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Effects of Alkaline Salts and Acid Concentrations in *Tragopogon latifolius*

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Özet: Dünya çapında küresel kıtlık yayılış gösterirken gıda ya da ilaç olarak tüketilen yabani bitkilerin çevresel stres koşulları altında fizyolojik ve biyokimyasal özelliklerinin belirlenmesi önemlidir. Bu sebeple alkaline tuz ve asit konsantrasyonlarının yenilebilir yabani bir bitki olan *Tragopogon latifolius'*un çimlenme, süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktiviteleri üzerine etkileri araştırılmıştır. Alkaline tuzlarının (KNO_3 ve NaCl) ve asid (H_2SO_4) konsantrasyonlarının çimlenmeyi uyardığı, ancak kontrolle karşılaştırıldığında SOD ve CAT enzim aktivitelerini etkilemediği belirlenmiştir. H_2SO_4 uygulaması ile çimlenme gerçekleşmiş olsa da 17 gün sonra çimlenme kesintiye uğramıştır. Testlerden elde edilen sonuçlar stres koşulları altında *Tragopogon latifolius*'un bazı ekofizyolojik özelliklerine ışık tutmaktadır.

Anahtar kelimeler: Alkalin tuzlar, Asit konsantrasyonlar, CAT, Çimlenme, SOD, *Tragopogon latifolius*

Alkaline Tuz ve Asit Konsantrasyonlarının (Tragopogon latifolius) Üzerine Etkisi

Abstract: When global famine spreads throughout the world, it is important to determine the physiological and biochemical properties of wild plants, which are consumed as food, under environmental stress conditions. Herewith the effects of alkaline salt and acid concentrations on germination, superoxide dismutase (SOD) and catalase (CAT) enzyme activities of *Tragopogon latifolius*, a wild edible plant, were investigated. It was determined that alkaline salts (KNO_3 and NaCl) and acid (H_2SO_4) concentrations induced germination, but did not affect the activity of SOD and CAT enzyme when compared to control. Although germination has occurred with application of H_2SO_4, it has stopped after 17 days. *Tragopogon latifolius* was not affected by the application of alkaline salts, but acidic concentrations led to interruption of germination. The data obtained from tests shed light on several eco-physiological characteristics of *Tragopogon latifolius* under stress conditions.

Key words: Acid concentrations, Alkaline salts, CAT, Germination, SOD, *Tragopogon latifolius*

Introduction

There are about 800.000 species of plant in worldwide and approximately 10000 species in Turkey. 3000 species are cultivated for nutrition purpose. However, the number of wild plants consumed as food is over 10,000 (Savran et al. 2002). Anatolia is one of the most important regions in the world in the terms of richness of vegetation, and many wild plants maintain the existence in this region; these plants are usually consumed as fresh salads or food (Demir 2006). Plants are important sources of food, pharmaceutics and medicine for humanity because of widely availability (Akcin 2007; Güneş and Özhatay 2011).

Tragopogon L. (Asteraceae) comprises from approximately 150 species native to Eurasia (Qureshi et al. 2008). *Tragopogon latifolius* is one of the edible wild plants, and is consumed as salad (Yucel et al. 2010; Simsek et al. 2002) or is cooked with oil and egg (Akcin 2007). It is locally known as Yemlik (Yucel et al. 2012). Chemical structure, amounts of active ingredients, and features should be determined for effective and sufficient usage while using plants (Baytop 1984; Yapıcı et al. 2009). Salinity and acidity are environmental stresses in soils. Therefore, understanding the mechanisms of plant's tolerance to salinity and acidity has gained importance (Yücel et al. 2008). Abiotic stresses such as drought and salinity cause a series of physiological and biochemical changes that adversely affect plant life. Different

abiotic stresses such as salt, drought, heat and oxidative stresses are accompanied by the formation of ROS (Reactive Oxygen Species), which leads death (Gill ve Tuteja 2010; Abbaspour 2012). Plants have developed several anti-oxidation strategies to minimize these toxic compounds (Sevengor et al 2011). Antioxidants (ROS scavengers) include enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Wang et al. 2003; Abbaspour 2012). The wild plants which are widely used as food in Turkey are especially preferred by people living in rural areas. Also, the increasing consciousness about the health in recent years, the insufficiency of synthetic medicines to increasing diseases, and their adverse effects detected led the trend of using of natural products to increase (Yücel et al. 2012).

Since the natural plants have become an increasingly popular treatment option, cultivation of wild edible plants become more important. Recently, cultivation of some important medicinal and aromatic plants has started in Turkey. However, biochemical and physiological properties of many edible plants when exposed to environmental stress conditions remain unknown. The aim of this study is to ascertain the seed germination characteristics and biochemical activities of *Tragopogon latifolius*, an edible and medicinal plant, and to investigate the effects of different concentrations of KNO_3, NaCl and H_2SO_4, on its seed germination and enzyme activities. There is no adequate information about the germination of *Tragopogon latifolius*.

Material and Methods

Tragopogon latifolius has been collected in and around Eskisehir, and were identified and placed in the Herbarium of the Department of Biology Faculty of Science, Anadolu University. Its seeds were collected when plants were fresh. Each germination experiment comprised experimental series of 4x100 seeds (4x100 seeds mean that each treatment is replicated four times). In climate chamber, filter-paper was used, a constant temperature (+25ºC) was maintained throughout the experiment, and the photoperiod of 8 hours light and 16 hours darkness was implemented (Yücel and Yılmaz, 2009). Four main treatment series and control group were tested (potassium-nitrate (KNO_3), salt (NaCl), sulfuric acid (H_2SO_4). In each series, seeds were treated with 1 M, 2 M and 3 M concentrations of KNO_3, NaCl and H_2SO_4. They were treated with the salt, nitrate and acid for 17 days. Pure distilled water was used in control group. It was agreed that seeds were germinated when both the plumule, and radicle were extended more than 2 mm from their junctions. Germination tests were performed with four replicates in a Petri dish (9cm diameter lined with two discs of filter paper). Seeds were considered to be germinated when the radicle touched the filter paper.

Seedlings were homogenized in extraction buffer, 50 mM sodium phosphate buffer (pH 7.6) containing 10 mM EDTA and 10% (w/v) PVPP. Homogenates were centrifuged at 12000 g for 15 minutes. The samples were collected for protein estimation after the implementation of Bradford method (1976) using BSA as standard. Superoxide dismutase (SOD) enzyme activity was measured by using a SOD Assay Kit (Cayman, 706002) according to the manufacturer's instructions. Each treatment was replicated three times. Catalase (CAT) enzyme activity was determined spectrophotometrically as a decrease in absorbance at 240 nm for 1 minute after the decomposition of H_2O_2. The 1 mL of reaction mixture contained 50 mM phosphate buffer (pH 7.0), 100 mM H_2O_2 and 20-60 mL of enzyme was extracted at 25°C. The activity was calculated using the extinction coefficient ($40mM^{-1}cm^{-1}$) for H_2O_2 (Chance and Maehly 1955). Each treatment was replicated three times. All the data from the experiment were statistically analyzed using the Anova; and all statistical significance was set at the level of $P<0.05$. Duncan test was used for determination of significance level.

Results

Germination of seeds was induced by treatments of NaCl, KNO_3 and H_2SO_4 when compared to control group (Table 1). Three homogenous groups arose and these groups were statistically different ($P < 0.05$) (Table 2).

There was no statistically important difference between treatments and control for SOD enzyme activities (Table 3). When H_2SO_4 was applied, germination occurred but then was interrupted. At the end of 17 days, no statistical analysis could be conducted because appropriate amount of samples couldn't be collected from H_2SO_4 implemented groups for measurement of SOD and CAT activity measurements.

Although there is no statistical difference between the treatment groups, KNO_3 and NaCl caused a slightly induction of SOD enzyme activities, especially 1M NaCl (Table 4).

Table 1. The results of analysis of variance according to the percentage of germination

ANOVA

Germination Percentage (%)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	739,525	9	82,169	2,882	0,014
Within Groups	855,250	30	28,508		
Total	1594,775	39			

Table 2. Germination percentage of seeds with treatment of KNO_3, NaCl and H_2SO_4 (Values followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test)

Groups	**N**	**Germination percentage (%)**
Control	4	77.75^{a}
1M KNO_3	4	91.50^{c}
2M KNO_3	4	82.00^{ab}
3M KNO_3	4	91.25^{c}
1M NaCl	4	87.00^{bc}
2M NaCl	4	91.25^{c}
3M NaCl	4	89.00^{bc}
1M H_2SO_4	4	89.00^{bc}
2M H_2SO_4	4	84.00^{abc}
3M H_2SO_4	4	88.00^{bc}

Tablo 3. The results of analysis of variance according to SOD Enzyme Activities

ANOVA

SOD Enzyme Activities	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4966,961	6	827,827	0,371	0,886
Within Groups	31258,126	14	2232,723		
Total	36225,088	20			

Table 4. SOD enzyme activities of seeds with treatment of KNO_3, NaCl (Values followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test)

Groups	**N**	**SOD ($U.mg^{-1}$)**
Control	3	161.31^{a}
1M KNO_3	3	175.81^{a}
2M KNO_3	3	187.44^{a}
3M KNO_3	3	171.78^{a}
1M NaCl	3	214.09^{a}
2M NaCl	3	182.95^{a}
3M NaCl	3	187.62^{a}

CAT enzyme activities had no changes among treatment groups (Table 5). Although there is no statistical difference between the treatment groups, as in SOD enzyme activity, KNO_3 and NaCl caused a slightly induction of CAT enzyme activities, especially 2M KNO_3 (Table 6).

Tablo 5. The results of analysis of variance according to CAT Enzyme Activities

ANOVA

CAT Enzyme Activities	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0,234	6	0,039	0,483	0,810
Within Groups	1,132	14	0,081		
Total	1,366	20			

Table 6. CAT enzyme activities of seeds with treatment of KNO_3, NaCl (Values followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test)

Groups	**N**	**Catalase ($U.mg^{-1}$)**
Control	3	0.52^a
1M KNO_3	3	0.66^a
2M KNO_3	3	0.81^a
3M KNO_3	3	0.50^a
1M NaCl	3	0.70^a
2M NaCl	3	0.71^a
3M NaCl	3	0.55^a

Discussion

Seed germination is an important and vulnerable stage in plant life cycle, and determines seedling establishment and plant growth (Bijeh Keshavarzi et al. 2011). Germination is delayed and inhibited under several stress conditions. However, salt stress depends on the species and concentration of the applied salt (Muhammad and Hussain 2010a). Germination of *Tragopogon latifolius* seeds was induced when exposed to KNO_3, NaCl and H_2SO_4. In parallel with the findings, high germination was determined at low NaCl concentrations (Yucel 2000). Effects of salinity on agricultural, forage and wood species have been investigated. However, there is little work that shows effects of salinity on wild edible plants and medicinal plants. Data obtained from research suggests that medicinal plants might be grown on saline soils (Muhammad and Hussain 2010b). KNO_3 has been described as growth-regulating substance (Yücel 2000). Pandey et al., (2000) showed that KNO_3 enhanced germination of some medicinal herbs. It was shown that membrane permeability increased with high NaCl and was reduced by supplementary $Ca(NO_3)_2$ and KNO_3 (Kaya et al. 2003; Kaya et al. 2007). H_2SO_4, known to be a germination inhibitor, inhibits germination completely even at low concentrations (Yucel 2000). However, data showed that sulfuric acid led to highest germination of five *Senna* species (Teketay 1996). Differences in germination percentage under H_2SO_4 may depend on plant species, exposure time or concentrations of acid.

There are conflicting results in some previous studies about changes of SOD and CAT enzyme activity. Activities of SOD and CAT are induced under salt stress in pumpkin, pistachio, senna (*Cassia angustafolia)* (Sevengor et al. 2011; Abbaspour 2012; Agarwal and Pandey 2004). But Jaleel et al. (2007) showed that a decrase of SOD activity was found in leaves under high salinity when CAT activity increased. Pan et al. (2006) also showed that activities of SOD were induced by salt and drought stress, while CAT activity decreased in liquorice. The changes in antioxidant enzyme activities depend on the intensity of stress, exposure time and species abundances in polluted area (Shim et al. 2003; Dazy et al. 2009).

In our findings KNO_3 and NaCl caused slightly induction of SOD and CAT activity. As a conclusion, *Tragopogon latifolius* was tolerant when exposed to KNO_3 and NaCl but not H_2SO_4. Although germination occurred, growth of seedlings was not enough to live under treatment H_2SO_4. Identifying species' resistance to several environmental conditions has gained a great amount of importance. It is thought that the information about the eco-physiological characteristics of *Tragopogon latifolius* will serve as a database for cultivating edible plants.

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