

## THE PROLINE ACCUMULATION OF SOME HALOPHYTES IN THE VICINITES OF THE SALT LAKE

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### ABSTRACT

In this study, accumulation of proline amino acid which is known to accumulate in the stress conditions has been examined in some halophytes. For this purpose, some species which are widespread, dominant and forming associations in the environs of the Salt Lake have been used; *Frankenia hirsuta* L., *Arthrocnemum fruticosum* (L.) Moq., *Salicornia europaea* L., *Taraxacum farinosum* Hausskn et Bornm., *Camphorosma monspeliaca* L. ssp. *monspeliaca*, *Halocnemum strobilaceum* (Pall.) Bieb., *Halimione portulacoides* L. Aellen. In these halophytes having a zonal distribution around the Salt Lake, it has been found that the amount of proline was different according to the severity of stress. It has been found out that the species of *Frankenia hirsuta* L. has the most proline content and that of *Camphorosma monspeliaca* L. ssp. *monspeliaca* has the least.

### INTRODUCTION

The environmental stresses such as salinity, drought, low and high temperatures have a limiting effect on the plant growth and development, and correspondingly on the yield. The proline is known to accumulate in plant tissues in response to environmental stresses (ASPINALL and PALEG, 1981).

As often happens in the other stresses, the plants react against to osmotic pressure changes taking place within the cell with the osmotic adjustments (HELLEBUST, 1976; ASPINALL and PALEG, 1981). For this reason, the plants accumulate a variety of compounds including carbohydrates (WAISEL, 1972), organic acids (OSMUND, 1963) and sugar alcohols (LEWIS and SMITH, 1967). The proline which is an amino acid is one of this kind of compound and it has been shown that it accumulates the highest contents in halophytes (STEWART and LEE, 1974; TREICHEL, 1979) and also in non-

halophytes (MUKHERJIE, 1974; BAR-NUN and POLJAKOFF-MAYBER, 1977) in salinity stress. There is some evidence that salts, particularly  $\text{Na}^+$  and  $\text{Cl}^-$ , are sequestered in the vacuole (FLOWERS, TROKE and YEO, 1977) and that proline is located mainly in the cytoplasm of cells in stressed tissue (GREENWAY, 1967; LEIGH, AHMAD and WYN JONES, 1981; PAHLICH, KERRES and JAGER, 1983).

The water potential which is low in saline medium has complicated water uptake of plant. The proline which has osmoregulatory property (STEWART and LEE, 1974; ASPINALL and PALEG, 1981; TREICHEL, 1979) has produced the low osmotic potential through being accumulated in cytoplasm (STEWART and LEE, 1974; HANDA, BRESSAN, HASEGAWA and HANDA, 1982) to balance solute potential taking place due to accumulation of salts in vacuole (FLOWERS, TROKE and YEO, 1977). In addition to its osmoregulatory role, proline is related with enzymes because it has protective role in cytoplasm (BAR-NUN and POLJAKOFF-MAYBER, 1977; SCHOBERT, 1977; WRENCH, WRIGHT, BRADY and HINDE, 1977). Proline which is soluble amino acid has not affected to the activities of most enzymes in high concentrations (STEWART and LEE, 1974).

In this study, proline accumulation in some halophytes growing under the natural conditions in the vicinities of the Salt Lake has been examined.

## MATERIAL AND METHOD

In this study, some halophytes collected from the environs of Salt Lake were used. Samples were taken from the areas between Yavşan saltern and Bozan village which is located in the north-west of the lake, in the late summer when the plants reach their optimum development stage, in 1987. The halophytes which are widespread and dominant were preferably taken from the region (Table 1) and were immediately brought to laboratory and they were conserved in the deepfreeze until the analysis.

The proline extraction and determination were made according to BATES, WALDREN and TEARE (1973). Extraction was made by using the tissues of leaves. The proline amount obtained in the extract was estimated spectrophotometrically using the ninhydrin method. Purified proline was used for standardization. The method was applied with details as follows:

Table 1: Some halophytes collected from the vicinities of the Salt Lake  
(Between Yavşan saltern and Bozan village)

Family	Species
<i>Frankeniaceae</i>	<i>Frankenia hirsuta</i> L.
<i>Compositae</i>	<i>Taraxacum farinosum</i> Hausskn et Bornm.
<i>Chenopodiaceae</i>	<i>Arthrocnemum fruticosum</i> (L.) Moq.
<i>Chenopodiaceae</i>	<i>Salicornia europaea</i> L.
<i>Chenopodiaceae</i>	<i>Halimione portulacoides</i> L. Aellen.
<i>Chenopodiaceae</i>	<i>Comphorosma monspeliaca</i> L. ssp. <i>monspeliaca</i>
<i>Chenopodiaceae</i>	<i>Halocnemum strobilaceum</i> (Pall.) Bieb.

1- Approximately 0.5 g of plant material (leaf) was homogenized in 10 ml 3 % aqueous sulfosalicylic acid and the homogenate filtered through Whatman 2 filter paper.

2- Two ml of filtrate was reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction terminated in an ice bath.

3- The reaction mixture was extracted with 4ml toluene mixed vigorously with a test tube stirrer to 15-20 sec.

4- The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank.

5- The proline concentration was determined from a standard curve and calculated on a fresh basis as follows:

$$[(\mu\text{g proline/ml} \times \text{ml toluene}) / 115.5 \mu\text{g}/\mu\text{mole}] / [(g \text{ sample} / 5)] = \mu\text{moles proline/g of fresh weight material.}$$

The results related with proline content are average values at least 3-4 samples of each species.

## RESULTS

MUSTAFA (1982) has declared that the soils around Yavşan saltern and Bozan village, the area from which the plant samples were taken, were very salty and alkaline. Halophytes which are growing on this area soils, have adapted these conditions. In the great part of these soils, the principal cations are  $\text{Na}^+$ , then  $\text{Mg}^{++}$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$  respectively (DRIESSEN, 1970; MUSTAFA, 1982). The main anion is  $\text{Cl}^-$  (MUSTAFA, 1982).

In this region, annual excessive evaporation which is more than rainfall has caused the salt accumulation inside and surface of the soils

in vast part of the area. This accumulation is also seen on the leaves and stems of plants.

The results related with proline accumulation in leaf tissues of halophytes collected in the vicinities of the Salt Lake are seen in Table 2. These results have shown that proline accumulated in much amount in the species of *Frankenia hirsuta* L. (202.54  $\mu$ moles/g fwt). The *Salicornia europaea* L. (121.50  $\mu$ moles/g fwt), *Taraxacum farinosum* Hausskn et Bornm. (71.99  $\mu$ moles/g fwt) and then the *Halimione portulacoides* L. Aellen. (43.25  $\mu$ moles/g fwt), *Halocnemum strobilaceum* (Pall.) Bieb. (26.18  $\mu$ moles/g fwt), *Arthrocnemum fruticosum* (L.) Moq. (24.20  $\mu$ moles/g fwt) and *Camphorosma monspeliaca* L. ssp. *monspeliaca* (20.25  $\mu$ moles/g fwt) follow it respectively.

It is seen that proline content which is the major component of the amino acid accumulation in halophytes is very variable among the species.

Table 2: Proline analysis results obtained from some halophytes collected from the vicinities of the Salt Lake (Between Yavşan saltern and Bozan village) (Average  $\pm$  standard error)

Species	Proline content ( $\mu$ moles/g fresh wt)
<i>Frankenia hirsuta</i> L.	202.54 $\pm$ 2.83
<i>Taraxacum farinosum</i> Hausskn et Bornm.	71.99 $\pm$ 2.49
<i>Arthrocnemum fruticosum</i> (L.) Moq.	24.20 $\pm$ 1.72
<i>Salicornia europaea</i> L.	121.50 $\pm$ 3.54
<i>Halimione portulacoides</i> L. Aellen.	43.25 $\pm$ 1.95
<i>Camphorosma monspeliaca</i> L. ssp. <i>monspeliaca</i>	20.25 $\pm$ 2.11
<i>Halocnemum strobilaceum</i> (Pall.) Bieb.	26.18 $\pm$ 1.37

## DISCUSSION

The proline accumulation mechanism in stress conditions such as salinity, cold and drought in plants has been tried to explain by the studies of STEWART and LEE (1974), CAVALIERI and HUANG (1979), ASPINALL and PALEG (1981).

The proline accumulation as a response to various stress conditions and especially to soil salinity which is subject of our investigation can play an important role on the adaptation of halophytes to the saline conditions.

In all of the halophytes, it is found that proline accumulations are also, in high amounts in our study (Table 2). Especially, in the spe-

cies of *Frankenia hirsuta* L., *Salicornia europaea* L., *Taraxacum farinosum* Hausskn et Bornm., the proline content is more than the other species. Just as in the study made by ÇAKIRLAR and TOPÇUOĞLU (1987), it was found that proline accumulation in leaf tissue of *Frankenia hirsuta* L. species collected in the vicinities of the Salt Lake (Şerefli-koçhisar-Adana Highway 130 km) is more than the other species. Our proline values for the species of *Frankenia hirsuta* L., *Halocnemum strobilaceum* (Pall.) Bieb. and *Salicornia europaea* L. are more than the values of ÇAKIRLAR and TOPÇUOĞLU (1987). This depends on the capacity of accumulation of proline in different species due to the formation of various saline soils by the ground water in various depth. Thus, the capacity of proline accumulation is different among the families and species, and it creates a situation peculiar to the species. For this reason, there may be a zonation as regards distribution of species according salt concentration in the soil around the lake (BİRAND, 1961). STEWART and LEE (1974) have found that the proline accumulation is very high in the shoots of halophytes. CAVALLIERI and HUANGIN (1979) have explained that proline accumulated in marsh halophytes and BUHL and STEWART (1983) have found that proline accumulated in NaCl-treated excised barley leaves. VOETBERG and STEWART (1984) have also found an increase in proline content as a response to high Na<sup>+</sup> and K<sup>+</sup> concentration in barley leaves. These results have supported ours.

The high concentration of proline present in the shoots of some halophytes could be interpreted as being indicative that this compound acts as a storage compound for reduced carbon and nitrogen, as has in fact been postulated for water stressed plants (BARNETT and NAYLOR, 1966; STEWART, MORRIS and THOMPSON, 1966). This rise in proline level due to the increase of salinity, could simply be a stress reaction to physiological drought rather than any kind of adaptive response (STEWART and LEE, 1974). The accumulation of high concentrations of proline in some halophytes under saline conditions suggests that it might have a role in osmotic adjustment. Certainly proline meets the requirements of a compound with this function; it has a high solubility (162 / 100 ml at 25°C), it is a neutral compound and at high concentrations has little effect on enzyme activity.

In saline conditions, the reason of proline accumulation in halophytes is that the major products of dark fixation are amino acids (WEBB and BURLEY, 1965) or it is a consequence of impaired pro-

tein synthesis (GOAS, 1971). In each case, what it is important is that the proline accumulation adjusts the cell sap concentration with osmoregulatory mechanism in stress conditions.

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