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Effects of Salinity and Temperature on Germination of Three Millet Varieties

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ABSTRACT

An experiment was conducted to determine the effects of salinity and temperature on the germination responses of three of the more commonly cultivated millet varieties in South-East regions of Iran, including common millet (*Panicum miliaceum*), pearl millet (*Pennisetum galucum*) and foxtail millet (*Setaria italica*). Seeds were exposed to different salinity levels equal to six osmotic potentials of 0 to -1.25 with -0.25 MPa intervals and three constant temperature treatments (15, 25 and 35°C). Cumulative germination percentage and germination rate were analyzed under different exerted treatments. Germination rate was calculated as the reciprocal of time to 50% germination in each combination of treatments. Our results indicate that temperature is more important than salinity level to obtain more favorable germination. The highest values of germination percentage and germination rate were obtained for higher temperatures with lower salinities. Also, decreased temperature alleviated the detrimental effects of salinity on studied traits, which indicates a synergistic effect of higher temperatures on detrimental effects of salinity. The lowest values of studied characters also were associated with lower temperatures, independent of salinity level. Results indicate that germination of seeds at high temperatures with low salinity levels, and high temperatures and high salinity levels was better than when seeds were germinated at low temperatures (independent of the salinity conditions). Also, foxtail millet had the highest germination percentage and germination rate at all salinity levels and temperatures while the lowest values belonged to pearl millet.

Keywords: abiotic stress, arid regions, germination rate

INTRODUCTION

Germination is a complex and dynamic stage of plant ontogeny, with a number of interactive metabolic processes changing from a storage phase to a mobilization phase (Bewely and Black 1994). One of the first physiological disorders taking place during seed germination under salt stress is a decrease in water uptake by seed due to low water potential of the germination medium. In addition to causing various structural changes at different levels of organization in the seed, a slow rate of imbibition may lead to a series of metabolic changes, including up-regulation or down-regulation of enzyme activities (Filho and Sodek 1998; Guerrier 1988; Ashraf *et al*. 2002), perturbance in the mobility of inorganic nutrients to developing tissues (Yapsanis and Domiandou 1994; Ashraf and Wahid 2000), an imbalance in the levels of plant growth regulators (Khan and Rizvi 1994), reduction in hydrolysis and utilization of food reserves (Mondal *et al*. 1988; Ahmad and Bano 1992), and accumulation of compatible osmotica such as soluble sugars, free proline, and soluble proteins (Poljakoff-Mayber *et al*. 1994; Zidan and Elewa 1995; Ashraf *et al*. 2003). These processes may lead to poor or complete lack of seed germination under saline conditions (Poljakoff-Mayber *et al*. 1994). Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions (Ungar 1995). In these regions, germination occurs during rainy seasons when soil salinity levels are usually reduced (El-Keblawy 2004). Optimal germination in seeds of halophytes often occurs under fresh-water conditions and germination of most species is reduced and delayed with an increase in salinity, so the response may vary greatly depending on the species. Temperature and salinity can interact in determining salinity tolerance during germination. Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at optimum temperature. The detrimental effect of salinity was found to be severe at higher temperatures in some halophyte species (Delesalle and Blum 1994; de Villiers *et al*. 1994; Khan and Ungar 1998; Aiazzi *et al*. 2002; Khan *et al*. 2002), but at lower temperatures for other species (Gul and Weber 1999; Khan *et al*. 2000; Gulzar and Khan 2001; Khan and Ungar 2001). Millet varieties are traditionally grown in marginal and unsustainable environments to sustain growth of other cereal crops (FAO and ICRISAT 1996). In order to extend arable lands by sustaining marginal lands in stressful and fragile environments, especially in arid and semi-arid regions of the world, it is necessary to evaluate the ability of favorable crops to these regions and millet can be considered in this respect as a C_4 crop with high potential yield. Arid and semi-arid regions are faced by high temperatures and in some cases soil salinity. Information about the interaction of temperature and salinity on germination responses of millet varieties (to obtain successful establishment of individuals) is scarce. In one report, Ashraf and Idree (1992) noted that salinity stress caused a reduction in the germination percentage and delayed germination of pearl millet. To promote cultivation of millet varieties in arid and semi-arid regions that are faced by high temperatures overall and salinity levels in many cases, knowing the germination responses of different varieties is essential. In this work, we attempt to assess the effects of various temperatures and salinity regimes on germination responses of three common cultivated millet varieties in South-East Iran.

MATERIALS AND METHODS

This study was carried out with three of the more commonly cultivated millet varieties in South-East regions of Iran, including common millet (*Panicum miliaceum*), pearl millet (*Pennisetum glau-*

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cum) and foxtail millet (*Setaria italica*). Seeds were obtained from South East Iran (Birjand, 59° 12′ N longitude, 32° 52′ E latitude, 1491 m asl). All empty seeds were cleaned by soaking in water for two minutes. Germination test was carried out in the laboratory in Petri dishes containing Whatman filter paper No 10 imbibed with distilled water (5 ml) or one of five osmotic potentials, varying from -0.25 to -1.25 MPa with -0.25 MPa intervals. The seeds were incubated in the dark at three temperatures (15, 25 and 35°C). Four replicates of 50 seeds each were used for each treatment. Salinity and temperature levels were arranged in a factorial experiment with a CRD design. After the beginning of germination, seeds with a radicle equal to 2 mm were scored as having germinated and removed. Germinated seeds were counted at different time intervals based on the observed rate under different temperatures, with shorter and longer periods for higher and lower temperatures, respectively. Counting was considered complete when no more seeds germinated during two consecutive days. The time from the beginning of incubation to last germination was considered as the total time to maximum germination. The collected data were used to plot mean germination percentage curves against time. From these curves, time to 50% germination was determined by interpolation (Dumur *et al*. 1990). The reciprocal of time to 50% germination was considered as the germination rate (GR). The results of the assays were subjected to analysis of variance, according to an experiment with factorial structure of treatments and CRD design with four replications. Mean comparisons were done by Duncan's multiple range test at 5% probability. All statistical analysis were conducted using the SAS program version 8e.

RESULTS

Germination rate and percentage

ANOVA results indicated that salinity, variety, temperature and their interactions had significant effects on germination rate and percentage (**Table 1**). Seeds of all varieties germinated rapidly in control conditions at 35°C, reaching maximum germination after less than 40 hours in all varieties. Increased temperature from 15 to 35°C hastened germination rate significantly (**Table 2**). Germination percentage was also affected by an increase in temperature ($p<0.01$). High germination percentage at 35°C was accompanied by a higher germination rate (**Table 2**). Cumulative germination trends showed that the time to reach maximum

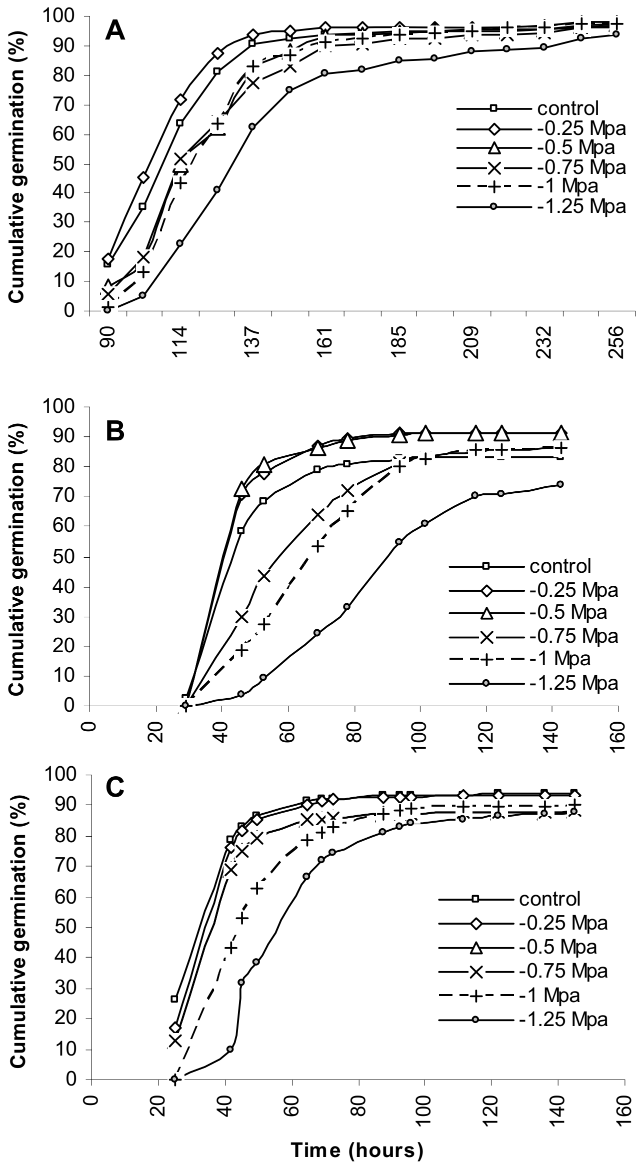

Fig. 1 Mean germination percentage as a function of time and salinity levels at different temperatures: 15°C (**A**), 25°C (**B**), 35°C (**C**).

Table 1 Mean squares of ANOVA table for germination percentage (GP) and germination rate (GR).

S.V	df	GP (%)	GR (1/D50)
R	3	0.002^{ns}	0.00006^{ns}
Temperature(T)	2	0.233**	0.0059**
Variety(V)	2	0.471**	0.0002**
Salinity(S)	5	0.036**	0.0004**
V×S	10	0.007**	0.00002**
T×S	10	0.013**	0.00095**
T×V	4	0.103**	0.0001**
T×V×S	20	0.009**	0.0004**
Error	56	0.0163	0.043
CV	---	6.34	6.2

ns: non significant
**: $p<0.01$
* means with common letter are not different significantly

Table 2 Mean comparisons for germination percentage (GP) and germination rate (GR) for different varieties and temperature regimes.

Variety	GP (%)	GR (1/D50)
Pearl Millet	0.819 c	0.016 c
Common millet	0.941 b	0.019 b
Foxtail millet	0.972 a	0.002 a
Temperature (ºC)		
15	0.85 c	0.009 c
25	0.913 b	0.0199 b
35	0.966 a	0.027 a

* means with common letter are not different significantly (based on DMRT).

germination percentage at different temperatures and salinity levels decreased as temperature increased (**Figs. 1A-C**). On the other hand, as temperature increased, the time to reach maximum germination percentage decreased at different salinity levels (**Figs. 1A-C**). Also, with passing time, increasing temperature caused an obvious decrease in germination percentage at high salinity levels (**Figs. 1A-C**). In this case, germination percentage at 25 and 35°C decreased dramatically in comparison with the same salinity levels at 15°C.

Our results indicated that although the temperature × salinity interaction on germination was significant ($p<0.01$) for all studied characters (**Table 1**), an increase in salinity levels did not affect germination rate of varieties at 15°C, while at all salinity levels, the germination rates at higher temperatures (25 and 35°C) were significantly different when at 15°C (**Fig. 3**). Increasing salinity from control to -0.5 MPa at 25°C did not affect germination rate significantly, while an increase in salinity of more than -0.5 MPa resulted in a significant decrease in germination rate compared to lower salinity levels. This was also true for germination percentage. Increasing salinity from control to higher levels at 35°C resulted in a significant reduction of germination rate, confirming the synergistic effects of higher temperatures with detrimental effects of salinity stress.

These results indicated that lower temperatures decreased the detrimental effects of salinity on germination

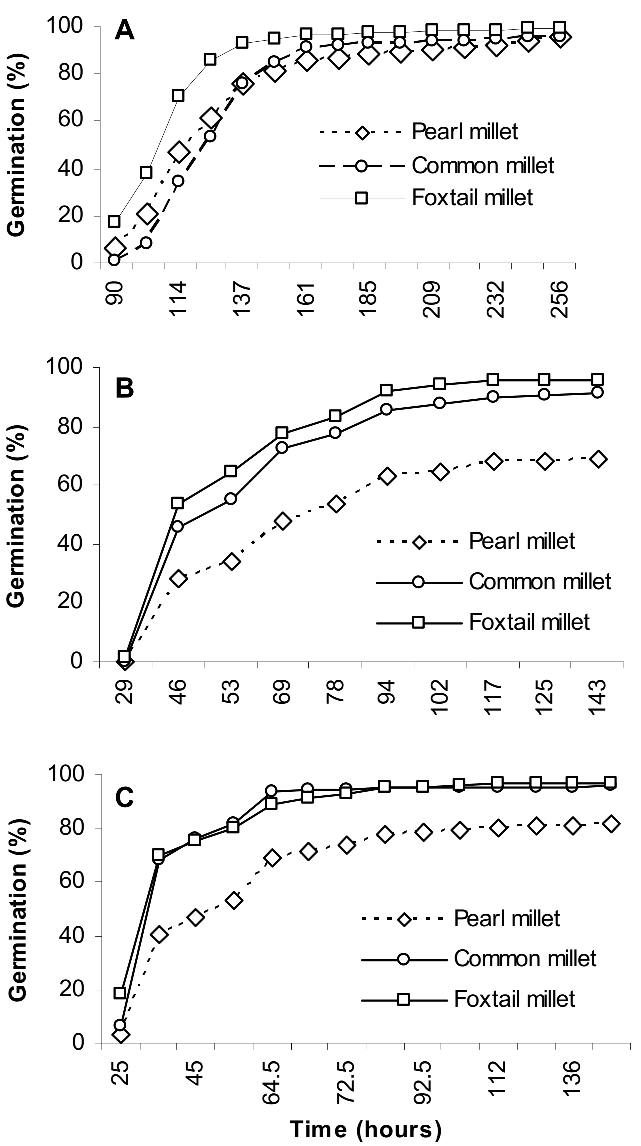

Fig. 2 Mean germination percentage as a function of time and variety at temperatures: 15°C (**A**), 25°C (**B**), 35°C (**C**).

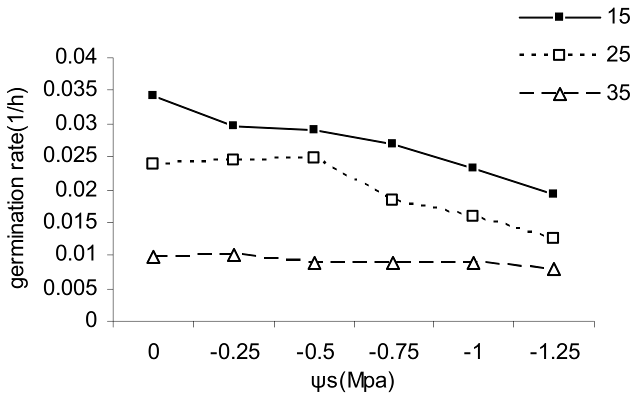

Fig. 3 Germination rates at different temperatures (ºC) and salinity levels.

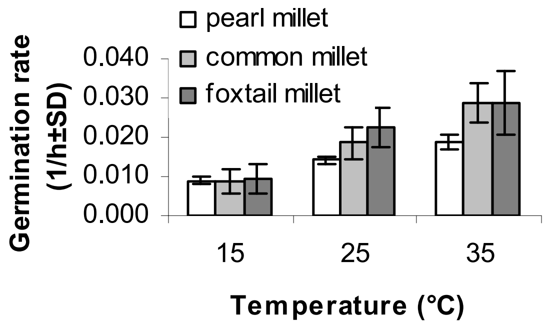

Fig. 4 Observed germination rates of millet varieties under different temperature treatments (based on MRT).

rate and germination percentage of millet varieties in comparison with same salinity levels at higher temperatures. These results also confirmed that although lower temperatures exert negative effects on germination rate and germination percentage, considerable differences between different salinity levels were eliminated as all salinity levels had similar trends.

Response of varieties to salinity and temperature

Cumulative germination percentage of all varieties followed a close trend at 15°C (**Fig. 2A**), while increasing temperature to 25 and 35°C resulted in a clear difference between pearl millet and common and foxtail millets (**Figs. 2B, 2C**). Although the time to reach maximum germination decreased with increasing temperatures, pearl millet was significantly different to the two other varieties with respect to final germination percentage and cumulative percentage (**Fig. 2A-C**). Also results indicated that germination rate of all varieties increased as temperature increased (data not shown). This revealed that temperatures closer to optimum germination temperatures for these varieties resulted in a higher germination rate and germination percentage. Kamkar *et al.* (2006) calculated cardinal temperatures of all three studied varieties and reported optimum temperatures of 38.8, 40.1 and 37°C for pearl, common and foxtail millet, respectively. Our results also indicated that germination rate responds to temperature linearly up to 35°C.

Decreasing germination rate and germination percentage at higher temperatures (25 and 35°C) revealed a synergistic effect of higher temperatures on detrimental effects of salinity.

Our results indicate that different varieties have different responses to salinity and temperature. Germination rate of pearl millet was not affected significantly by an increase in temperature, but its average germination rate at 25 and 35°C was significantly different to common and foxtail millets (**Fig. 4**). As temperature shifted from optimum to base temperature for all varieties, germination rate and germination percentage of common and foxtail millets decreased more than pearl millet. Also, foxtail millet had the highest germination percentage and germination rate at all salinity levels and temperatures and the lowest values were those of pearl millet. The response of all studied varieties to exerted temperatures is shown in **Fig. 4**.

Therefore, although temperature can alter responses to salinity in a synergistic manner, it can also increase germination rate and germination percentage, too. Esechie (2008) showed that seed germination of sorghum was more tolerant to salinity at germination temperature of 30-40°C than at 15-25°C.

DISCUSSION

General discussion

Salinity and temperature interact in their control of seed germination (Khan and Ungar 1999), with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature (Badger and Ungar 1989). This has reported in *Hordeum jubatum* (Badger and Ungar 1989), *Crambe abyssinica* (Fowler 1991), *Puccinellia ciliata* (Myers and Couper 1989), *Halopyrum mucronatum* (Noor and Khan 1995), and *Urochondra setulosa* (Gulzar *et al.* 2001). Yildiz and Casap (2007) in comparison of germination responses of cultivated wheat (*Triticum*) and its wild relative (*aegilops*) species under salinity, temperature and light reported that the inhibitory effect of high salinity on germination was greater at 25/35°C than at 15/25°C or 20/30°C. Willenborg *et al*. (2004) also reported that decreasing osmotic potential and temperature significantly reduced the germination of both coated and uncoated canola seeds.

Khan and Gulzar (2003) stated that salinity and temperature have differential effects on seed germination. Khan and Ungar (1999) stated that the dependence of the response to salinity on temperature may be due to an initial inhibition of germination at higher salt concentrations at some temperatures than others.

Other reports have also shown that high temperatures increase the detrimental effects and irreversible damage of salinity (Sharma 1976; Khan and Ungar 1984; Potter *et al*. 1986; Ungar 1995; Kahan and Ungar 1996). Aiazzi *et al*. (2002) also showed that the susceptibility of *Atriplex cordobensis* seeds to salinity increased at higher temperatures. Alkhateeb (2006) in study on the effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk, reported that Seed incubated under high temperatures with high NaCl concentration seemed to be subjected to more environmental stress, which is indicated by delayed germination. Under such condition, changes in incubation temperature particularly under high salt concentration may result in malfunctioning of enzymatic systems. This situation would lead to limitations in many physiological processes vital to seed germination.

Khan and Ungar (1997, 1998) also stated that temperature interacts with salinity to modify the speed of germination and total germination percentage, probably due to a physico-chemical effect, both osmotic and toxic (Khan and Ungar 1997, 1998). Thus, based on these results, it seems that millet seeds will germinate better at higher mean temperatures and lower salinity levels (condition prevalent in some arid and semi-arid regions) which is consistent with Sincik *et al*. (2004) which in a study on the effect of low temperatures on germination of different field pea genotypes stated that generally temperature below the optimum results in progressively poorer germination.

Overview on varieties

The effect of different temperatures on germination response of various species has been described for many species such as *Atriplex* spp. (Potter *et al*. 1986; Wang *et al*. 1997). Flores and Brions (2001) studied the effects of soil water potential (SWP) and temperature on seed germination of six different life-form coexisting species of an inter-tropical desert and reported that In all species except *P. laevigata* germinability increased and the germination time and t_{50} decreased as temperature increased. These species showed 60-80% germination at 20 and 25°C but germination dropped to 10-40% and 5-25% at the extreme temperatures of 10 and 40°C, respectively. Millet varieties used in this research are cultivated in South-East Iran, with saline soils, high mean temperatures and low soil moisture content. Therefore, based on these results, the most acceptable germination along with non-delayed germination will be achieved at higher temperatures with lower salinity severity. This is true, because temperature effects on improved seed germination were more obvious than detrimental effects of salinity. At 15°C, increased salinity did not affect germination rate compared to the control treatment and germination rate was at the lowest level at 15°C and all salinity levels (**Fig. 3**). Also, although the slope of germination rate which decreased in response to salinity levels increased at higher temperatures (**Fig. 3**), at all salinity levels, germination rate was higher at higher temperatures. Therefore, it seems that best germination situation will occur with a combination of higher temperatures and lower salinities. Undoubtedly, higher temperatures beyond the optimum temperature will suppress germination rate and percentage (Kamkar *et al*. 2006).

Moderate response threshold to salinity (ψ_S>-075 MPa) at higher temperatures (**Fig. 3**) revealed that millet varieties with the ability to tolerate moderate-saline conditions at the germination stage can be considered as hopeful varieties to convert marginal to arable lands in arid and semi-arid regions of world, in general, and South-East Iran in particular. Our results confirmed that germination at low temperatures close to the base temperature, without considering salinity, is not favorable, because in addition to lower germination percentage (**Table 2**), the seeds also will experience delayed germination (lower germination rate) (**Fig. 4**). Therefore, germination of seeds at high temperatures with low salinity levels, and high temperatures and high salinity levels was better than when seeds were germinated at low temperatures (independent of the salinity conditions).

Favorable germination does not necessarily imply good establishment, too. Thus, it is advisable to study the ability of germinated seeds to establish on a soil surface too, which is dependent on water supply. Also it should be mentioned that the germination tolerance of plant species to salinity under laboratory conditions does not necessarily correlate with their response to salinity under field conditions, and may be many times lower.

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