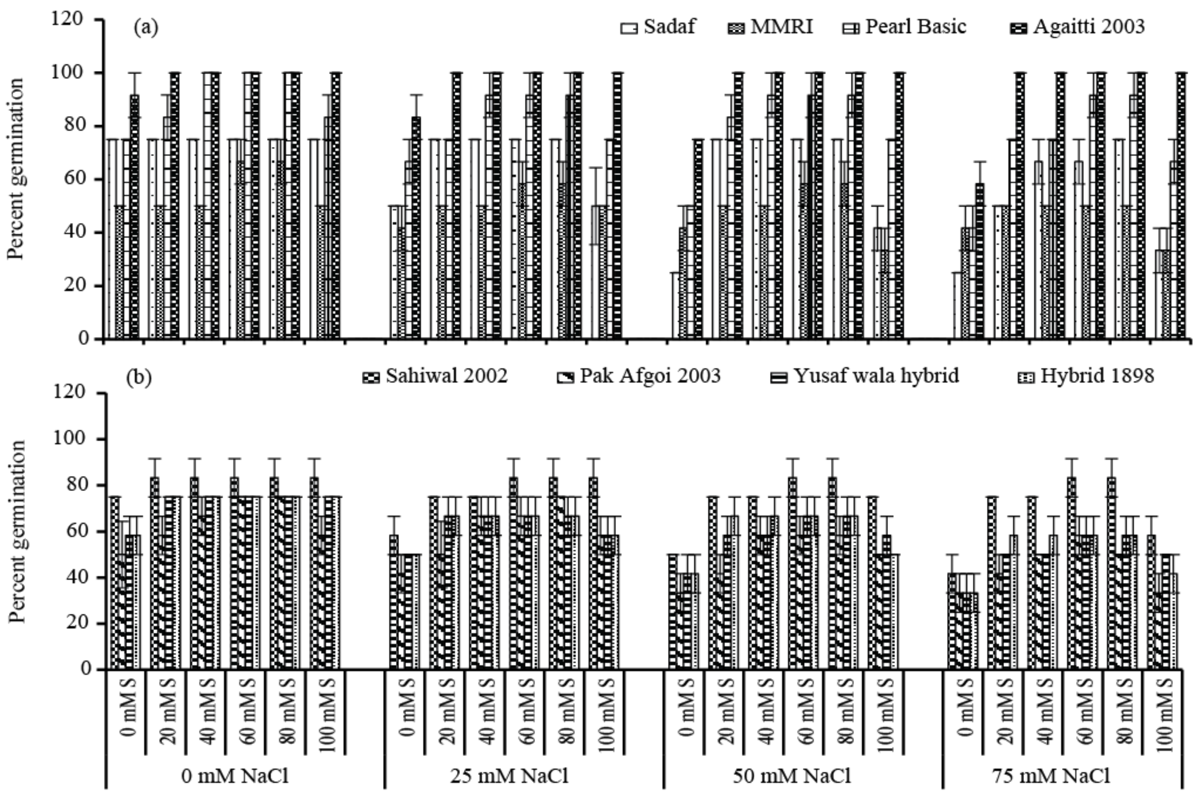

Fig. 2. Effect of different levels of exogenously applied sulfur (S) on percentage germination in maize (*Zea mays* L.) cultivars under saline conditions.

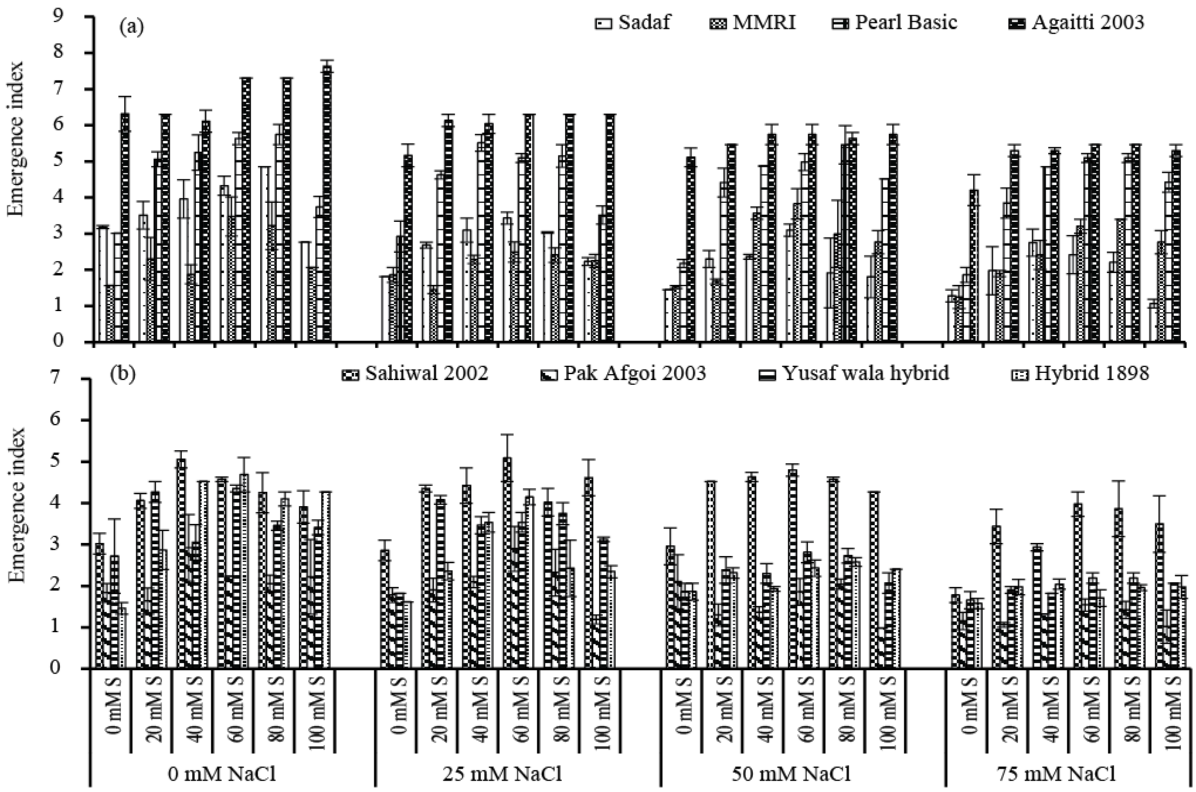

Fig. 3. Effect of different levels of exogenously applied sulfur (S) on emergence index in maize (*Zea mays* L.) cultivars under saline conditions.

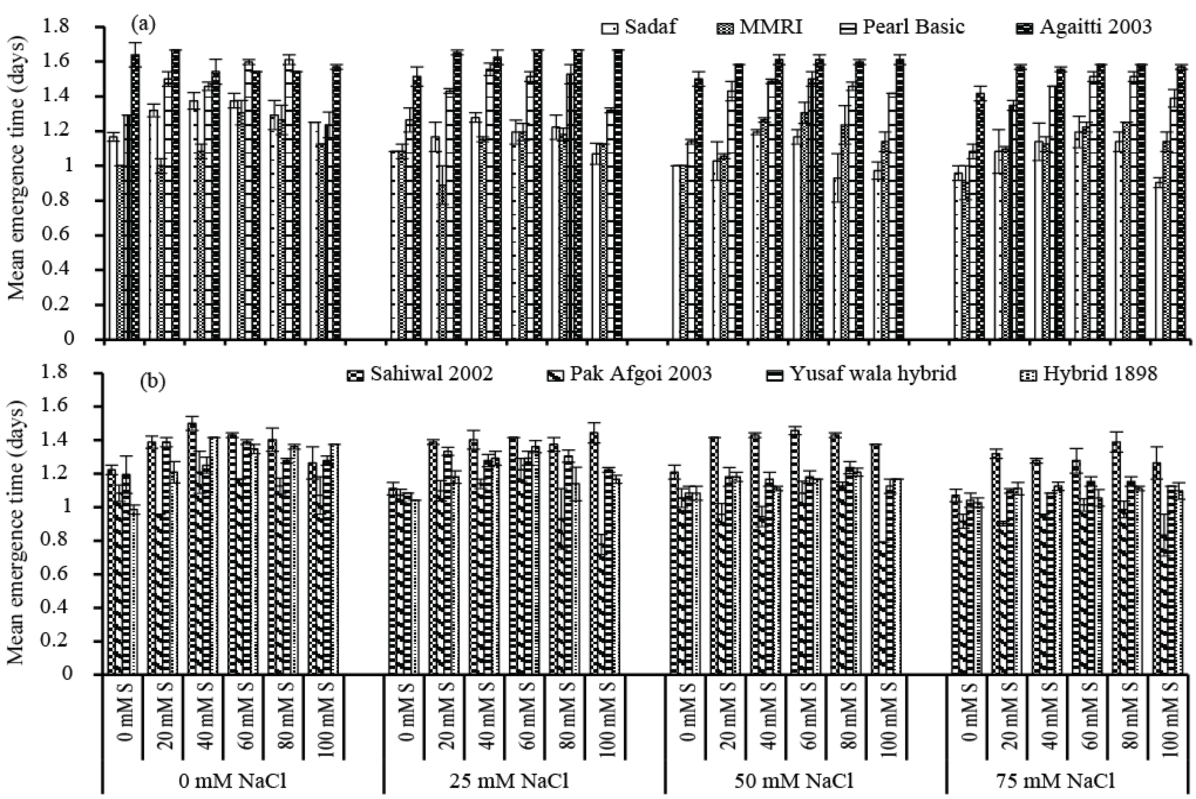

Fig. 4. Effect of different levels of exogenously applied sulfur (S) on mean emergence time in maize (*Zea mays* L.) cultivars under saline conditions.

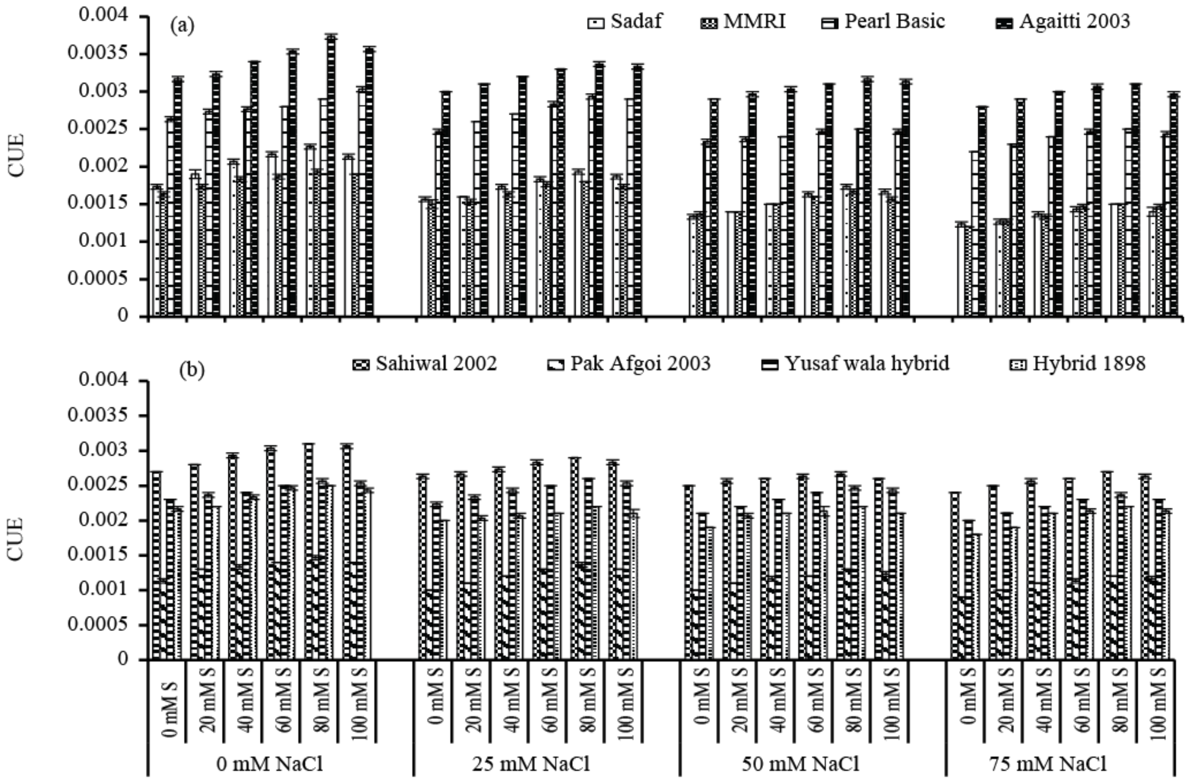

Fig. 5. Effect of different levels of exogenously applied sulfur (S) on coefficient of uniformity of emergence (CUE) in maize (*Zea mays* L.) cultivars under saline conditions.

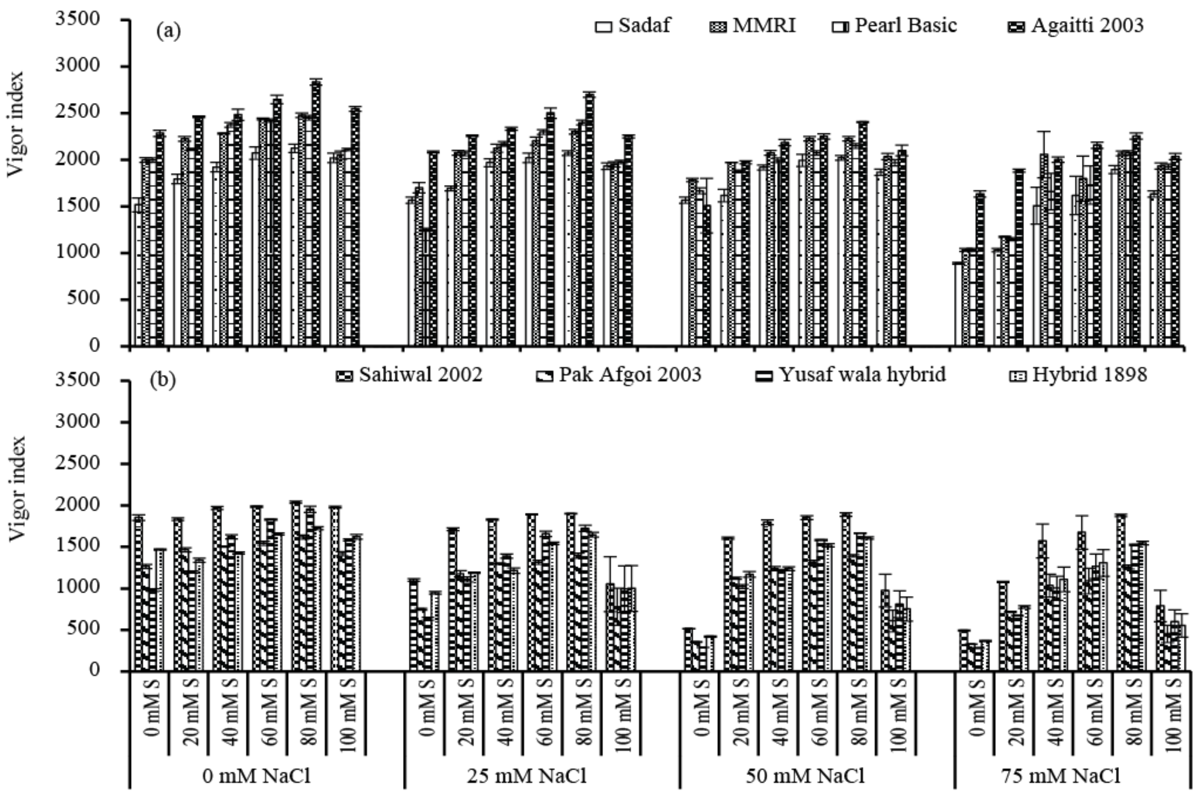

Fig. 6. Effect of different levels of exogenously applied sulfur (S) on vigor indexin maize (*Zea mays* L.) cultivars under saline conditions.

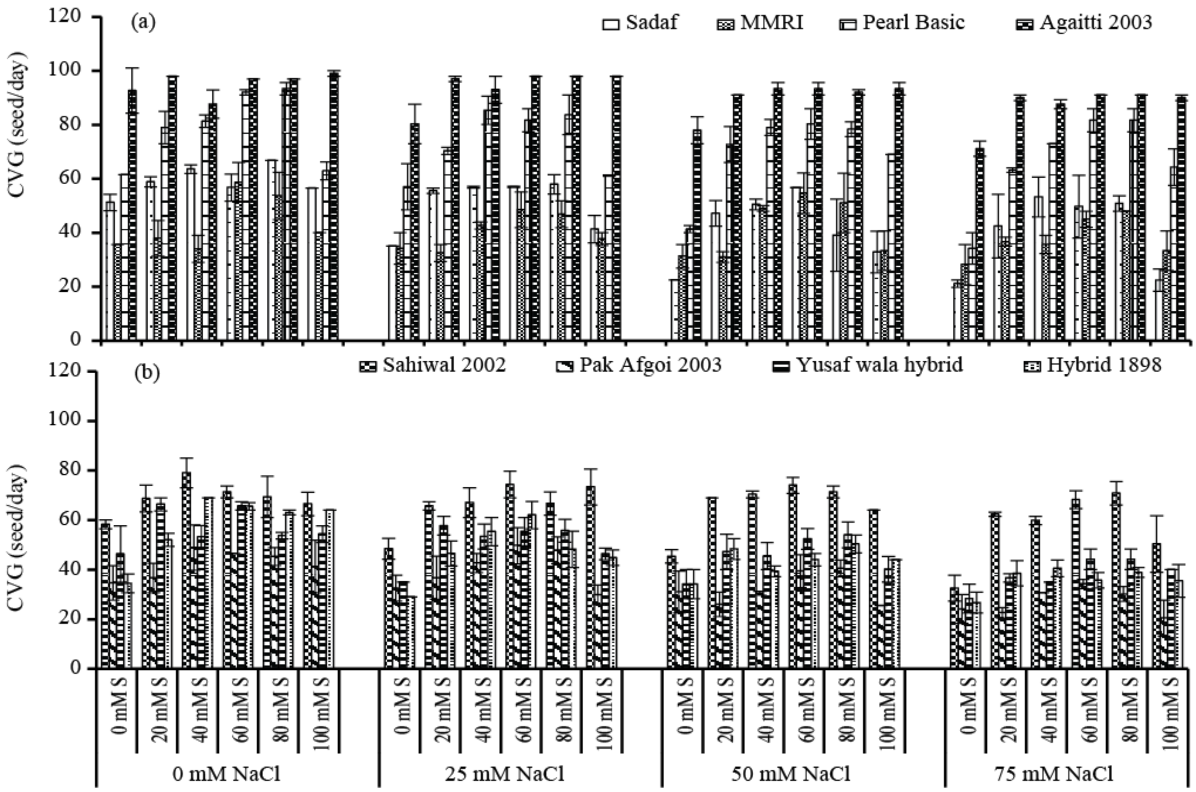

Fig. 7. Effect of different levels of exogenously applied sulfur (S) on coefficient of velocity of germination (CVG) in maize (*Zea mays* L.) cultivars under saline conditions.

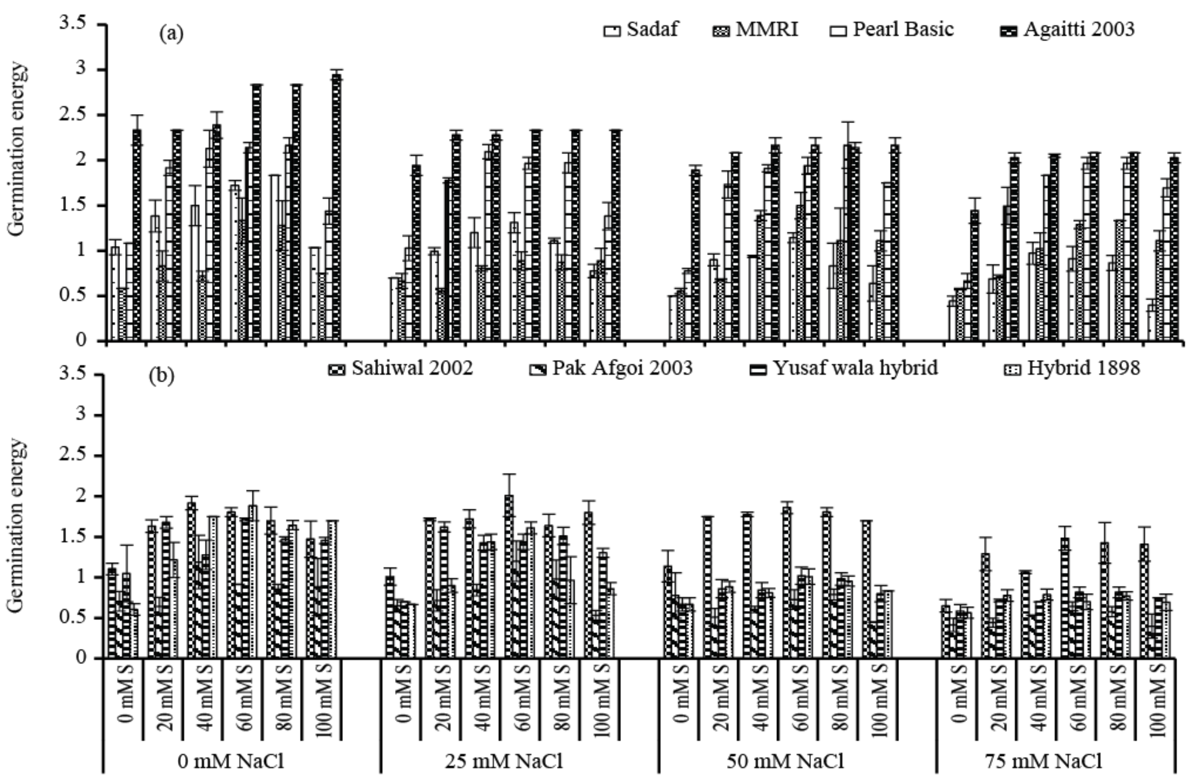

Fig. 8. Effect of different levels of exogenously applied sulfur (S) on germination energy in maize (*Zea mays* L.) cultivars under saline conditions.

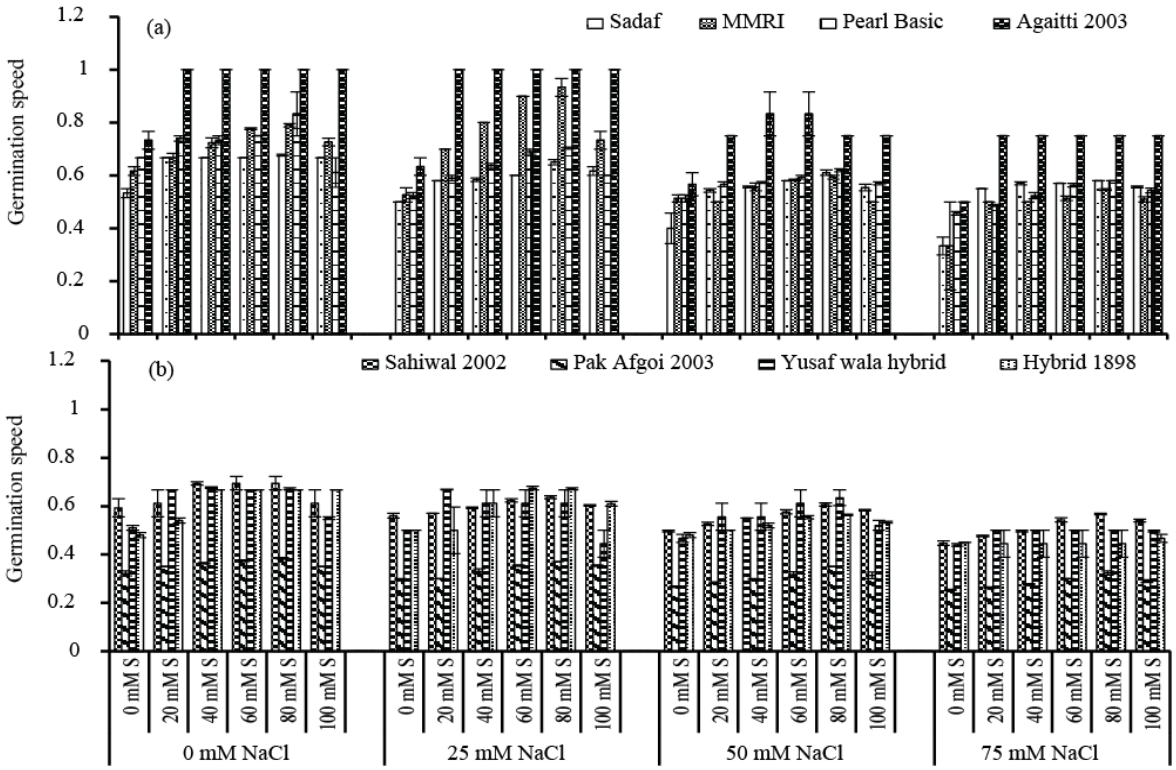

Fig. 9. Effect of different levels of exogenously applied sulfur (S) on germination speed in maize (*Zea mays* L.) cultivars under saline conditions.

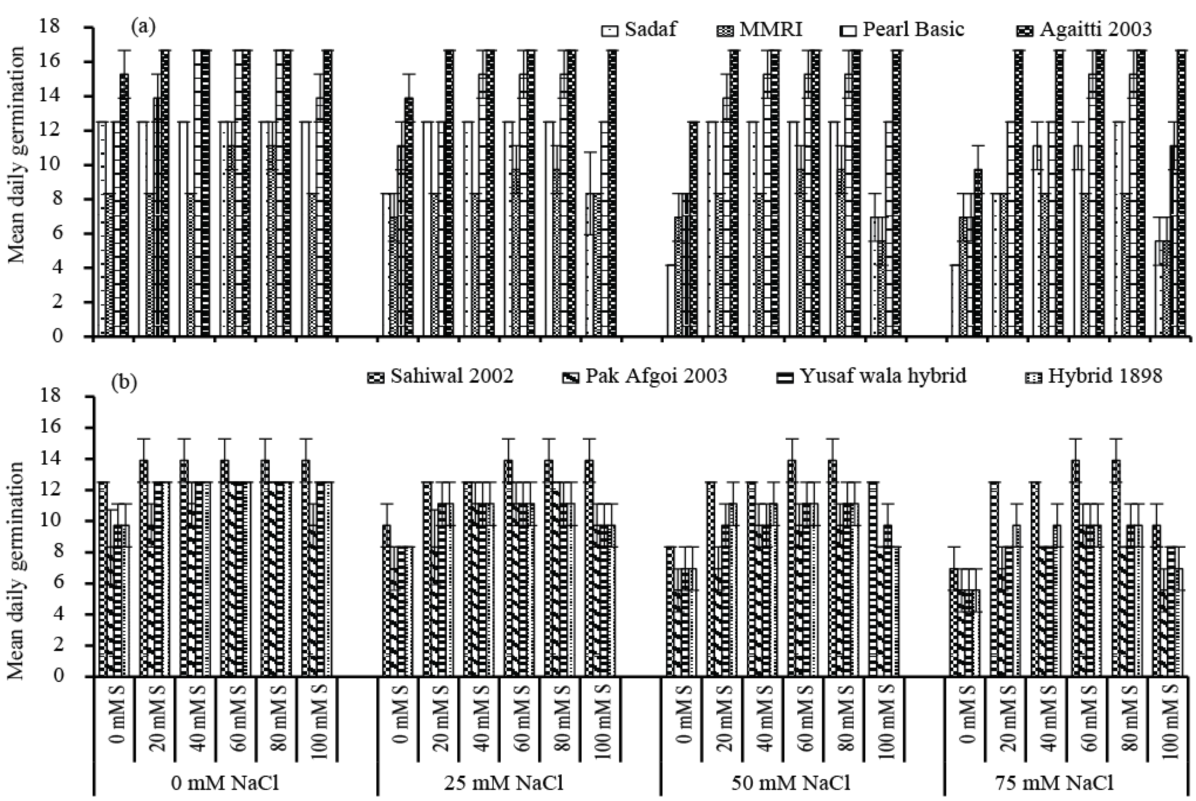

Fig. 10. Effect of different levels of exogenously applied sulfur (S) on mean daily germination in maize (*Zea mays* L.) cultivars under saline conditions.

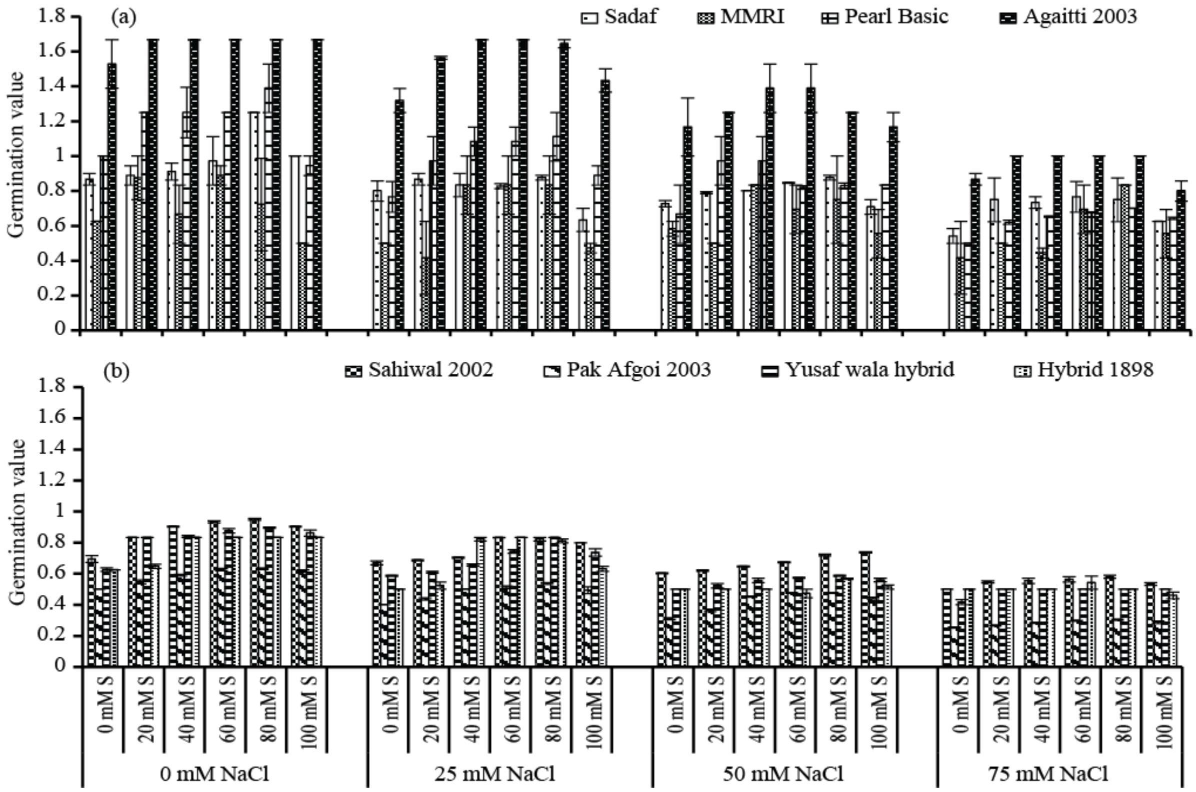

Fig. 11. Effect of different levels of exogenously applied sulfur (S) on germination value in maize (*Zea mays* L.) cultivars under saline conditions.

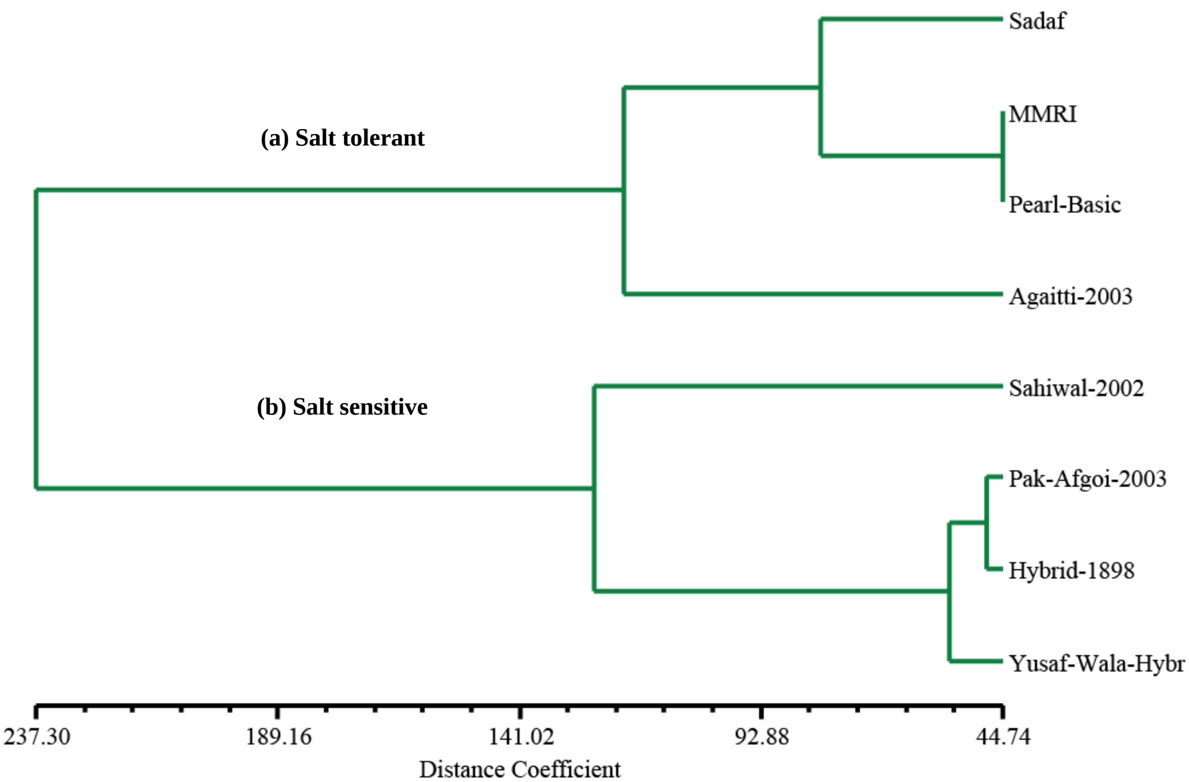

Fig. 12. The clustering based on distance matrixes generated for germination, growth and ionic contents of maize (*Zea mays* L.) cultivars as modulated by exogenously applied sulfur under saline conditions. The genotypes were clustered into (a) and salt sensitive (b) groups based on phylogenetic distance generated by NTS Sys PC.

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.

Hybrid	Cultivars	Distance coefficient	Salt tolerance
		237.30	**Tolerant group**
	Agaitti 2003	**119.12**	**Highly tolerant**
Group 1	Sadaf	80.33	Moderately tolerant
	MMRI	46.36	Least tolerant
	Pearl Basic	46.36	Least tolerant
		237.30	**Sensitive group**
	Sahiwal	123.88	Least sensitive
Group 2	Yusafwala hybrid	54.36	Moderately sensitive
	Pak Afgoi 2003	**46.29**	**Highly sensitive**
	Hybrid 1898	**46.29**	**Highly sensitive**

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 SAHN 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.

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-Niemi *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 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 germniability attributes. Thus it is recommended that for improving maize germination and seeding establishment grown in low to moderately saline media the sulfur can be applied at 60 mM concentration at germination stage.

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