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Effect of pre-chilling and environmental factors on breaking seed dormancy and germination of three foxtail species

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ABSTRACT

The effect of wet and dry pre-chilling duration, pH, osmotic stress, salt stress and planting depth on seed germination and seedling emergence of three foxtail species (*Setaria glauca*, *S. verticillata* and *S. viridis*) was investigated in a series of laboratory and greenhouse experiments. Both wet and dry pre-chilling for 45 days promoted seed germination of *S. glauca* compared with the control. Pre-chilling was not significantly effective in seed dormancy breaking of *S. viridis* and *S. verticillata*. The maximum germination of foxtails (*S. verticillata, S. viridis*) was obtained when seeds were treated with pH 7 buffer solution. Increasing of osmotic and salt stress decreased seed germination of foxtails. *Setaria verticillata* seed germination was more tolerant than those of *S. glauca* and *S. viridis* to high water stress condition. *Setaria glauca* and *S. verticillata* seed germination were more tolerant to high salinity stress than *S. viridis*. Seedling emergence decreased with increasing the burial depth and no germination observed at 8 cm soil depth.

Key words: osmotic and salt stress, seed depth burial. germination, *S. glauca*, *S. verticillata* and *S. viridis*

IZVLEČEK

UČINKI HLADNEGA PREDTRETIRANJA IN OKOLJSKIH DEJAVNIKOV NA PREKINITEV DORMANCE IN KALITEV TREH VRST MUHVIČEV

Učinki trajanja mokrega in suhega hladnega predtretiranja, pH, ozmotskega in solnega stresa ter globine setve na kalitev in vznik so bili preučevani pri treh vrstah muhviča (sivozeleni muhvič- *Setaria pumila* (Poir.) Roem. & Schult.(= *S. glauca* auct.)., vretenčasti muhvič - *S. verticillata* (L.) P. Beauv. in zeleni muhvič - *S. viridis* (L.) P. Beauv.) v rastlinjaku in laboratoriju. Mokro in suho hladno predtretiranje v trajanju 45 dni je pospešilo kalitev semen sivozelenega muhviča v primerjavi s kontrolo. Isto obravnavanje ni imelo značilnega vpliva na prekinitev dormance semen pri zelenem in vretenčastem muhviču. Največja kalitev muhvičev (*S. verticillata, S. viridis*) je bila dosežena pri semenih, tretiranih s pufersko raztopino s pH 7. Povečanje ozmotskega in solnega stresa je zmanjšalo kalitev vseh vrst muhvičev Semena vretenčastega muhviča so bila bolj odporna na večji sušni stress kot semena zelenega in sivozelenega muhviča. Semena sivozelenega in vretenčastega muhviča so bila bolj odporna na velik slanostni stress kot semena zelenega muhviča. Vznik vseh treh vrst muhvičev se je zmanjševal s povečevanjem globine setve, na globini 8 cm kalitve ni bilo.

Ključne besede: ozmotski in solni stress, globina setve, kalitev, *Setaria pumila*, *S. verticillata* and *S. viridis*

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1 INTRODUCTION

Foxtail species (*Setaria pumila* (Poir.) Roem. & Schult. = *Setaria glauca* auct., *S. verticillata* (L.) P. Beauv. and *S. viridis* (L.) P. Beauv.); are member of Poaceae family and native to the Eurasia. Foxtails are the worst weeds that are interfering with world agriculture (Holm *et al*., 1977) and are considered as problematic in many crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), potato (*Solanum tuberosum* L.) and sugarcane (*Saccharum officinarum* L.). Due to high competitive ability and reproductive potential, these species are expected to be a serious threat to summer crops and orchards in north of Iran.

Seed germination is one of the critical phases in plant life cycle (Shoab *et al.*, 2012) which is affected by dormancy and environmental factors such as light, soil pH, osmotic potential, salt stress, temperature and burial depth. The foxtails seeds are characterized by dormancy and a long period of survival in soil. *Setaria glauca*, *S. viridis* and *S. verticillata* are able to survive in soil for 13 to 39 years (Dekker, 2003).

Dormancy can be defined as an inhibition mechanism of seed germination of intact viable seed to optimize the distribution of germination over time (Bewley and Black, 1983; Hilhorst, 1995) and is one of the survival mechanisms of invasive annual weeds (Baskin *et al.* 2004). Seed dormancy is categorized as physical, physiologic, morphologic, morpho-physiologic and combined dormancy (Baskin and Baskin, 2004). For breaking dormancy and enhancing of seed germination, moist chilling or cold stratification has been widely used in different plants (Schopmeyer, 1974; AOSA, 1992; ISTA, 1999; Wang and Berjak, 2000).

Investigation of germination requirements show how a species germination process is adapted to habitat conditions and regulated by environmental factors (Van Assche *et al*., 2002). Thus, finding of some information about factors effective in seed dormancy breaking and optimal condition of germination and seedling growth are necessary for management of weeds. The aim of the present study is promotion of seed germination of foxtail species by means of moist and dry chilling and also investigation of effect of environmental factors such as soil pH, water and salt stress on seed germination and burial depth on seedling emergence.

2 MATERIALS AND METHODS

2.1 Plant materials and experimental conditions

Laboratory and greenhouse experiments were carried out at Department of Agronomy, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran during 2013.

The mature seeds of *Setaria glauca*, *S. verticillata* and *S. viridis* were collected from more than 200 plants in 2012, Qaemshahr, Iran. Seeds were cleaned and stored in paper bags in darkness at room temperature (23±2 °C) until start the experiments.

Prior start laboratory experiments, seeds were surface-sterilized by soaking in 1 % sodium hypochlorite (NaOCl) for 1 min and subsequently rinsing with distilled water. Twenty five seeds were placed in Petri dishes (8 cm diameter) lined by two sheets of filter paper (Whatman No. 1). The filter paper was moistened with 5 ml of distilled water or treatment solutions. Petri dishes were sealed with Parafilm to reduce the water loss and placed in germinator with 16/8 h (day/night) photoperiod and 25/15 °C (day/night) fluctuating temperature with a light intensity of 300 µmol m^{-2} s^{-1}. Germinated seeds with at least 2 mm long seminal root were counted after a period of four weeks. The percentage of germinated seeds was calculated using following equation: number of germinated seeds/number of total seeds × 100.

2.2 Pre-treatment by cold stratification

To verify the effectiveness of the pre-chilling treatments, moisturized seeds with distilled water and dry seeds were placed in refrigerator at temperature of 4 °C for 15, 30 and 45 days. After the pre-chilling period; seed germination was tested as in the plant materials and experimental conditions section described.

The best pre-treatment of wet or dry pre-chilling that promoted the maximum germination was considered as pre-treatment for further experiments (pH, osmotic stress, salt stress and seed burial depth).

2.3 Effect of pH on seed germination

The effect of pH on seed germination was determined by using different buffer solutions of pH 3, 5, 7, 9 and 11 prepared according to the method described by Chachalis and Reddy (2000). Seed germination tested by method described in the plant materials and experimental conditions section.

2.4 Effect of osmotic stress on seed germination

To evaluate the effect of osmotic stress, different levels of osmotic potential including 0, -0.1, -0.25, -0.5, -1 and -1.5 MPa were prepared by dissolving of 0, 99.4, 157.1, 222.2, 314.2, 384.8 g polyethylene glycol 6000 (Merck, Germany) in 1 L distilled water, respectively. Five ml of polyethylene glycol 6000 solution was added to the Petri dishes. Seed germination was tested according to the plant materials and experimental conditions section.

2.5 Effect of salt stress on seed germination

The effects of salt stress on seed germination were evaluated by soaking seeds in solution of 0, 10, 20, 40, 80, 160 and 320 mM sodium chloride (NaCl) (Merck, Germany). Germination was tested according to the plant materials and experimental conditions section.

2.6 Effect of seed burial depth on seedling emergence

Fifty seeds of foxtails were planted in soil in 16-cm-diameter plastic pots (30 cm height) at the depths of 0, 2, 5 and 8 cm in greenhouse. Soil used for experiment was a clay soil (clay, 61.4 %; silt, 7.9 %; sand, 30.7 %) with 7.49 pH and 2.58 % organic carbon. The temperature of the greenhouse was set at 28/20 °C (day/night) with a natural photoperiod. Pots were irrigated as needed to maintain soil moisture in field capacity. Seedling emergence was recorded as coleoptiles appeared. Seedling emergence counted every 7 days for 28 days.

2.7 Statistical analysis

All experiments were conducted in a complete randomized design with four replicates. Analysis of variance was performed on transformed (Arcsine transformation) data. Significant difference of means were identified by protected LSD test ($p = 0.05$) and standard error bars. The SAS program (SAS Institute Inc., Cary, NC, USA) was used for the statistical analyses.

3 RESULTS AND DISCUSSION

3.1 Effect of wet and dry pre-chilling pre-treatment on seed dormancy breaking of foxtails

Germination of foxtails seeds was significantly affected by wet and dry pre-chilling durations. Initial germination (control treatment) of *S. glauca*, *S. verticillata* and *S. viridis* was 41.54 %, 47.89 % and 53.78 %, respectively (Table 1). Both wet and dry pre-chilling for 45 days increased seed germination of *S. glauca* about 32.35 % and 48.61 % compared with the control, respectively (Table 1). In contrast, seed germination of *S. verticillata* and *S. viridis* did not significantly increase by both wet and dry pre-chilling in comparison with control (Table 1). Therefore, for continuing the experiments just *S. glauca* seeds were dry pre-chilled for 45 days prior pH, osmotic potential, salt stress and seed burial depth experiments. Positive effects of wet and dry pre-chilling on seed germination of different weed species was previously reported by Wartidiningsih *et al*. (1994) and Baskin *et al*. (1992). For Poaceae family it is well known that the duration of stratification from 3 days to 6 weeks improve seed germination (Williams 1983, Smith-Jochum and Albrecht 1988, Matus-Cádiz *et al*. 2001, Matus-Cádiz and Hucl 2003). The cold pre-chilling may change the hormonal balance of seed and increase germination through enhancement of gibberellic acid and cytokinin activity and/or the decline of abscisic acid (Copeland and McDonald, 2001). The wet pre-chilling provides enough moisture to activate the hydrolytic enzymes that make seeds ready to germinate once they were moved to the warm temperature.

Table 1: Effect of wet and dry pre-chilling duration on germination of foxtail seeds

Germination (%)					
Wet pre-chilling			Dry pre-chilling		
S. glauca	*S. verticillata*	*S. viridis*	*S. glauca*	*S. verticillata*	*S. viridis*
41.54±0.81^{c}	47.89±0.99^{a}	53.78±0.91^{a}	41.54±0.81^{b}	47.89±0.99^{a}	53.78±0.91^{a}
52.60±1.03 ^{b}	48.45±0.47^{a}	60.35±1.59^{a}	46.16±1.42^{b}	33.08±1.12^{b}	49.70±1.75^{ab}
56.83±0.64^{ab}	39.22±0.48^{b}	34.43±1.50^{b}	52.92 ±3.27^{b}	29.83±1.22^{b}	41.94±2.15^{b}
61.40±0.65^{a}	34.28±1.38^{b}	57.00±1.50^{a}	80.83±2.68 ^{a}	19.53±1.70^{c}	59.22±2.19^{b}

Notes: Mean±Se; Values in each column followed by different superscripted letters indicate significant differences ($p < 0.05$).

3.2 Effect of pH on seed germination of foxtails

Seed germination of foxtails was significantly influenced by pH solution. Approximately, 40 % of seeds of *S. glauca* germinated at pH 3, 5 and 7 (Fig. 1a). The maximum seed germination of *S. verticillata* and *S. viridis* occurred at pH 7 (Fig. 1b and 1c). The pH 9 and 11 had an inhibitory effect on both *S. glauca* and *S. verticillata* seed germination (Fig. 1a, 1b) However, about 25 % of *S. viridis* seeds germinated at pH 9 (Fig. 1c). Inhibition of germination at pH 9 and 11 shows that soil pH is a limiting factor for seed germination. Wilson (1979) and Singh and Achhireddy (1984) indicated that a narrow range of pH from 6 to 7 was needed for germination of canadian thistle (*Cirsium arvense* L.) and strangler vine (*Morrenia odorata* Lindl.). Chachalis and Reddy (2000) showed that trumpet creeper (*Campsis radicans* L.) germination was limited by extreme pH range (pH 4 or 10). In contrast, Chachalis et al. (2008), Fani Yazdi et al. (2013) and Rezvani and Fani Yazdi (2013) reported that seed germination of venice mallow (*Hibiscus trionum* L. HIBTR), sheep sorrel (*Rumex acetosella* L.) and black nightshade (*Solanum nigrum* L.) occurred over a wide range of pH, that shows pH should not be a limiting factor for germination of some plants.

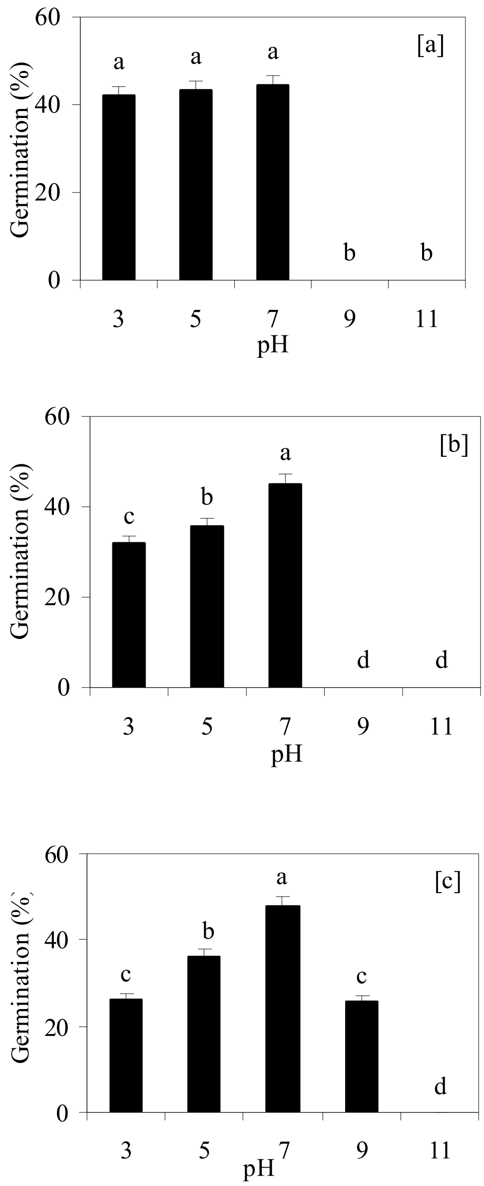

Figure 1: Effect of pH on germination of foxtails seed. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means.

3.3 Effect of osmotic stress on seed germination of foxtails

Osmotic stress significantly affected seed germination of *Setaria glauca, S. verticillata* and *S. viridis*. With increasing osmotic stress a decline in seed germination of foxtails was recorded (Fig. 2a, 2b and 2c). At osmotic stress -0.5 MPa, *S. verticillata* seed germination was higher than those of *S. glauca* and *S. viridis* (Fig. 2a, 2b and 2c). Seed germination of *S. verticillata* was more tolerant to high water stress than *S. glauca* and *S. viridis*. About 40.35 % of *S. verticillata* seeds germinated at -0.5 MPa (Fig. 2b). Seed germination of *S. glauca* and *S. viridis* was 33.05 % and 29.09 % at -0.5 MPa osmotic potential, respectively (Fig. 2a and 2c). Germination of foxtail seeds was inhibited when seeds were exposed to osmotic potential lower than -0.5 MPa. These results show that foxtails seeds are sensitive to high osmotic stress and germination is favored by a moist environment. Therefore, low moisture of soil from mid spring up to summer could be a limiting factor of emergence of foxtails in the north of Iran. Manthey and

Nalewaja (1987) showed that germination of *S. glauca* and *S. viridis* was reduced by water stress and *S. viridis* germinated more rapidly than *S. glauca*.

Germination of horseweed (*Conyza canadensis* (L.) Cronquist) decreased as osmotic potential increased from 0 (distilled water) to -0.8 MPa (Nandula *et al.,* 2006). Our results are in agreement with the work of Shaw *et al.* (1991) on redvine (*Bunnichia* sp.) and Reddy and Singh (1992) on hairy beggarticks (*Bidens pilosa* L.). Low osmotic potential was found to inhibit germination of trumpet creeper (Chachalis and Reddy, 2000), texasweed (*Caperonia palustris* (L.) A. St. Hil.) (Koger *et al*., 2004) and cadillo (*Urena lobata*) (Wang *et al*., 2009). Rezvani and Fani Yazdi (2013) and Fani Yazdi *et al.* (2013) reported that low water stress significantly reduced the germination of both black nightshade and sheep sorrel.

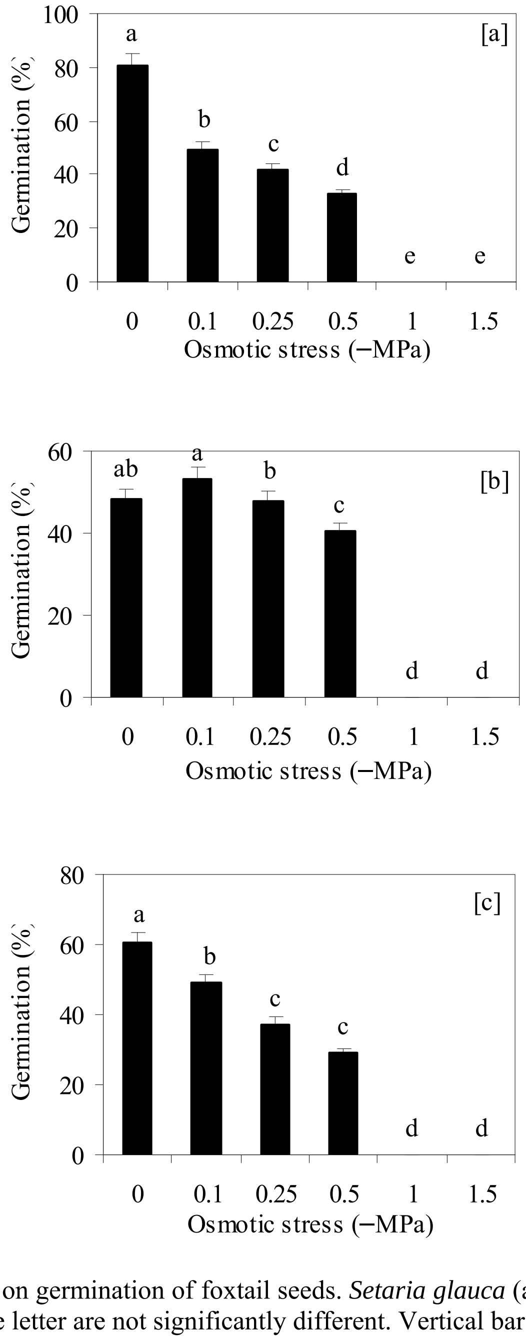

Figure 2: Effect of osmotic stress on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

3.4 Effect of salt stress on seed germination of foxtails

The germination of *S. glauca* seeds was not significantly differed among treatments in the range of 10 to 80 mM NaCl concentration. When seeds were treated with 320 mM NaCl, seed germination was inhibited (Fig. 3a). Similarly, seed germination of *S. verticillata* was not significantly differed in comparison with the control as salt stress increased from 10 to 80 mM NaCl. No germination occurred as seeds were treated with 320 mM NaCl (Fig. 3b). The germination of *S. viridis* decreased significantly as salt concentrations increased and no seed germination was observed at 160 mM NaCl (Fig. 3c). These data show that foxtails seeds were fairly tolerant to salt stress. Zia and Khan (2004), Koger *et al*. (2004), Nandula *et al*. (2006) and Lu *et al*. (2006) also reported negative effect of salt stress on seed germination of different plants. Rao *et al*. (2008) showed that germination of American sloughgrass was inhibited at 300 mM NaCl concentration.

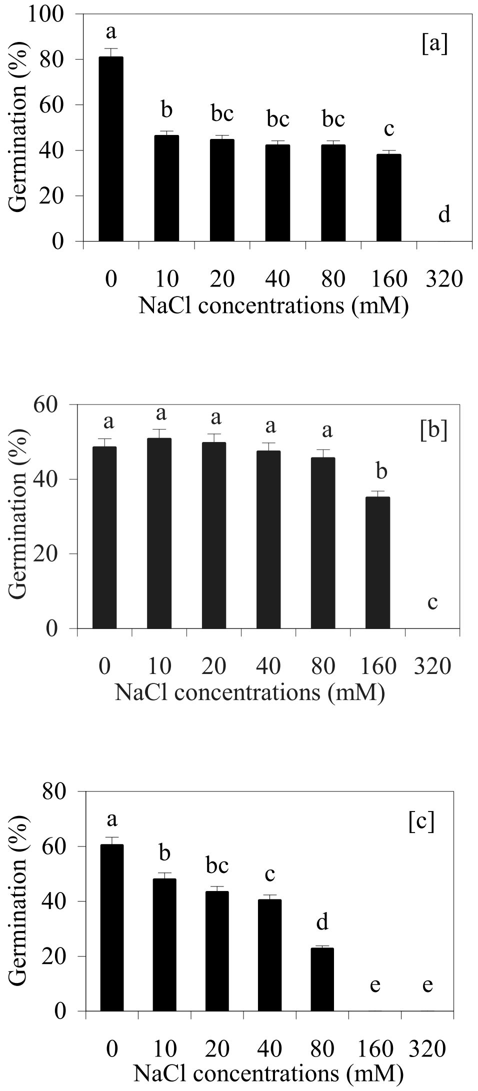

Figure 3: Effect of NaCl concentration on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

3.5 Effect of seed burial depth on seedling emergence of foxtails

Seed burial depth markedly influenced on seedling emergence of *Setaria glauca, S. verticillata* and *S. viridis*. The maximum seedling emergence of foxtails was found where seeds were sown on the soil surface. The lowest seedling emergence was obtained where planting depth was 8 cm (Fig. 4a, 4b, 4c). Decreasing in seedling emergence due to increasing burial depth could be linked primarily to seed energy reserves (Mennan and Ngouajio, 2006). Seeds of bigger size have higher energy reserve than those of smaller seed size; therefore can emerge from higher depth. Chauhan *et al.* (2006) indicated that light and seed size generally limit seedling emergence from deep in the soil. Also, low emergence from bigger depths because of the lack of oxygen diffusion and the presence of CO_2 deriving from soil biological activity has been reported by Benvenuti and Macchia (1995).

Decreased emergence by increasing in planting depth has been reported in several weed species, including hairy beggarticks (Reddy and Singh, 1992), horse purslane (*Trianthema portulacastrum* L.) (Balyan and Bhan, 1986), stranglervine (Singh and Achhireddy, 1984), and horseweed (Nandula *et al.*, 2006).

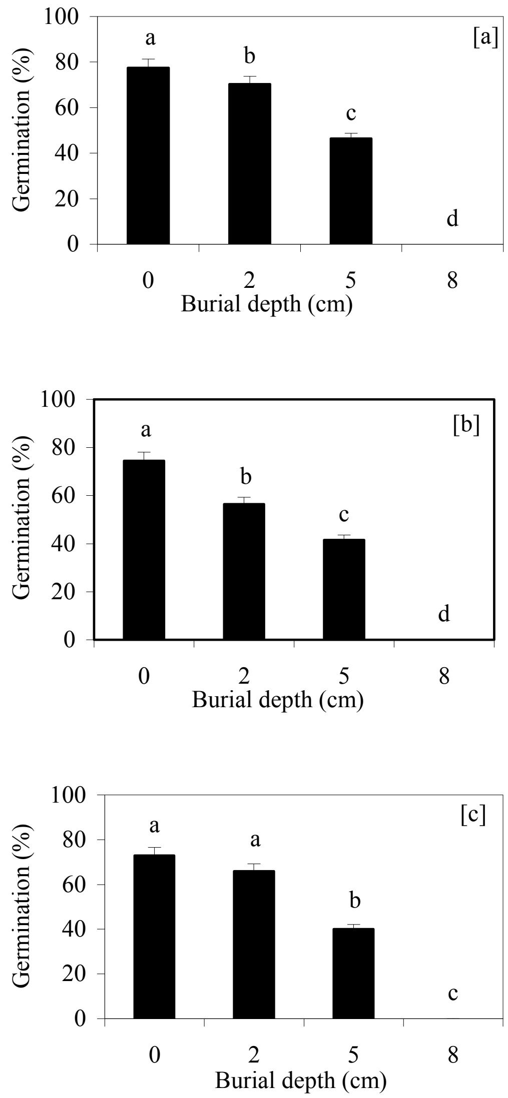

Figure 4: Effect of burial depth on germination of foxtail seeds. *Setaria glauca* (a), *S. verticillata* (b) and *S. viridis* (c). The columns with the same letter are not significantly different. Vertical bars are standard error of the means

4 CONCLUSIONS

Seed dormancy of foxtails species showed different responses to pre-chilling treatments. However, dry pre-chilling was only significantly effective in seed dormancy breaking of *S. glauca*. Moreover, pH, salt and osmotic stress and seed planting depth significantly altered seed germination of foxtails. The results of the experiments could be effective in understanding the distribution of foxtails in agricultural ecosystems in the future. High seedling emergence of these species on the soil surface could be suggesting light requirements. Therefore, spreading of foxtails would be higher in fields with no-tillage or minimum-tillage practices. Our results suggest that increasing of tillage depth could be an effective weed management strategy to reduce seedling emergence of foxtails.

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