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## Seed Germination of Populations of Wild Wheat Species, *Aegilops biuncialis* and *Ae. triuncialis*: Effects of Salinity, Temperature and Photoperiod

Mustafa Yildiz, Hakan Terzi and Evrim Suna Arıkan  
Department of Biology, Faculty of Science and Arts, Afyon Kocatepe University,  
ANS Campus, 03100 Afyonkarahisar, Turkey

**Abstract:** In this research, seed germination of populations of wild wheat (*Aegilops*) species (*Bi-1* and *Bi-2* populations of *Ae. biuncialis* and *Tri-1* and *Tri-2* populations of *Ae. triuncialis*) was investigated under different salinities (0-675 mM NaCl), temperatures (15/25 and 25/35°C, night/day) and photoperiods (in dark and in 12 h light: 12 h dark), using a completely randomized block design. No seeds germinated at 675 mM NaCl. Seed germination was partly or completely inhibited in all populations with an increase in salinity and this inhibition was more important at 25/35°C than in 15/25°C temperature regime. The highest germination percentages occurred at 15/25°C for all populations. The effect of light differed depending on population, temperature and salinity. Seed germination of *Ae. biuncialis* populations was similar in light under salinity and temperature interactions. Seed germination of *Bi-2* population was higher than *Bi-1* population in dark. Differences between *Tri-1* and *Tri-2* populations were remarkable in both photoperiods at 25/35°C temperature regime. At 15/25°C, the detrimental effect of light was determined in all populations at high salinity levels. Seed germination of only *Bi-1* population in light was significantly greater than in dark in all NaCl concentrations at 25/35°C.

**Key words:** *Aegilops* L. species, population, germination, salinity, temperature, photoperiod

### INTRODUCTION

The genus *Aegilops* (wild wheat) is characterized as a Mediterranean-Western Asiatic element and its center of diversity follows the central part of the Fertile Crescent arc in West Asia. The ecological distribution of *Aegilops* species is seen along roadsides, edges of cultivation, dry-hillsides and grassy steppes, where it tolerates disturbances (Van Slageren, 1994). The wild relatives (*Aegilops*) of cultivated wheat are suggested as potential gene resources for abiotic stress tolerance of cultivated wheat (*Triticum*) species (Hegde *et al.*, 2002; Baalbaki *et al.*, 2006). The individual effects and/or their interactions of abiotic stresses such as salinity, temperature and light on germination were studied in many glycophytic and halophytic species (Prakash and Sastry, 1992; Gulzar *et al.*, 2001; Almansouri *et al.*, 2001; Alam *et al.*, 2002; Perez-Garcia *et al.*, 2002; Socolowski and Takaki, 2004; Tobe *et al.*, 2004; Jamil *et al.*, 2005). Salinity and high temperature stresses are primary limiting environmental factors which adversely effect the most critical germination phase of many crops (Basnizki and Mayer, 1985; Fowler, 1991). Seed germination shows optimal germination in distilled water, but differs in their ability to germinate at different salinities, temperatures

and light levels (Khan and Gulzar, 2003; El-Keblawy and Al-Rawai, 2005). Perez-Garcia *et al.* (2002) reported that the main source of variation in germination response to environmental stresses was population variability. However, some researchers suggested that studies of the germination characteristics of a pine species should consider different populations.

Currently, the literature is unavailable on the individual effects of salinity, temperature and photoperiod and their interactions on the seed germination of populations of wild wheat (*Aegilops* L.) species. In the present study, two each populations of *Ae. biuncialis* and *Ae. triuncialis* comprise an experimental set to test some questions related to germination behaviors, such as: (a) What are the individual and combination effects of salinity, temperature and light on germination, taking into account populations of *Aegilops* species? (b) Is there any differences in germination behaviors between populations of the same *Aegilops* species collected from different localities?

### MATERIALS AND METHODS

In this research, seeds of natural populations of two wild wheat (*Aegilops* L.) species were used. Ears

Table 1: Geographical distribution of populations of *Ae. biuncialis* and *Ae. triuncialis* species

Populations	Latitude	Longitude	Altitude
Bi-1	38°47' 44, 65" E	37°13' 45, 81" N	575
Bi-2	38°32' 28, 05" E	37°20' 42, 62" N	705
Tri-1	38°37' 40, 99" E	36°49' 18, 81" N	453
Tri-2	38°32' 28, 05" E	37°20' 42, 62" N	705

of *Ae. biuncialis* Vis. populations (*Bi-1* and *Bi-2*) and *Ae. triuncialis* L. populations (*Tri-1* and *Tri-2*) were collected in July 2003 from Şanlıurfa (Southeast Anatolia Region), Turkey. The localities of populations were given in Table 1. Seeds were separated from the spikelets. Seeds were selected for uniform size, shape and color.

Seeds were surface-sterilized with 3% Clorox (sodium hypochlorite) solution for 10 min. Seeds showed 100% germination in distilled water in a viability test. Seeds were imbibed in distilled water (control); in 150, 225, 300, 375, 450, 525, 600 and 675 mM NaCl solutions at room temperature in dark for three hours. The imbibed seeds were germinated in their solutions under alternating temperature regimes (15/25 and 25/35°C, night/day) in 24 h dark and 12 h photoperiod (110 µmol m<sup>-2</sup>s<sup>-1</sup>, Sylvania Gro-Lux fluorescence lamp, F18W/GRO) in germination cups. Six replicates of 15 seeds were used for each treatment. Fifteen seeds for each of six cups on two layers of solutions soaked filter papers were used throughout. Germination experiments were replicated three times for each treatment. Germination was recorded at the end of the fifth days. A seed was considered to be germinated with the emergence of the radicle (Bewley and Black, 1994).

Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS, version 10.0. The effects of population, salinity, temperature and photoperiod on the germination percentage were evaluated using analysis of variance (ANOVA). Duncan Multiple Range and Student t-tests were used for means comparison. All statistical tests were conducted at p<0.05.

## RESULTS AND DISCUSSION

A four-way ANOVA indicated significant (p<0.05) individual effects of population, salinity, temperature, photoperiod and their interactions on seed germination (Table 2). No germination was observed at 675 mM NaCl in all populations. Thus, this concentration was not added to statistical evaluation.

The test populations of wild wheat (*Aegilops*) species differed greatly in their response to salinity. At both temperature regimes, seed germination was 100% in distilled water for all populations and also was 100% at 150 mM NaCl for *Bi-2* and *Tri-2* populations.

Table 2: Results for four-way analysis of variance of characteristics by population (P), salinity (S), temperature regime (T) and photoperiod (L). All values are significant at the p<0.05 level

Source	Type III		Mean Square	F	Significance
	Sum of Squares	df			
P	13.278	3	4.426	367.994	0.000
S	222.136	7	31.734	2638.375	0.000
T	37.870	1	37.870	3148.556	0.000
L	0.595	1	0.595	49.456	0.000
P×S	9.603	21	0.457	38.019	0.000
P×T	1.866	3	0.622	51.717	0.000
S×T	15.978	7	2.283	189.776	0.000
P×S×T	8.598	21	0.409	34.039	0.000
P×L	1.041	3	0.347	28.862	0.000
S×L	1.528	7	0.218	18.147	0.000
P×S×L	1.235	21	5.881E-02	4.889	0.000
T×L	2.236	1	2.236	185.870	0.000
P×T×L	0.112	3	3.734E-02	3.105	0.026
S×T×L	0.871	7	0.124	10.347	0.000
P×S×T×L	2.078	21	9.896E-02	8.227	0.000

However, seed germination decreased with increasing salt concentration (Fig. 1). This decrement in germination percentage has been reported by some workers for three cultivars of durum wheat (Almansouri *et al.*, 2001), three species of *Brassica* (Jamil *et al.*, 2005), three cultivars of maize (Yildiz *et al.*, 2006) and many halophytes (Khan and Rizvi, 1994; Katembe *et al.*, 1998; Gul and Weber, 1999; Khan *et al.*, 2001; Khan and Gulzar, 2003). This decrease is probably the result of a physico-chemical effect (Bewley and Black, 1994), either osmotic stress or ion cytotoxicity (Zhu, 2002). Elevated salinity slowed down water uptake by seeds, thereby inhibiting the germination process.

In this study, seed germination of *Ae. biuncialis* populations in light was similar under salinity and temperature interactions (Fig. 1). Except for this result, there were significant differences in seed germination behaviors between populations of *Aegilops* species collected from different localities (shown with asterisks in Fig. 1). Schütz and Rave (1999) suggested that habitat differences may be responsible for the differences in germination requirements between the species of a single genus. Present results show that the each population of a species responds to the abiotic condition(s) in a unique manner. Bayuelo-Jimenez *et al.* (2002) reported that seed germination among and within 4 wild *Phaseolus* species and their 24 accessions obtained from different localities showed differences in response to salinity.

Many glycophytes such as wheat could germinate at NaCl concentrations above 300 mM (Malcolm *et al.*, 2003), while many halophytic species could not germinate at NaCl concentrations higher than 400 mM (Khan, 2002). The seeds of *Aegilops* populations germinated in high salt concentrations (525 or 600 mM NaCl) in dark at only 15/25°C (Fig. 1): *Bi-2* and *Tri-2* populations showed high

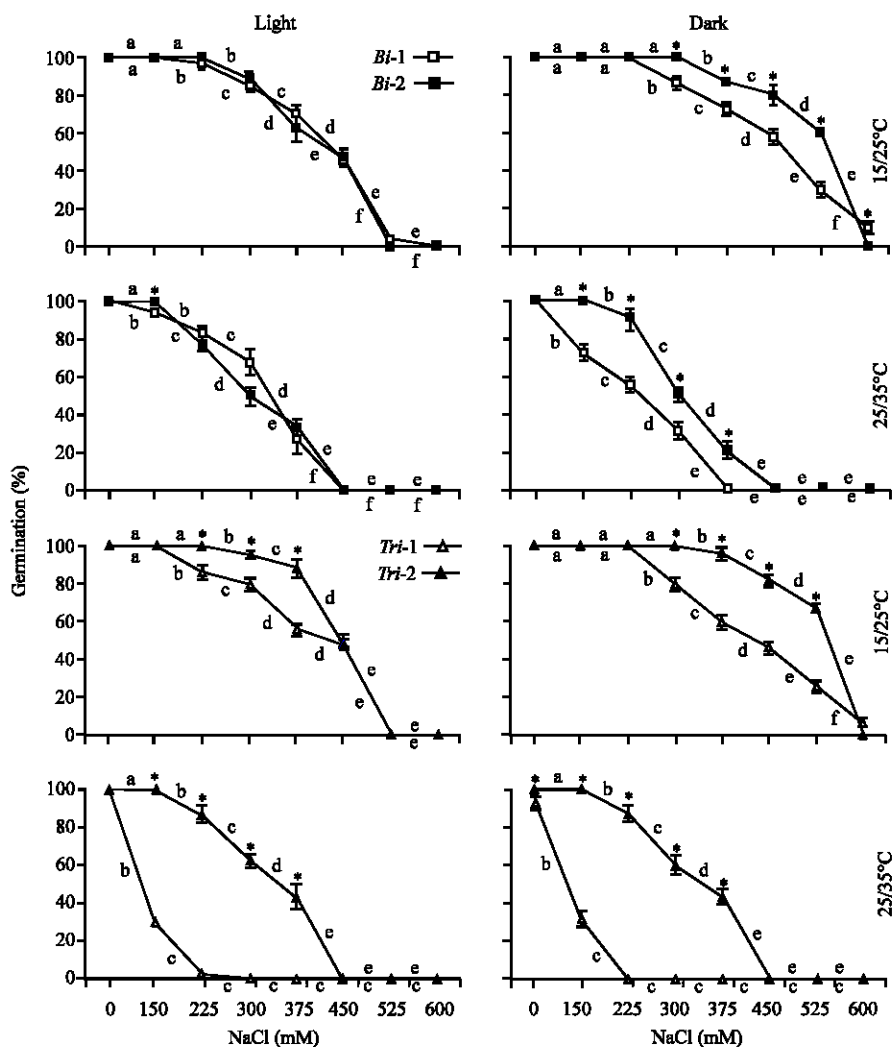


Fig. 1: Mean percent germination (%) of *Ae. biuncialis* populations (*Bi-1* and *Bi-2*) and *Ae. triuncialis* populations (*Tri-1* and *Tri-2*) in various salinity, temperature and photoperiod treatments. Different letters indicate significant differences ( $p < 0.05$ ) among NaCl concentrations for each population (Duncan Multiple Range test). Asterisk (\*) indicates significant difference ( $p < 0.05$ ) between two populations at each NaCl concentration (Student-t test). Bars represent means  $\pm$  SE

germination percentage up to 525 mM NaCl concentration. The germination percentage of these populations was higher even at 525 mM NaCl (60.0 and 66.7%, respectively), while germination was completely inhibited at 600 mM NaCl. On the other hand, seed germination of *Bi-1* and *Tri-1* populations was progressively affected up to 600 mM NaCl (Fig. 1). Macke and Ungar (1971) reported that halophytes show the characteristic germination behavior in response to increased salt concentration, with higher resistance up to certain critical concentration level and then a rapid decreased in final germination beyond this level; instead glycophytes show a concomitant

reduction in germination with increasing salinity. In the light of this knowledge, *Bi-2* and *Tri-2* populations can be evaluated as close to halophytes due to showing high germination up to 525 mM and then a complete inhibition beyond this level. On the other hand, *Bi-1* and *Tri-1* can be evaluated as close to glycophytes, as their germination was progressively affected by increasing salt concentrations (Fig. 1). The germination behaviors of *Bi-2* and *Tri-2* populations would therefore imply adaptive mechanisms to saline habitats, while *Bi-1* and *Tri-1* populations, such mechanisms would not present. Similar results were reported by Villagra (1997) for *Prosopis*

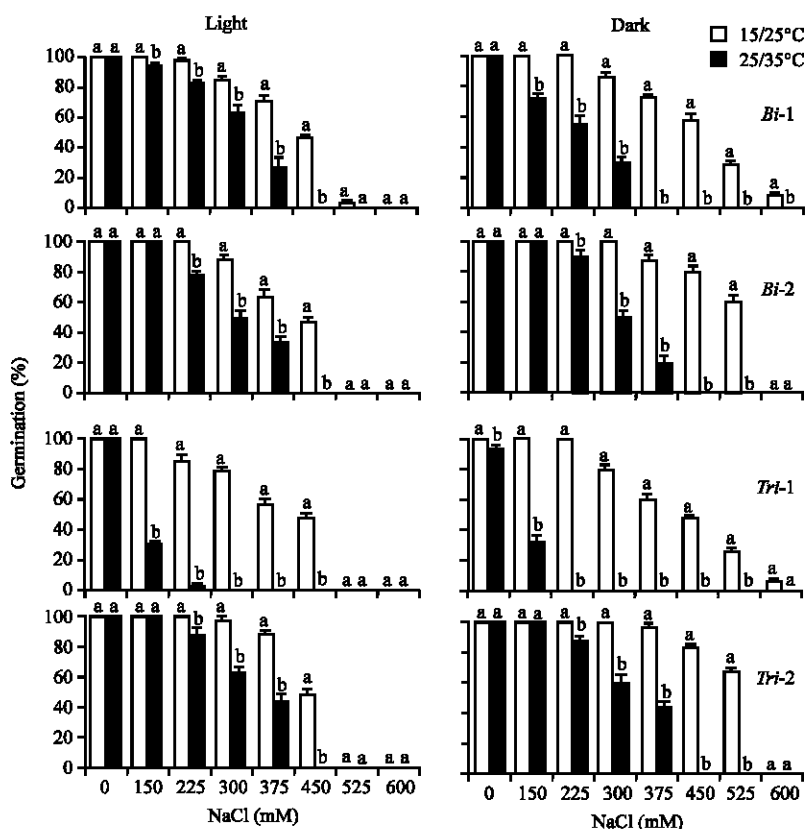


Fig. 2: Effect of different temperature regimes on seed germination of *Ae. biuncialis* populations (*Bi-1* and *Bi-2*) and *Ae. triuncialis* populations (*Tri-1* and *Tri-2*) in various salinity and photoperiod interactions. Different letters at each NaCl concentration indicate significant differences ( $p < 0.05$ ) between 15/25°C and 25/35°C temperature regimes (Student-t test). Bars represent means  $\pm$  SE

species at both 25 and 35°C constant temperatures: *P. alpataco* was evaluated as close to halophytes, while *P. argentina* was evaluated as close to glycophytes.

Temperature shifts may affect a number of processes determining the germinability of seeds, including membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994). The effect of salinity on germination varies considerably with temperature and the firstly inhibition of seed germination occurs at different salt concentrations (Khan and Ungar, 1997; Gulzar *et al.*, 2001). The highest germination percentages occurred at 15/25°C for all populations (Fig. 2). The present study showed that 100% of the control seeds of *Aegilops* populations [except *Tri-1* (93.3%) in dark] germinated at 25/35°C. This temperature had no effect on percent germination in non-saline conditions. Some workers also reported this phenomenon in spring wheat cultivars (Lafond and Baker, 1986; Hampson and Simpson, 1990). Due to high seed germination percentages observed in distilled water, the

high temperature on germination did not cause to high temperature dormancy. Temperature and salinity interactions were highly significant (Table 2). High temperature amplified the deleterious effects of salinity stress on germination. The significant differences in germination responses between populations of each species were determined at lower salt concentrations by arising temperature to 25/35°C (Fig. 2). High test temperature reduced the ability of seeds to germinate at lower levels of salinity. An increase in temperature reduced seed germination of some halophytic species at control and salt treatments (Gulzar *et al.*, 2001; Khan and Gulzar, 2003), while high temperature appeared to stimulate seed germination of some salt tolerant halophytic species over a wide range of salinity (Gul and Weber, 1999; Khan *et al.*, 2001, 2002).

In many plant species, light is one of the most important environmental regulatory that interacts with temperature to regulate seed germination (Baskin and Baskin, 1998). However, the light sensitivity interacted

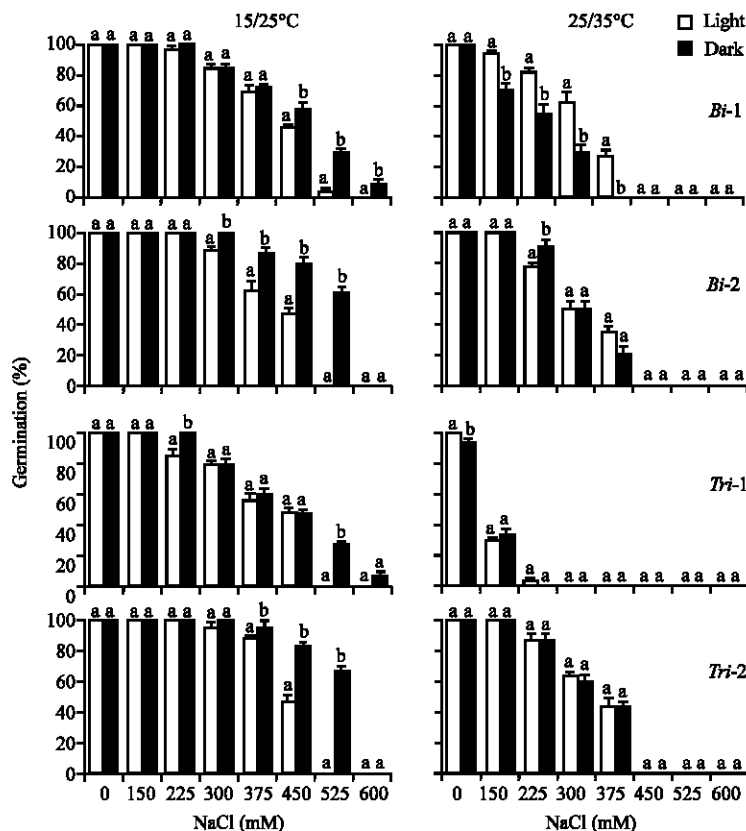


Fig. 3: Effect of photoperiod on seed germination of *Ae. biuncialis* populations (*Bi-1* and *Bi-2*) and *Ae. triuncialis* populations (*Tri-1* and *Tri-2*) in various salinity and temperature interactions. Different letters at each NaCl concentration indicate significant differences ( $p < 0.05$ ) between light and dark (Student-t test). Bars represent means  $\pm$  SE

with salinity or water stress during germination in several species (Gul and Weber, 1999; Takaki, 2001). In this study, the seeds of the populations germinated equally well in light and dark treatments in distilled water (Fig. 3). When seeds of *Aegilops* populations were exposed to salinity stress at 15/25°C temperature regime, some significant differences were observed between light and dark treatments (Fig. 3). Increase in salinity affected light germination more adversely than seeds germinated in the dark. The germination in light differed with an increase in temperature regime. In this study, seed germination of *Aegilops* populations except *Bi-1* population did not show significant difference between light and dark treatments at 25/35°C temperature regime (Fig. 3). Many researchers reported that there was no significant difference between light and dark germination for *Prosopis juliflora* at 15 and 25°C (El-Keblawy and Al-Rawai, 2005) and for *Haloxylon ammodendron* at 5-30°C temperatures (Huang *et al.*, 2003). On the other

hand, the effect of light promoting the germination under 150-375 mM NaCl concentrations was observed in only *Bi-1* population at 25/35°C temperature regime (Fig. 3). Similar result was reported that the germination of *P. juliflora* in light was significantly greater than in dark at 40°C for control and 100 mM NaCl (El-Keblawy and Al-Rawai, 2005). Socolowski and Takaki (2004) reported that light had no effect under water stress condition at optimum temperature (25°C), but the effect of light promoting the germination under water stress was observed at 20 and 30°C.

The seeds of populations of a species may show differences in germination response to environmental stresses. We think the population variability is the main source of variation in germination response to environmental stresses. In salinity and temperature interactions, the seeds of *Bi-2* and *Tri-2* populations may be evaluated as most tolerant and also the seeds of *Tri-1* population may be evaluated as most sensitive. The

populations of *Aegilops* species must be screened as potential gene resources for abiotic stress tolerance of cultivated wheat (*Triticum*) species.

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