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Research Article

Proline accumulation in three closely related *Salsola* L. taxa

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Abstract

Halophytes, gypsicoles and serpentinicoles are adapted to specialized edaphic conditions at arid and semi-arid regions. These arid and semi-arid areas possess physical and chemical stress factors for all plants. For these plants, one of the most important parameter that provides stress tolerance is the proline accumulation. Proline is a water-soluble amino acid generally accumulated under stress and behave like an indicator for adaptation of plants against extreme conditions. In this study, the amounts of proline accumulation in three different taxa, *Salsola boissieri* subsp. *serpentinicola*, *Salsola boissieri* subsp. *boissieri* and *Salsola turcica*, were determined. These taxa are phylogenetically close to each other but adapted to different soil types. The highest proline accumulation measured in leaves of *S. turcica* with the value of 2.510 ± 0.020 µmol g^{-1} FW and the lowest accumulation measured in leaves of *S. boissieri* subsp. *serpentinicola* with the value of 0.996 ± 0.024 µmol g^{-1} FW. As a result of these proline accumulations, it can be concluded that the high amount of proline accumulation in halophytic *S. turcica* is a response against stress conditions but the low proline accumulation of *S. boissieri* subsp. *serpentinicola* means this species may have other adaptations against the stress factors that caused by serpentinicolous soils. Both of the studied taxa accumulate proline and the findings show that proline accumulation can be a marker in the assessment of stress tolerance of *Salsola* species.

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1. Introduction

Environmental conditions like aridity, salinity, extreme changes in temperatures, extreme precipitations etc. cause abiotic stress factors and influence the growth and propagation of

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plants (Taiz and Zeiger, 2010). Under abiotic stress conditions, plants made some physiological and metabolic changes to adapt themselves against any harm at growth and development (Kalefetoğlu and Ekmekçi, 2005).

Proline, a water-soluble amino acid, is one of the most important parameter in determining the tolerance of plants to stress in saline and arid habitats and used as an indicator for plants adaptation to these harsh conditions (Bian et al., 1988). Proline accumulation is important for balancing osmotic pressure in plants (Iba, 2002; Ashraf and Harris, 2004), protecting the cell membrane by reducing lipid peroxidation in arid conditions (Molinari et al., 2007), adjusting cytosolic pH and regulating hydroxyl radicals (Matysik et al., 2002), photosynthetic activity and preservation of mitochondrial functions, chlorophyll molecules and enzyme activities (Rai et al., 2011).

Like halophytic plants, both gypsicole and serpantinicole plants generally distributed at arid and semi-arid areas and these areas contain physical and chemical stress factors in many aspects. Serpentine soils restrict plant growth and propagation because they have high Fe and Mg and low Ca concentrations, they are rich in heavy metals like Ni, Cr and Co, and also they are poor soils especially for some nutrients like N, P and K (Gordon and Lipman, 1926; Vlamis and Jenny, 1948; Walker, 1954; Proctor and Woodell, 1975; Brooks, 1987; Avcı, 2005). Plants on serpentine soils are also exposed to secondary water stress because of the presence of heavy metals. They have to adapt heavy metal toxicity, water stress and also lack of nutrients. Gypsaceous soils are also poor in organic matter and increase in gypsum cause low cation exchange capacity. Ca concentration of gypsaceus soils is high and decrease Mg and K availability, also cause increase in Ca:Mg ratio in plant tissues (FAO, 1990). Increase in gypsum also causes decrease in water availability so plants face with water stress (Llinares et al., 2015).

Salsola L. genus from Chenopodiaceae/Amaranthaceae family has a distribution area at arid regions of Central and Southeastern Asia, northern Africa and Mediterranean region (Guma et al., 2010). In Turkey, genus *Salsola* L. represented with 18 species and totally 23 taxa (Yaprak, 2012). Species of genus *Salsola* L. generally have adaptations to live on saline or semi-saline areas (Aellen, 1967). Even though *Salsola turcica* Yıldırımlı, *Salsola boissieri* Botsch. subsp. *boissieri* and *Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila are phylogenetically closely related, they are adapted on different edaphic conditions and became habitat specialists which make them more vulnerable against habitat loss and degradation (Pueyo et al., 2008).

Salsola turcica Yıldırımlı is member of steppe vegetation with gypsaceous and saline soils at 950-1000 m (Yıldırımlı, 2010). It is endemic for Turkey and halophytic and gypsicole ecotypes were studied. *Salsola boissieri* Botsch. subsp. *boissieri* prefers rocky and stony slopes at 900-2500 m around Sivas and Kahramanmaraş provinces (Aellen, 1967). *Salsola boissieri* Botsch. subsp. *serpentinicola* (Freitag & Özhatay) Freitag & Uotila geographically separated from the other subspecies and prefers serpentine rocks and rock cracks between 1600 and 2000 m around Burdur and Muğla provinces, it is also endemic for Turkey (Güner et al., 2000). According to the literature surveys there is not any record about the proline accumulation of studied taxa. In this study, the authors aimed to determine the proline accumulation of leaf tissues of the aforementioned *Salsola* taxa and whether these conditions cause stress over the plants or they are adapted to these harsh environmental conditions.

2. Materials and Methods

2.1. Collection of Plant Specimens

The distribution and sampling areas of three taxa were given at Table 1. From each locality leaf samples were taken from 5 different individuals in 2016 and preserved at -20°C at the laboratory.

Table 1. Taxa, collection number of specimens and localities

Taxon	Collection number of specimen (IBÇınar)	Locality
S. turcica (gypsicole ecotype)	1055	Ankara, Beypazarı, about 14 km west of Beypazarı, on the left side of Beypazarı-Nallıhan highway
	1074	Ankara, Beypazarı, about 14 km west of Beypazarı, on the right side of Beypazarı-Nallıhan highway
	1075	Eskişehir, Sivrihisar, Aşağıkepen village
S. turcica (halophytic ecotype)	1088	Ankara, Şereflikoçhisar south of Akin village, Tuz Lake
	1096	Konya, Cihanbeyli, around Yavşan Saltpan, saline steppe
	1105	Konya, Cihanbeyli, Bolluk Lake, saline alkaline areas
S. boissieri subsp. *serpentinicola* (serpentinicole subspecies)	1110	Muğla, Fethiye, Beyağaç, Köyceğiz, Sandras Mountain
	1111	Burdur, Altınyayla, Dirmil pasture, serpentine soils
S. boissieri subsp. *boissieri* (glycophyte subspecies)	1115	Sivas, Yıldızeli, Yusufoğlan village
	1131	Kahramanmaraş, Ahır Mountain, southern slopes

2.2. Determination of Free Proline Accumulation

Proline accumulation was determined by ninhydrin assay according to the method of Bates et al. (1973). At a wavelength of 520 nm, the absorbance of the proline extract in toluene was determined by Perkin Elmer 1420 Multilable Counter VICTOR3V. The accumulation of proline was calculated by comparison with a standard calibration curve previously made by using different concentrations of proline.

2.3. Statistical Analysis

Analysis results were calculated as mean values for each *Salsola* taxa. Measured proline values were analysed by repeated measures analysis of variance (One way ANOVA). Variance analysis was conducted by using SPSS 20 software. Based on the variance analyses conducted for proline, the difference was found to be statistically significant ($p<0.05$). Data used in the statistical analysis are mean values ± standard error measures for three replicates (n = 3).

3. Results and Discussion

Plants generally have two main mechanisms of tolerance against stress factors like salinity and extreme temperatures. First one is the avoidance from the stress factor by making some morphological and/or chemical modifications. The second mechanism is the resistance against stress factors by making cellular and tissue level modifications (Avcıoğlu et al., 2003). Against salinity stress plants accumulate some organic molecules like proline, glycine, betain and sucrose to protect osmotic potential of cytoplasm (Taban et al., 1999). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). It is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses (Choudhary et al., 2005; Munns, 2005; HongBo et al., 2006; Ashraf and Foolad, 2007; Saed-Moucheshi et al., 2013; Sharma et al., 2014; Hunt et al., 2017; De Freitas et al., 2019; Trovato et al., 2019).

In this study, specimen with collection number IBÇınar1096 was used as a control group because most of the taxa of genus *Salsola* are halophytic. In the present work, proline amounts in gypsicoles (1055, 1074, 1075) and halophytic species (1088, 1105) were found to be higher than subspecies of *S. boissieri* (1110, 1111 and 1131) ($p<0.05$) (Table 2). In *S. boissieri* subsp. *boissieri* (1115), it was found that there was an increase in the amount of proline compared to the control group ($p<0.05$) (Table 2). According to the results the highest proline accumulation was found in *S. turcica* (1105) with the value of 2.510 ± 0.020 µmol g^{-1} and the lowest was found in *S. boissieri* subsp. *serpentinicola* (1111) as 0.996 ± 0.024 µmol g^{-1} ($p<0.05$) (Table 2). In *S. turcica* (1105) species, compared with control group, the increase in proline level were approximately 136.8 % ($p<0.05$).

Table 2. Proline amounts in Salsola species (Values are means ± SE, n = 3)

Taxa	Collection Number	Free Proline
		μmol g^{-1} FW
S. turcica (Gypsicole ecotype)	1055	1.826 ± 0.035
S. turcica (Gypsicole ecotype)	1074	1.890 ± 0.135
S. turcica (Gypsicole ecotype)	1075	1.713 ± 0.023
S. turcica (Halophytic ecotype)	1088	1.893 ± 0.066
S. turcica (Halophytic ecotype)	1096	1.060 ± 0.025
S. turcica (Halophytic ecotype)	1105	2.510 ± 0.020
S. boissieri subsp. *serpentinicola*	1110	1.336 ± 0.018
S. boissieri subsp. *serpentinicola*	1111	0.996 ± 0.024
S. boissieri subsp. *boissieri*	1115	2.000 ± 0.046
S. boissieri subsp. *boissieri*	1131	1.316 ± 0.016

All plants including glycophytes synthesize proline as a response to abiotic and biotic stress and it is also true for halophytes (Flowers and Hall, 1978; Tipirdamaz et al., 2006; Grigore et al., 2011). In this study, the high proline accumulation of gypsicole and halophytic species may be related to the osmotic stress caused by the aridity and salinity of the soil. The osmotic stress caused by gypsum depending on salinity was seen as a stimulating factor for proline biosynthesis in plants (Boscaiu et al., 2013). Osmotic stress caused by salt stress activates proline synthesis may have had. Increased proline under salt stress conditions causes an increase in cellular osmotic pressure and may have helped osmotic adjustment in the cytoplasm, which may help increase tolerance. Moreover, the ability to retain water under saline conditions with proline accumulation can increase salt tolerance by eliminating an excess ion concentration by a dilution effect. Saradhi et al. (1995) and Hare and Cress (1997) reported that proline amino acid, as one of the most common osmolite, was accumulated in cytosol as a response to abiotic stress factors like salinity, aridity, extreme temperatures, nutrient deficiencies etc. According to Boscaiu et al. (2013) the main trigger of proline synthesis is the lack of water at gypsaceous and saline soils as in arid and semi-arid areas. The lowest value measured at *S. boissieri* subsp. *serpentinicola* may be the result of adaptation of this taxon to serpentine soils. Proline accumulation as an osmoregulator occurs due to water stress in plants growing in serpentine soils with high metal content (Avcı, 2005). However, the amount of proline can vary between species. Also, these different reactions can be associated with different genotypes.

4. Conclusion

In nature, there are so many stressful factors and their influences change from species to species, and many plants have developed some adaptation mechanisms through their evolutionary processes and do not show any sign of stress under unfavourable conditions. By having these adaptation mechanisms, plants can invade extreme habitats. According to the proline levels it can be said that *S. boissieri* subsp. *serpentinicola* successfully adapted to its habitat or have some other adaptation mechanisms as well as proline synthesis. But the soil and climatic conditions cause stress over especially both ecotypes of *S. turcica* and *S. boissieri* subsp. *boissieri* (1115) according to their proline accumulation and the presence of proline help them to survive at these unfavourable conditions.

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