

## ORIGINAL ARTICLE

### Effect of Different Ethanol Concentrations on Seed Germination of Three Turfgrass Genera

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M.R. Salehi, F. Ashiri and H. Salehi,: Effect of Different Ethanol Concentrations on Seed Germination of Three Turfgrass Genera, *Adv. in Nat. Appl. Sci.*, 2(1): 6-9, 2008

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#### ABSTRACT

This investigation was conducted in two separate experiments. The first experiment was conducted to study the effect of different ethanol concentrations on seed germination of three turfgrass genera. In this experiment, effects of ethanol with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2 and 3% (v:v) along with control (distilled water) treatments on seed germination of three turfgrass genera namely: *Cynodon dactylon* [L.] Pers., *Festuca arundinacea* Schreb. and *Lolium perenne* L. were investigated. The second experiment was conducted to study the effect of different soaking times (4, 8, 12, 16, 20 and 24 h along with control treatment without soaking) of seed in 10% (v:v) ethanol on seed germination of the seeds used. Experiments were conducted in a complete randomized design with factorial arrangement, four replications in each treatment and continued for 15 days. Results showed that in *Cynodon*, germination percentage was increased with higher concentrations of ethanol up to 3% (v:v). However, the same concentration manner had negative effect on *Lolium* seed germination percentage. Soaking the seeds of both genera used in 10% ethanol did inhibit the germination percentage.

**Key words:** *Cynodon*, Ethanol, *Festuca*, Germination percentage, *Lolium*, Seed

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#### Introduction

Worldwide, the cultivated area under grassland is estimated to be twice that under crops. Grasses are important for grazing, stabilization of soil for agricultural purposes and improvement of environmental quality through multiple uses, such as forage, conservation and turf (Forster and Spangenberg, 1999). Turfgrasses appear as green masses of vegetation composed of individual herbaceous plants. These plants have distinct characteristics, such as low growth habit, prostrate creeping tendency, high shoot density and coarse-to-fine leaf texture (Beard, 1973, Alderson and Sharp, 1994). Less than 50 grass species are used as turf because of their ability of persist under regular mowing (Christians, 2004). The turfgrasses industry has undergone rapid growth in its attempts to meet the public's increasing demands for products and services (Turgeon, 2002). Among turfgrass genera, common bermudagrass (*Cynodon dactylon* [L.] Pers.), tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) are perennial species widely used in landscape. Common bermudagrass, a member of poaceae family, eragrostioideae sub-family and chlorideae tribe is a native of eastern Africa (beard, 1973) and was introduced into the United States in the mid 1700s (Hanson et al., 1969). It is widely used on lawns, roadsides, parks, school grounds, athletic fields, golf courses, and other areas where a close-mown, dense turf is desired (Christians, 2004). Bermudagrass is sensitive to cool temperature and will stop growing, lose its chlorophyll, and take on a brown-tan color when soil temperature fall below 10°C. The plant remains in this winter-dormant condition until soil temperatures at the 4-in. depth rise and remain above 10°C. Root and rhizome growth will substantially increase when soil temperature reach 15 to 20°C, with optimum growth occurring in soil temperatures of 24 to 29°C (Christians and Engelke, 1994). The fescue

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includes approximately 450 species that vary greatly in appearance (Clayton and Renvoize, 1986). One of the fescues is tall fescue that is known for tolerance of wear, heat and drought. It is also fairly well-adapted to shaded conditions. The ryegrasses are group of eight species in the genus *Lolium* and they are closely related to the grasses in the genus *Festuca*. Perennial ryegrass is known for its rapid germination and establishment. It is useful for quickly reestablishing damaged area on lawns, athletic fields, and golf courses. The rate at which a new turf becomes established is influenced by specific procedures used during propagation (Turgeon, 2002).

Direct seedling is the least expensive and most efficient method for revegetation large parcels of land with herbaceous species such grasses (Trask and Pyke, 1998). Different turfgrass species vary substantially in the normal percentage of viable seed contained in a seed lot (Simpson, 1990, Turgeon, 2002). Previously, germination characteristics of some perennial grasses have been studied (Young *et al.*, 1977, Beckman *et al.*, 1998, Tigabu and Oden, 2001, Salehi and Khosh-Khui, 2005). The present investigation was undertaken to study the effect of different ethanol concentrations on seed germination of three turfgrass genera and effect of different soaking times of seed in ethanol on seed germination of them. The findings can be used in tissue culture and biotechnological and genetic transformation studies, where the highest germination percentage is needed.

## Materials and methods

In the present investigation three turfgrass genera, common bermudagrass, tall fescue and perennial ryegrass were used. The investigation was conducted in two separate experiments.

### *Experiment 1: different ethanol concentration treatments*

In the first experiment, to study effects of different ethanol concentrations on seed germination, concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2 and 3% ethanol (v:v) along with control (0%) were used. The solutions were made using distilled water. Then Whatman no. 1 filter papers were sterilized in an oven at temperature of 72°C for 48h and placed in 2 parted petri dishes (9 cm diam.). Hundred seeds were placed in one part supplemented with 1 ml of solution and 3 ml of the same solution was added to the other part. For prevention of solution evaporation, petri dishes were sealed with parafilm and placed in a phytotron at 25± 2°C temperature with a 16h/8h light/dark photoperiod.

### *Experiment 2: different soaking times with 10% (v:v) ethanol*

In the second experiment, concentration of 10% ethanol (v:v) was made with distilled water. Then, seed were soaked in ethanol with different durations of 4, 8, 12, 16, 20 and 24 h along with control treatment (without soaking). Then, the seeds were air-dried for 1 h. Hundred seeds were placed in the same petri dishes as above and daily wetted with 3 ml distilled water. Petri dishes were placed in the phytotron with the same conditions as experiment 1.

In both experiments, four replications of 100 seeds were used for each treatment. They were monitored every day and moistened with distilled water when needed. Seeds were considered germinated when the radicles were about 3mm long and discarded after counting. Germination tests were continued up to 20 days. In each genus and treatment, the final germination date was the time in which seed germination percentage (GP) was completed, or when the seeds failed to germinated in three previous days.

### *Data recording and statistical analysis*

The number of germinated seeds was daily recorded in all the experiments. Final GPs were calculated for each trial. Experiments were conducted as complete randomized design with factorial arranging. Data were analyzed using MSTAT-C program and the mean comparisons were made following Tukey's test at P= 0.01.

## Results and discussion

The results of experiment 1 showed that germination percentage was increased with increasing ethanol concentration up to 0.5% and 0.3% for *Lolium* and *Festuca*, respectively. However, in both genera with increasing ethanol concentration up to 3% germination percentage was decreased. In *Cynodon* with increasing ethanol concentration up to 3%, germination percentage was increased (Table 1). In one experiment on effect of ethanol on *Erythrina caffra* Thunb. seed germination (Chris *et al.*, 1989), it was shown that low

**Table 1:** The effect of different ethanol concentrations on seed germination of three turfgrass genera.

Ethanol concentration (%)	Seeds germination percentage		
	Turfgrass genera		
	<i>Cynodon</i>	<i>Lolium</i>	<i>Festuca</i>
0	17.50d <sup>†</sup>	89.50a	87.00a
0.1	13.00e	90.50a	88.00a
0.2	18.75de	93.50a	88.00ab
0.3	27.25cde	92.00a	86.50ab
0.4	29.25cde	89.25a	86.00ab
0.5	33.5bcd	88.00a	79.00abc
0.6	38.75bc	85.25ad	71.25abc
0.7	37.75bc	82.75abc	65.50abc
0.8	44.75abc	70.00bc	61.00abc
0.9	49.25ab	60.50c	55.00abc
1	50.75ab	55.25c	49.75cd
2	61.25a	3.25d	10.75d
3	63.00a	1.00d	4.00d

<sup>†</sup>In each column, means followed by the same letters are not significantly different according to Tukey's test at 1% level.

**Table 2:** Effect of different seed soaking times in 10% (v:v) ethanol on seed germination of three turfgrass genera.

Ethanol soaking times (hr)	Seeds germination percentage		
	Turfgrass genera		
	<i>Cynodon</i>	<i>Lolium</i>	<i>Festuca</i>
0	17.50a <sup>†</sup>	89.5a	87.00a
4	5.00b	5.75b	6.00b
8	6.00b	4.25b	5.25b
12	5.00b	2.50b	4.00b
16	6.25b	1.50b	2.50b
20	5.50b	0.25b	0.00b
24	4.25b	0.00b	0.00b

<sup>†</sup>In each column, means followed by the same letters are not significantly different according to Tukey's test at 1% level.

concentration of ethanol increased seed germination but higher concentrations inhibited that. In other experiments acetone application increased seed germination (Crozier *et al.*, 1972, Newell *et al.*, 1996). Effect of ethanol on increasing seed germination of *Cynodon* may be resulted from breakdown of carbohydrate reserves or affecting the endogenous plant hormones level (Vivian *et al.*, 1976). Inhibition of *Lolium* and *Festuca* seed germination in higher ethanol concentrations may cause embryo damage or death.

The results of experiment 2 showed that in *Cynodon*, *Lolium* and *Festuca* soaking seeds in 10% ethanol for 4 hr and more inhibited seed germination compared to control treatment. This result showed the effect of high concentration of ethanol on embryo death (Table 2).

### Conclusions

Based on the present investigation the treatments of 3% and 0.1-0.2% ethanol resulted in the highest germination percentage in *Cynodon*, *Festuca* and *Lolium*, respectively. Exposure to ethanol time longer than 4 hr lowered the seed germination percentage. These findings can be used in tissue culture and genetic engineering studies, where the highest germination percentage is needed.

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