Sensitivity of Seed Germination to Salt Stress in Teff [*Eragrostis tef* (Zucc.) Trotter]

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BulletinUASVM Horticulture 76(1) / 2019
Print ISSN 1843-5254, Electronic ISSN 1843-5394
DOI:10.15835/buasvmcn-hort: 2019.0004

Abstract

Teff is an annual grass originated in Ethiopia, grown for both human food and animal feed. Salinity and moisture stress are the main limiting factors of agricultural development on arid and semi-arid regions. The aim of this study was to investigate the effect of salinity due to NaCl on germination of teff. A set of experiments was conducted under a completely randomized design with four replications of 200 seeds at two different suboptimal germination temperatures (15 and 25 °C) in the dark for 10 days. In order to create salt stress conditions during the germination process, seven solutions were used (0 as control, 80, 160, 240 and 320, 400 and 480 mM NaCl). The germination performance was evaluated by final germination percentage and mean germination time. The presence of NaCl reduced germination, especially above 240 mM for 15 °C and 400 mM for 25 °C. The mean germination time increased with higher NaCl levels.

Keywords: germination percentage, germination time, NaCl, salinity, teff

Introduction

Climate change is the greatest environmental threat facing humanity worldwide. Areas of South-East Europe and the Mediterranean basin are expected to be among the most vulnerable countries to climate change. Furthermore, in the semi-arid regions of the Mediterranean region water and salinity stresses are increasingly becoming the primary limiting environmental conditions which restrict successful establishment of crops. As reported by Daliakopoulos *et al.* (2016), soil salinization in the Mediterranean region affects 25% of irrigated agricultural land at a significant level and about 9% of the 1.4 Mha of irrigated land in Greece is affected by soil salinization due to seawater intrusion. The establishment of new crops with better performance under stress conditions could be an alternative solution for modern farmers and an opportunity to cultivate innovative crops for alternative uses (Bilalis et al., 2017).

Teff [*Eragrostis tef* (Zucc.) Trotter] is an annual C4 grass crop that originated in Ethiopia and has been introduced to other countries such as India, Africa, Western Europe, Australia and the U.S.A. mainly as a forage crop (Bedane *et al.*, 2015). It is grown for both human food and animal feed with an attractive nutritional profile, being high in dietary fibre, iron, calcium and carbohydrate, bearing also high levels of phosphorus, copper, aluminium, barium and thiamine (Hager *et al.*, 2012). It has an excellent balance of amino acid composition (including all 8 essential amino acids for humans) making it an excellent material for malting and brewing (Gebremariam *et al.*, 2014). It is also considered to be a healthy food since its grain is free of gluten, making it suitable for people suffering from celiac disease (Assefa *et al.,* 2015). It grows in a wide range of environmental conditions and is more tolerant to drought, water logging and disease in comparison with other common cereals (Yihun *et al.*, 2013).

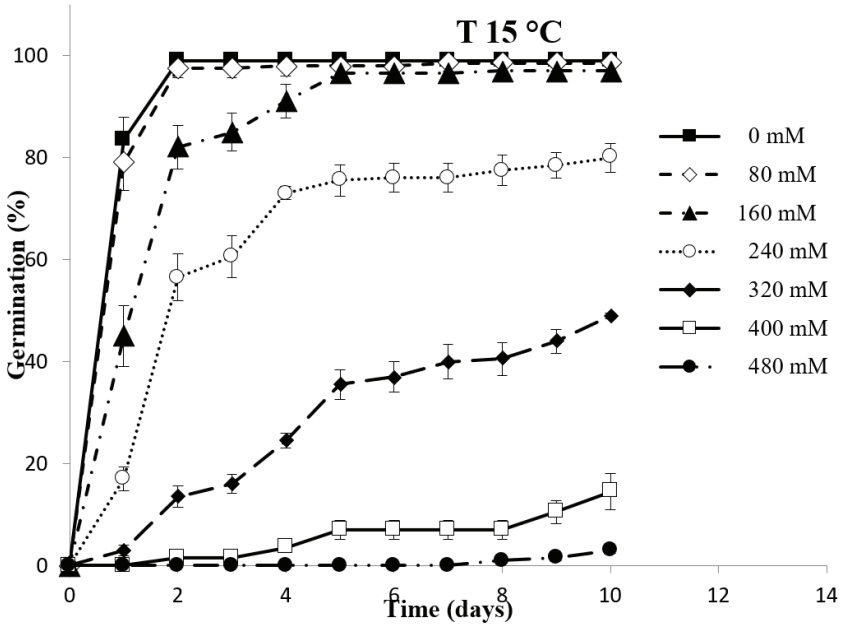

Figure 1. Cumulative germination of teff seeds exposed to NaCl solutions at 15 °C. Vertical bars represent the standard error.

Germination of high-quality seeds may be delayed or prevented by various abiotic stresses (Ashraf and Foolad, 2005). Seed germination and seedling growth may be significantly reduced and delayed by the decreasing rate of water absorption, when saline water is used for irrigation or when soil salinity is high. Under these conditions, salt stress may also cause excessive uptake of ions (Murillo-Amador *et al.*, 2002). There is evidence that teff shows moderate tolerance to salinity stress (Kebebew and McNeilly, 1995). However, relative data are rather inadequate (Mamo *et al.*, 1996; Abusuwar and Abbaker, 2009; Asfaw and Itanna, 2009).

The aim of the present study was to investigate the effect of salinity due to NaCl on germination of teff seeds.

Materials and Methods

Seeds of teff were surface sterilized in a 1% sodium hypochlorite solution, rinsed in distilled water and dried before the experiment (4.4% seed moisture). A set of experiments was conducted under a completely randomized design with four replications. Seeds were germinated at two constant temperatures 15 °C and 25 °C and maintained in a thermostatically controlled incubator (±1 °C) for 10 days. Samples of 800 seeds (four replicates of 200 seeds each) were placed in covered 9-cm Petri dishes between two Whatman No. 1 filter papers moistened with 7 mL of NaCl solutions. Salt solutions were used in germination tests to imbibe seeds. Seven solutions were prepared, dissolving different concentrations of sodium chloride (NaCl) in distilled water (0 as control, 80, 160, 240, 320, 400 and 480 mM). Petri dishes were hermetically sealed with parafilm to prevent evaporation and then randomized in an incubator in the dark. The seeds were kept in the dishes to assess germination. This was scored when the radicle approached approximately 2 mm length and data were collected daily until no additional germination had occurred for 48 h.

At the end of the tests the final percentage of germination was calculated. Using the daily counts, the mean germination time (MGT) was calculated for each lot using the formula cited by Ellis and Roberts (1980) given below, $MGT = \sum n.D / \sum n$ where n= number of seeds newly germinated at time D; D= days from the beginning of the germination test, $\sum n$= final germination. Data of the final germination percentage (arcsine transformed) and mean germination time were statistically analyzed by a completely randomized two-way analysis of variance (ANOVA) using the StatSoft software (1999). The analysis of variance was conducted considering osmotic potential and temperatures as fixed factors. When 'F' ratios were significant, means were separated by the Student-Newman-Keuls (SNK) test at ≤ 0.05.

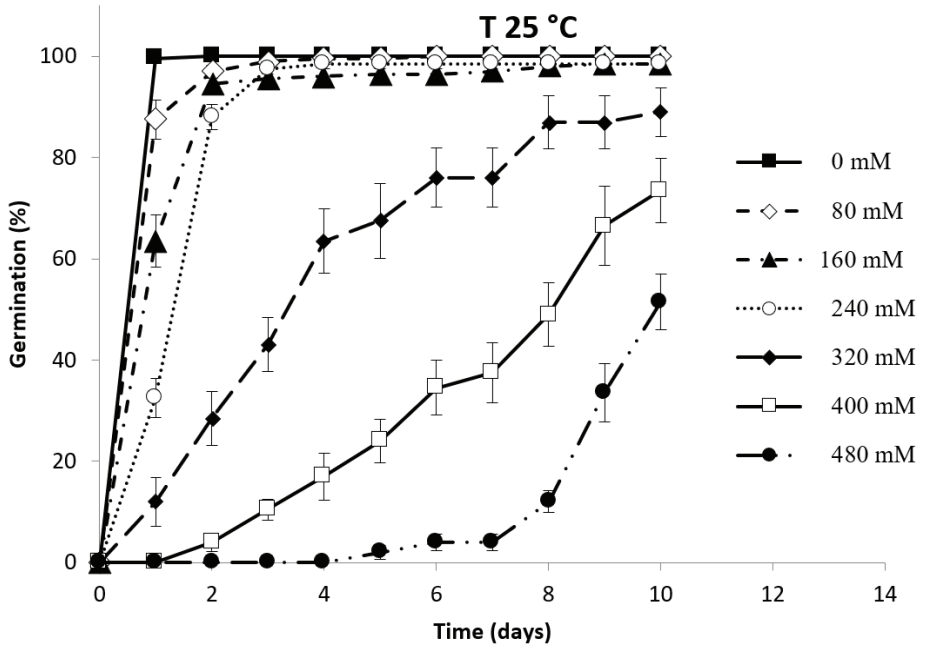

Figure 2. Cumulative germination of teff seeds exposed to NaCl solutions at 25 °C. Vertical bars represent the standard error.

Results and Discussion

The cumulative seed germination time courses during imbibition at different concentrations in NaCl solution are shown in Figures 1 and 2. Final germination percentage of the controls was 99% and 100% at 15 °C and 25 °C, respectively. Comparable results were reported by Vecchio et al. (1996) who found that germination percentage of teff seeds starting from 15°C was above 90%. Several authors have reported that salinity stress affects seed germination either by decreasing the rate of water uptake (osmotic effect) and/or by facilitating the intake of ions (ion toxicity), which may change enzymatic or hormonal activities inside the seed (Murillo-Amador *et al.*, 2002; Ashraf and Foolad, 2005). In general, seeds from the controls showed a higher rate of final germination percentage and a lower mean germination time compared to seeds from the salt treatments. The effect of salinity on seed germination percentage and mean germination time was significant at $p<0.001$ (Tab. 1). The presence of NaCl regardless of temperature reduced germination. This reduction was more severe at 15 °C (49%, 15% and 3% germination in case of 320, 400, and 480 mM concentration respectively) than at 25 °C (74% and 52% at 400 mM and 480 mM salinity level) (Figs. 1 and 2, Tab. 1). Mean germination time ranged from 1 to 2 days at lower salt concentration. Excessive salinity caused a significant delay in mean germination time (7 and 9 days at 400 mM and 480 mM concentration, respectively) regardless of temperature (Tab. 1). Similar effects were observed in seed germination, seedling emergence and seedling growth of teff in the laboratory and the field (Kebebew and McNeilly 1995; Mamo *et al.*, 1996).

Moreover, our results were in agreement with the findings of Abusuwar and Abbaker (2009), Asfaw and Itanna (2009) and Mamo *et al.* (1996) who found that high levels of salinity can significantly inhibit seed germination in teff. In particular, increasing of salinity from 4 to 8 dS m^{-1} reduced the seed germination percentage and delayed germination time of various teff genotypes and no germination occurred in the highest salinity stress of 16 dS m^{-1}. Saline soil causes physiological and metabolic disorders in seed germination, morphological characteristics of plants, affecting development, growth, yield, and quality of plants (Gorham, 1990; Asfaw and Itanna 2011). Salinity sensitivity of teff varied in different growth stages, from germination to harvest (Mamo *et al.*, 1996). According to this experiment, varieties that showed the highest mean germination percentage and relative seedling shoot dry weight also had the highest mean relative shoot dry weight at the late vegetative stage and others that had a slow

Table 1. Results from an analysis of variance showing the effects of NaCl solutions on final germination percentage and mean germination time of teff seeds germinated at 15 °C and 25 °C.

	Germination (%)		Mean germination time (days)	
C (mM)	15 °C	25 °C	15 °C	25 °C
0	99.0 a	100 a	1.16 a	1.00 a
80	98.5 a	100 a	1.23 a	1.18 a
160	97.0 ab	98.5 a	1.89 a	1.52 a
240	80.0 b	98.5 a	2.62 b	1.79 a
320	49.0 c	89.0 a	4.83 c	3.98 b
400	14.5 d	73.5 b	6.81 d	6.70 c
480	3.0 e	51.5 c	8.88 e	8.94 d
Average	63 A	87.3 B	3.92 A	3.59 B
Significance				
C	***		***	
T	***		**	
C x T	***		ns	

C, salt concentrations, T, temperatures. ns, not significant; significant at *p < 0.05, **p < 0.01, ***p < 0.001. Percentage data were transformed before analysis; actual values (means of the main factors) are shown. Values belonging to the same characteristic with different lower-case letters within a column indicate significant differences between osmotic potential mean values and different upper-case letters within a row indicate significant differences between the means of temperatures tested according to Student-Newman-Keuls test at p < 0.05.

start at germination and seedling stages exhibited the highest mean relative root dry weight at a later stage. These results confirm that whether the degree of emergence and seedling vigour was positively correlated with relative dry matter yield at maturity depended on the varieties. This observation supported the general belief that salt tolerance is not a constant trait over the plant's life cycle but varies with the environment and growth stage of the plant.

The overall results of this experiment showed the non-inhibitory effects of salt stress on seed germination parameters of teff in lower salt concentration. The lowering of the temperature makes the seeds more sensitive to salinity and at 15 °C seed germination below 320 mM declines rapidly.

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