

Comparative Effects of Drought and Salt Stress on Germination and Seedling Growth of *Pennisetum divisum* (Gmel.) Henr.

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Abstract: Problem statement: Water stress due to drought and salinity is probably the most significant abiotic factor limiting plant and also crop growth and development. Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent. Water deficit affects the germination of seed and the growth of seedlings negatively. Temperature is an exceedingly important factor in seed germination. It directly affects whether a plant can sprout and, if so, how long it will take to emerge from the ground. **Approach:** The objective of this investigation was to determine the effect of four alternating temperature regime, drought and salt stress on germination characteristics of *Pennisetum divisum*. Seeds were germinated at four alternating temperatures (10/20, 15/25, 20/35 and 25/40°C at 12 h light). Seeds were also germinated with the iso-osmotic concentrations of sodium chloride (NaCl) or in polyethylene glycol PEG8000 (0, -0.2, -0.4, -0.6 and -0.8 MPa) for 14 days. Concentrations were applied to determine their effects on seed germination and seedling growth under laboratory conditions. The effects of different osmotic concentrations of NaCl and PEG were compared to distilled water (control). **Results:** Optimum germination was attained at 15/25°C which corresponds to temperatures prevailing during spring time. The highest values of germination parameters were obtained with no osmotic potential (0 MPa) under 15/25°C. The final germination percentage and rate of germination in the *Pennisetum divisum* treated seeds were decreased with the increase of the osmotic potential. At treatment by PEG, the germination was severely decreased at -0.6 MPa. While, no germination occurred at -0.8 MPa by NaCl. The results of the effects of the different osmotic potential of NaCl and PEG on the Radicle Length (RL) and the Hypocotyl Length (HL) mm of the tested *P. divisum* seeds were retarded when compared to the control. **Conclusion:** Results indicated germination sensitive to both the stresses. However, seedling growth was more sensitive to NaCl than was germination. However, seedling growth was more sensitive to NaCl than was germination responses to water stress induced by PEG and NaCl. Results also indicated that seed germination of *P. divisum* is less sensitive to osmotic potential indicating that the seeds of the species are efficient in osmotically adjusting to soluble salts. This suggests the possibility of revegetating moderately salt affected soils.

Key words: Water potential, germination rate, *Pennisetum divisum*, polyethylene glycol, temperature

INTRODUCTION

Grasses play an important role in land stabilization and animal nutrition due to their protein, carbohydrates, fats, fibers and mineral contents, soil protection and sand dune fixation. Therefore, planting grasses constitutes an important part of any rangeland rehabilitation program. *Pennisetum divisum* (Gmel.) Henr. Gramineae grasses with inflorescence a dense, spicate, panicle terminal, widely distributed and most drought resistant grasses of the Saudi desert in coastal sands and sands around rocky ground places. This is a useful grazing plant, much browsed by

camels (Mandaville, 1990). Despite the importance of *P. divisum*, there is no available information on its autecology. Also, there is no available information browse production of the species. Plants are constantly confronted with various biotic and abiotic stress factors such as low or high temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity (Mahajan and Tuteja, 2005; Achuo *et al.*, 2006; Jaleel *et al.*, 2007). Water availability and temperature are important factors that determine whether or not dispersed seeds germinate. For the seeds to germinate, they must imbibe water under a favorable temperature. However, salts and other solutes in the medium cause osmotic

inhibitory effects on the seed's water uptake and retard and/or suppress germination. One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity (Almansouri *et al.*, 2001), which are widespread problems around the world (Soltani *et al.*, 2006). Salinity and drought affect the plants in a similar way (Katerji *et al.*, 2004). Reduced water potential is a common consequence of both salinity and drought (Legocka and Kluk, 2005). Water stress acts by decreasing the percentage and rate of germination and seedling growth. NaCl and Polyethylene Glycol (PEG) compounds have been used to simulate osmotic stress effects in Petri dish (*in vitro*) for plants to maintain uniform water potential throughout the experimental period (Misra and Dwivedi, 2004).

Among the stages of the plant life cycle, seed germination and seedling emergence and establishment are key processes in the survival and growth of plants (Hadas, 2004). Seed germination and early seedling growth are critical stages for the establishment of plant populations under saline conditions (Perez *et al.*, 1998; Khan and Gulzar, 2003). Grasses differ in their upper limit of salinity tolerance and an increase in salinity concentration usually delays and reduces seed germination (Gulzar *et al.*, 2001). Seed germination under saline conditions occurs after high precipitation where soil salinity is usually reduced due to leaching (Khan and Ungar, 1986). The degree to which salinity affects germination by an osmotic effect or a specific ion toxicity and whether salt tolerance varies in different species is still a subject of study. Solutions of high molecular weight polyethylene glycol are often used to control water potential in seed germination studies.

Solutions of high molecular weight polyethylene glycol are often used to control water potential in seed germination studies (Hardegree and Emmerich, 1990). The PEG-induced inhibition of germination has been attributed to osmotic stress (Dodd and Donovan, 1999; Sidari *et al.*, 2008). Temperature plays a crucial role in many biological and physiological processes of plants (Al-Ahmadi and Kafi, 2007; Berti and Johnson, 2008). The temperature changes have major impact on a number of processes which regulate seed germinability, including membrane permeability and the activity of membrane-bound as well as cytosolic enzymes (Tlig *et al.*, 2008) and its interaction with the variable soil water content in the surface layers of the soil. Salinity-temperature interaction, in particular, determines seed germination pattern in many salt-affected environments (Al-Khateeb, 2006; Song *et al.*, 2006).

Plants have evolved various biochemical and physiological mechanisms to combat water stress (Sakamoto and Murata, 2002; Sadeghian and Yavari, 2004). One such mechanism that is ubiquitous in plants is the accumulation of certain organic metabolites of lower molecular weight especially during seed germination and early stages of growth (Bewley and Black, 1994). The aim of this study is to investigate the effects of osmotic stress generated by NaCl or PEG and combined effects of these stress factors with temperature on germination characteristics and seedling growth of *Pennisetum divisum*.

MATERIALS AND METHODS

Seeds of *P. divisum* were collected during April, 2009, from naturally growing stands in the sandy soil near Al-Jubail on coast of the Arabian Gulf of Saudi Arabia. Seeds were separated from inflorescences and stored dry at 4°C., During September 2009, stored seeds were sterilized with 0.5% sodium hypochlorite solution for 1 min. Thereafter, they were washed twice with distilled water.

Effect of temperature: Seeds were germinated on double-layered with Whatman No. 1 filter papers placed in 10cm diameter plastic dishes. All of the Petri dishes were kept in dark incubators maintained at room temperature at four alternating night and day temperature regime (10/20, 15/25, 20/30 and 25/40°C) representing the seasonal common temperatures prevailing in some selected meteorological stations in Eastern Saudi Arabia. Twenty-five seeds were germinated. The filter papers were moistened daily with the of distilled water.

Effect of water potential: Seeds of *P. divisum* were germinated at 15/25°C. Seeds were submitted to germination using osmotic potentials (0, -0.2, -0.4, -0.6 and -0.8 MPa) of sodium chloride (NaCl) and Polyethylene Glycol (PEG) 8000 solutions according to (Michel, 1983). Twenty-five seeds were germinated on double-layered with Whatman No. 1 filter papers placed in 10 cm diameter plastic dishes. The filter papers were moistened daily with the five different concentrations of the extracts used. Control experiments whose filter papers were moistened with distilled water were set (25 seeds each). Each experiment was replicated four times. All Petri dishes were kept in dark incubators maintained at room temperature at 15/25°C. Germination was monitored daily. The germinated seeds were recorded every day for a period of 14 days after sowing. Seeds were

considered germinated when the radicle had protruded 2 mm through the seed coat. After 14 days of incubation, germination was expressed as the Final Percentage of Germination (FG%) and Rate of Germination (GR) was calculated by (seeds day⁻¹) according to the method of Maguire's equation (Pezzani and Montana, 2006), the Radicle Length (RL), the Hypocotyl Length (HL).

Statistical analysis: Data were subjected to Analysis Of Variance (ANOVA), according to Gomez and Gomez (1984). Averages of the main effects and their interactions were compared using the revised Least Significant Difference test (LSD) at 0.05 level of probability. Computations and statistical analysis were done using SAS .

RESULTS

Germination: The germination percentage of seeds at different alternating temperature is shown in Fig. 1. The germination percentages increased with rise of temperature attaining their maximum at 15/25°C being lower at 25/40°C (80-30%) respectively (Table 1). Incubation temperature of 10/25°C was suitable for germination of *P. divisum* and was followed by 20/35. Seed germination was significantly lower under extreme temperatures of 10-20 and 20-35°C with no significant difference noticed between them (Fig. 1). Germination in the control commenced on day 2 under 15/25°C and on day 4 with higher temperatures at 25/40°C. Germination percentage decreased significantly ($p < 0.0001$) by increasing the osmotic potential of NaCl and PEG MPa especially at (-0.6 and -0.8 MPa). The germination percentages attaining their maximum at treatment NaCl and PEG at -0.2, was reached 58-73% respectively, the germination was severely decreased at -0.6 MPa reached 12-35% respectively, while under -0.8 MPa of NaCl and PEG was reached 0-10% respectively (Table 2). Germination treatment under NaCl and PEG -0.2,-0.6 MPa commenced on day 2. The germination was delayed for 2,3 day under PEG -0.6 and -0.8 MPa respectively. At treatments by NaCl, no germination occurred at -0.8 MPa and germination was very low at -0.6 MPa reached 12%. Highest germination rates were observed at temperatures of 20/30 and 15/25°C. Lowest germination rate occurred under 25/40°C. Although significant, GR differences were not substantial and ranged from 27.96-18.86 (% day⁻¹) for distilled water at 15/25-25/40°C respectively Fig. 2. The Rate of Germination (RG) of *P. divisum* under PEG was 28.94-19.66 % day⁻¹ for -0.4 and -0.8 MPa respectively

(Table 2), while NaCl was 33.87-0.00 day⁻¹ for - 0.4 and -0.8 MPa respectively (Table 1). Increasing the solutions of PEG-8000 in the growth medium had a highly significant ($p < 0.0001$) adverse effect on mean germination time in *P. divisum* lines. Interactions among all the factors were also statistically non-significant with respect to germination percentage (Table 1 and 2). Water deficit affects the germination of seed and the growth of seedlings negatively.

Seedling growth: Effect of the four different osmotic concentrations of NaCl and PEG 8000 on the Radicle Length (RL) mm of *P. divisum* is shown in Table 1 and 2. Significant ($p < 0.0001$) differences occurred in all observed germination characteristics in response to decreased matric potential (PEG osmotic solutions) (Table 2). In general, all measured germination characteristics decreased as water decrease. The highest radical length was recorded in the control treatment it reached 61.60 mm, then decreased by increasing osmotic potential of NaCl and PEG. The reduction in the RL at the 15/25°C under the moderate and highest osmotic potentials of NaCl (-0.2 and -0.8 MPa) varied between 33.40 and 0.00 mm, while comparable osmotic potentials in the PEG the reduction varied between 49.40 and 3.60 mm, respectively.

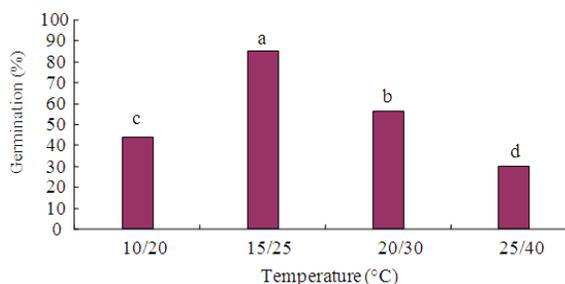


Fig. 1: Percentage of seed germination of *P. divisum* in response to four temperature regimes.

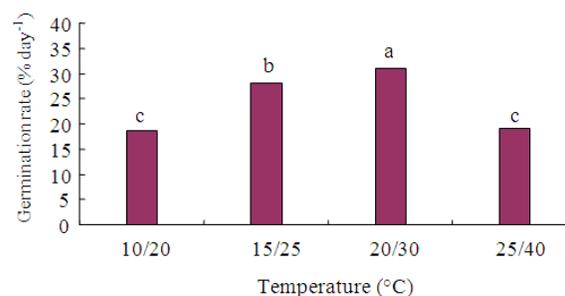


Fig. 2: Germination rate (% day⁻¹) of *P. divisum* in response to four temperature regimes.

Table 1: Effects of osmotic potential induced by sodium chloride NaCl MPa on seed germination of *P. divisum*.

Osmotic potential (MPa)	Final germination (%)	Germination rate (% day ⁻¹)	Radical length (mm)	Hypocotyl length (mm)
0	85.00 ^a	30.845 ^a	61.600 ^a	50.600 ^a
-0.2	58.00 ^a	29.4525 ^b	33.400 ^b	30.200 ^b
-0.4	41.00 ^b	33.8775 ^a	22.200 ^{bc}	16.000 ^c
-0.6	12.00 ^c	24.8725 ^c	5.400 ^{cd}	3.600 ^d
-0.8	0.00 ^d	0.0000 ^d	0.000 ^d	0.000 ^e

Table 2: Effects of osmotic potential induced by polyethylene glycol PEG8000 MPa on seed germination of *P. divisum*.

Osmotic potential (MPa)	Final germination (%)	Germination rate (% day ⁻¹)	Radical length (mm)	Hypocotyl length (mm)
0	85 ^a	30.845 ^a	61.600 ^a	50.600 ^a
-0.2	73 ^a	29.810 ^a	49.400 ^a	41.200 ^a
-0.4	60 ^b	28.948 ^b	27.400 ^b	17.400 ^c
-0.6	35 ^c	22.083 ^c	8.600 ^c	5.600 ^d
-0.8	10 ^d	19.665 ^d	3.600 ^c	2.800 ^d

The results of the effects of different osmotic potential of NaCl and PEG on the Hypocotyl Length (HL) mm of the tested *P. divisum* are shown in Table 1 and 2. It revealed that the growths of *P. divisum* in the different osmotic potential of NaCl and PEG seeds were retarded when compared to the control. The degree of retardation increased with the increase in the osmotic potential of NaCl and PEG. The highest growth appeared at the control treatment reaching 50.60 mm. The hypocotyl length was severely decreased of NaCl at 0.4,-0.6 MPa reached 16.0-3.60 mm respectively, while under -0.4,-0.6 MPa of PEG was reached 17.40-5.60 mm respectively. No growth of the HL was obtained of *P. divisum* at -0.8 MPa of NaCl (Table 1 and 2).

DISCUSSION

In this study, noticeable from Fig. 1 and 2 that significant differences existed in germination percentage among incubation temperatures. When seeds were moistened with deionized water of *P. divisum* showed a high percentage of germination over a wide temperature range. These results indicate that seeds of this specie in germinable in Saudi Arabia desert regions at any time between mid-spring to mid-autumn. When treatment by germination NaCl and PEG 8000, the germination percentage of *P. divisum* decreased significantly ($p < 0.0001$) by increasing the osmotic potential, germination was very low at -0.6 and -0.8 MPa osmotic potential and no germination occurred at -0.8 MPa of NaCl). *P. divisum* showed highest values of germination at osmotic potential zero, they were more sensible than PEG to salt imposed water deficit. However, the results obtained with NaCl did not match with the one observed for PEG and NaCl has higher effect than PEG, meaning that there was another factor acting in this case. According to Mayer and Poljakoff-Mayber (1989) results like this could be attributed to

absence of energy to start the germination process, as energy was obtained by increments in the respiratory pathway after the ambient and in low levels of water potential tax water absorption was processed slowly. Reduction in germination by an increase of salinity levels has been described by numerous authors (Breen *et al.*, 1977; Ungar, 1982; El-Tayeb, 2005; Azhdari *et al.*, 2010).

The inhibitory effects of NaCl on seed germination could be due to its direct affect on the growth of the embryo. Poljakoff-Mayber *et al.* (1994) found that the elongation of the embryonic axis of *Kosteletzkya virginica* was strongly inhibited by high levels of NaCl in irrigation solutions. Alternatively, NaCl may also increase the osmotic potential of the media causing inhibitory effects on seed imbibition (Almansouri *et al.*, 2001). There was significance ($p < 0.0001$) the interaction between *P. divisum* and NaCl induced water deficit, which led to reduction of shoot and root length, especially at -0.6 and -0.8 MPa. According to (Tobe *et al.*, 2001). When seeds were moistened with -5.0 MPa NaCl solution for 5 days, the seeds of three species completely lost their germinability. NaCl may be inhibitory to the activities of some enzymes that may play critical roles in seed germination.

For all treatments, NaCl was found to be more decrease to water uptake, especially at high concentrations, than iso-osmotic solutions of PEG. The first phase of water uptake by the seeds involves movement of water into the free space (apoplast) and does not depend on the osmotic potential of the surrounding solution (Bewley and Black, 1994). The second slower linear phase of water uptake involves the movement of water across cell membranes into the cells of the seeds and is determined by the difference between the osmotic potential of the seed and that of the medium (Bewley and Black, 1994).

The final germination percentage and rate of germination in the *P. divisum* -treated seeds were decreased with the increase of the osmotic potential, but this reduction in NaCl treatment were higher than PEG treatment. Similar results were found in *Salsola villosa* by Assaeed (2001) Mohammadkhani and Heidari (2008) in two Maize cultivars and Al-Taisan *et al.* (2010) in *Ephedra alata*. Differences in GR, followed a similar pattern of total germination percentage. Germination Rate (GR) also varied significantly ($p < 0.0001$) with temperature treatments. The decrease of GR was stronger at different levels of the NaCl and PEG solutions compared to the FG percentage. This result corroborates other studies showing that osmotic stress primarily reduces rate of germination rather than germination percentage (Kaya *et al.*, 2008). It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions (Hilhorst, 1995). Salinity stress can affect seed germination through osmotic effects (Welbaum *et al.*, 1990). Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Faheed *et al.*, 2005). Germination percentage also significantly decreased as the level of salinity of the medium increased (Mauromicale and Licandro, 2002). These results are similar in line with (Bayuelo-Jimenez *et al.*, 2002). They found that the mean time to germination of almost all Phaseolus species increased with the addition of NaCl and this increase in was greater in higher concentration as compared to low concentration. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant may be another reason for this decrease (Werner and Finkelstein, 1995).

The results of this study showed a greater reduction of HL and RL in comparison to germination phase with the increase of the osmotic potential with NaCl and PEG. This is in conformity with findings from Hosseini *et al.* (2002) in soybean and Kaya *et al.* (2008) in chickpea (*Cicer arietinum* L.) who observed that NaCl or PEG had greater inhibitory effects on seedling development than germination. Hosseini *et al.* (2002) suggested that cell division, which is a post-germination phenomenon responsible for seedling elongation and development, is more sensitive to the NaCl or PEG compared to cell expansion, which drives germination. Comparing the effect of both types of water potential, the present results indicate *P. divisum* seeds are efficient in

osmotically adjusting to NaCl solutions. Ions may enter the seed, lowering its osmotic potential and thus facilitating hydration (Sharma, 1973).

CONCLUSION

In the germination and early seedling growth stages the investigated showed different responses to water stress induced by PEG and NaCl. However, seedling growth was more sensitive to NaCl than was germination responses to water stress induced by PEG and NaCl. However, seedling growth was more sensitive to NaCl than was germination. Results also indicated that seed germination of *P. divisum* is less sensitive to osmotic potential indicating that the seeds of the species are efficient in osmotically adjusting to soluble salts. This suggests the possibility of revegetating moderately salt affected soils. Further study is needed to study the effect of interaction among the three environmental conditions on germination and seedling growth and establishment of *P. divisum*. This suggests the possibility of revegetating moderately salt affected soils.

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