Proline Accumulation Pattern in Species of an Inland Saline Habitat

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Seasonal and diurnal fluctuations were measured in the leaf proline content of three dicot species (*Limonium gmelini*, *Lepidium crassifolium* and *Camphorosma annua*), natives to an inland saline area of Hungary. Proline contents were negatively correlated to the mean monthly temperature in all three species. All the investigated species accumulated proline in February and/or March, while increases in temperature were followed by a drop in proline level by the beginning of April/May. Sodium accumulation in the leaves as well as soil water content influenced the amount of proline to a smaller extent than temperature in all of the investigated species. Accumulation of proline did not show significant correlations with the accumulation of sodium in the leaves. Diurnal pattern of leaf proline content changed during the growing season with a decreasing trend at the beginning (April) and increasing trend at the end (August) of the season.

Introduction

Plant productivity is greatly influenced by environmental stresses, of which osmotic stress, due to drought and salinity, is the most serious problem (Boyer, 1982). Salinity affects more than 40% of the world arable land (Wyn Jones and Gorham, 1986), and it is expected to be enhanced by the global climate change (Várallyai, 1994).

Depending on the degree of tolerance, plant cells under stress conditions accumulate certain metabolites, so-called compatible osmolytes, acting as osmoprotectants and as radical scavengers (Smirnoff and Cumbes, 1989; Yancey, 1994).

Proline is the most abundant compatible osmolyte (Yancey, 1994). Increase in the proline content in response to osmotic stress was reported for different organisms, including animals and bacteria (Csonka, 1989; Larher *et al.*, 1993). In higher plants, proline accumulation was described as a common metabolic response to water deficit, salinity and cold stress (Stewart and Larher, 1980; Delauney and Verma, 1993).

Recent advances in the research of salt-tolerance show that cells in culture express the stress response mechanisms similar to that by intact plants only partially (Adams *et al.,* 1992) and salt tolerance probably depends on a highly regulated network of plant organs. It has been argued (e.g. Radin, 1993) that examination of plants in their highly fluctuating natural environment is crucial for being able to develop a better understanding on the mechanism of salt tolerance.

In natural plant populations species are subjected to various fluctuating abiotic factors. This is especially essential since proline accumulation was previously observed in response to many abiotic stress factors; such as water stress, salinity, heat and cold stress (in detail see Samaras *et al.*, 1995).

We investigated species of a perennial inland salty vegetation which -in addition of soil salinity- is subjected to early spring freezes, chills and summer droughts. In order to characterize the importance of the abiotic factors in the observed (seasonal) proline accumulation patterns in species of this habitat, we have selected three characteristic dicot species which have previously been shown to have different abilities for proline accumulation. In these species, namely *Limonium gmelini*, *Lepidium crassifolium* and *Camphorosma annua*, we examined the seasonal (monthly intervals) and diurnal proline patterns in the leaves from February till August. Leaf proline accumulation was related to abiotic factors, such as: temperature, soil water content, leaf sodium content and potassium to sodium ratio in the leaf. The present work is a first report of a research programme on the functioning of osmotic adjustment in an inland saline vegetation of Hungary.

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Materials and Methods

The investigated species (*Limonium gmelini* (Willd.) subsp. hungaricum, *Camphorosma annua* Pall. and *Lepidium crassifolium* W et K.) are natives to a salt-affected area of the Danube valley (Kiskunság National Park) of Hungary, near the village Fülöpszállás (47.05 N. and 19.07 E.). The area lies in the warm temperate zone, i.e. climate "C" in the Köppen's system (Borhidi, 1993) and characterised by hot dry summers, an annual mean temperature of 11 °C, a large annual amplitude of temperature changes (22 °C); and a mean annual precipitation of about 450–550 mm.

According to the survey by the Research Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, the water soluble salt content in the upper 50 cm varies between 1.2 to 0.45% (solonchak) and 0.15 to 0.9% (solonchak-solonetz), with a high exchangeable sodium content; K:Na ratios range from 0.01 to 0.003. Most of the salts are $NaHCO_3$ and Na_2CO_3 (soda-type salinity). Because of the extremely alkaline pH (values from 9 to 10), the quantity of Ca^{2+} and Mg^{2+} ions are not significant. Slight changes in the microrelief result in an unequal salt accumulation and ultimately in a very heterogenous environment: salt concentration and humus content differs significantly in space and time. *Camphorosma annua* and *Lepidium crassifolium* are the characteristic species of the most salty sodic solonchak soil type, while *Limonium gmelini* occurs in the less-salty solonetzic meadow patches. Description of the plant associations formed according to the edafic conditions was given in detail by Bodrogközy (1962).

C. annua (Chenopodiaceae) is a therophyte, prostrate species with 5 to 40 cm long branching shoots. *L. crassifolium* (Cruciferae) is an overwintering biennial cryptophyte species, 10 to 15 cm tall plant with quite thick storing root. *L. gmelini* (Plumbaginaceae) is a hemicryptophyte, deep-rooting species, with leafless stem, and rosette-forming leaves at the basal part of the plants (individual leaves are 7 to 11 cm long). Leaves of *Limonium gmelini* appear in January and February and flowers in June. *C. annua* germinates at the end of February or beginning of March, and flowers in June. *L. crassifolium* germinates in February, flowers in May.

Climatic data has been provided by a regional meteorological station (Kunszentmiklós).

Plant samples (n=3 for proline, n=2 for ions) of similar exposition were collected at monthly intervals, from February/March. In the early phase of the growing season, when plant size was small, several individuals were taken in one sample to give a fresh weight of 2 to 3 grams. Later in the growing season, fully expanded leaves were chosen from the middle part of the plant/rosette. For daily measurements, samples were collected from mid-April, before dawn, at noon and after sunset. Collected samples were immediately frozen in liquid nitrogen. Freeze-dried samples were pooled, then manually grounded to fine powder. For determination of proline content, 50 milligrams of dry leaf powder was extracted in 2 ml of 5% sulpho-salicilic acid and then the method of Troll and Lindsey (1955) was used, as improved by Bates *et al.* (1973) and Magné and Larher (1992). Another fraction (50 mg) of each sample was used for determination of cation concentration, carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES), after an extraction in HNO_3 and H_2O_2 (*see in*: Tuba and Csintalan, 1993).

Proline concentration is expressed in millimoles per litre plant water, while concentration of the ions is expressed in milliequivalents (mEq) per litre plant water which gives the best reference to the actual concentration in the cell sap (Biebl and Kinzel, 1965).

Means were compared using Student's t-test.

Multiple linear regression was carried out on the data with proline-concentration as the dependent and temperature (°C) and sodium concentration ($mEq.dm^{-3}$ water) and potassium to sodium ratio as the independent variables. Standard regression coefficients for temperature (°C), sodium concentration ($mEq.dm^{-3}$ water) and potassium to sodium ratios were compared.

Results and Discussion

The selective accumulation of essential ions, decreasing water potential and retaining water can be a common property of salt and osmotic tolerance. Accumulation of K^+ under saline conditions appears to be one of the traits linked to stress tolerance in several higher plants. In our experiment,

Table I. Seasonal changes in the leaf sodium concentration and potassium to sodium ratios in *Limonium gmelini*, *Camphorosma annua* and *Lepidium crassifolium*. Sodium concentration is given in mEq.dm^{-3} water (Na^+_w); where the index w refers to water.

	Limonium gmelini		*Camphorosma annua*		*Lepidium crassifolium*	
Month	Na^+_w	K^+/Na^+	Na^+_w	K^+/Na^+	Na^+_w	K^+/Na^+
February					172±3	0.76
March	156±6	0.79	115±5	1.35	137±2	0.67
Apilr	150±3	0.80	174±5	0.85	133±8	0.44
May	145±5	0.61	180±15	0.67	151±14	0.39
July	157±10	0.66	200±3	0.54	145±5	0.34
August	150±2	0.62	201±7	0.42	156±6	0.29

during the vegetation period, on dry weight basis sodium concentration increased and potassium concentration decreased in the leaves (Table I). Therefore K^+/Na^+ ratios measured in the leaves diminished in all three species. Decrease of K^+/Na^+ ratios was found both on dry weight and on plant water content basis. It shows accumulation of sodium in the leaves, which results in lowering of the cellular osmotic potential.

In contrast to the previous reports on tissue samples, cell cultures, and microbes (Csonka, 1989; Larher *et al.*, 1993), there was no proline accumulation observed simultaneously to the changes in the sodium concentration. Rather, an important fall was found which correlated negatively to the temperature. Correlation between leaf proline content and temperature was statistically significant ($P \leq 0.01$) in all three species (Fig. 1). Proline has been shown to have a cryoprotective role in plant cell cultures (Duncan and Widholm, 1987; Santarius, 1992; Songstad *et al.*, 1990). We suggest that proline accumulated in the beginning of the growing season could confer cold tolerance to these plants, which appear in the field as early as February (*L. crassifolium)* and early March (*L. gmelini* and *C. annua*). It is interesting that even in *C. annua* which usually stores very low amounts of this amino acid, a relatively high amount of proline was found in March.

However, in addition to the cold tolerance, proline could serve as an important pool of nitrogen during development (in detail see Taylor, 1996). Furthermore, there is evidence that the degradation of proline in mitochondria is directly coupled to the respiratory electron transport system and ATP production (Elthon and Stewart, 1981).

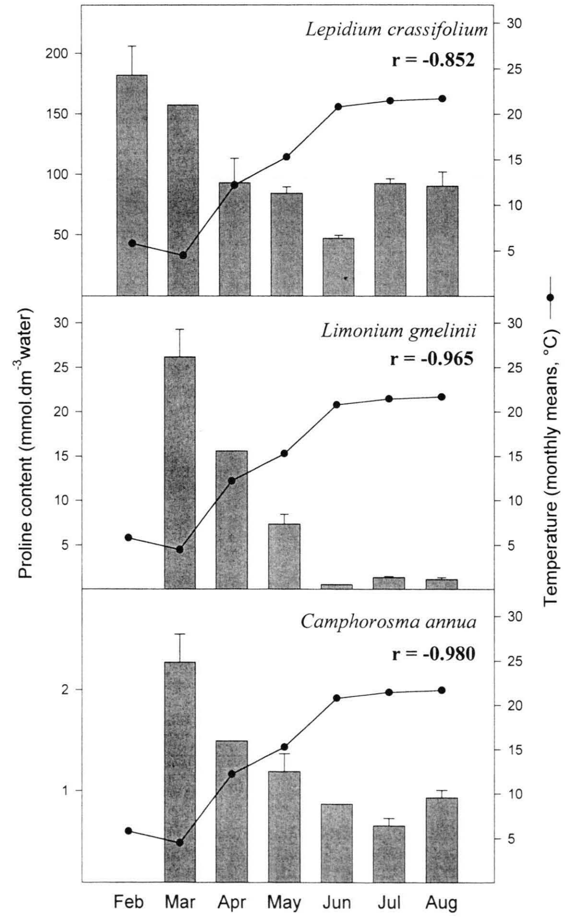

Fig. 1. Seasonal changes in leaf proline content (mmol.dm^{-3}) and monthly temperature (°C) on the example of *Limonium gmelini* and *Lepidium crassifolium*.

In our investigation, after an important drop of the proline content in April in the leaves of *L. crassifolium* and in May in those of *L. gmelini* and *C. annua*, as mean temperature values increased, a further, although less important decrease, was observed (Fig. 1). This occurred in May in *L. crassifolium* and in June in *C. annua* and *L. gmelini*, coinciding with the flowering time, and might have been caused by the enhanced energy requirement of flowering.

Diurnal changes in the concentration of proline were measured at monthly intervals from mid-April. Clear differences (Table II) were found between the diurnal proline patterns measured at the middle and at the end of the growing season. Here we present only values from April, when daily changes in the soil water content were small (between 100 to 88% of the saturation water content) and from August, when both temperature and soil water contents changed considerably during the day (between 20 to 50% of the saturation water content). In April the species have not showed a consistent diurnal proline pattern, while in August, leaf proline concentration dropped significantly during the dark in all species (Fig. 2).

In summary, the three investigated dicot species showed different proline accumulating pattern. The Cruciferae species *Lepidium crassifolium* is a proline-accumulator and maintains a relatively high proline level throughout the growing season. About 50 mmol.dm^{-3} proline was always present in the leaves of this species. *Limonium gmelini* accumulated proline to a much lesser extent, with high values only at the beginning of the growing season. The species *Camphorosma annua*, from the family Chenopodiaceae, although did not accumulate proline in important quantities, similarly to other members of this family (Popp, 1995), showed a similar seasonal and diurnal pattern in the leaf proline content as the other two species. Participation of proline in osmotic regulation (by mass action) seems likely in *L. crassifolium*, only. On the base of these results accumulation of proline is not necessarily a consequence of drought or salinity stress, while it shows strong correlation with temperature. There was no sign of sodium concentration dependent proline accumulation in any of the species.

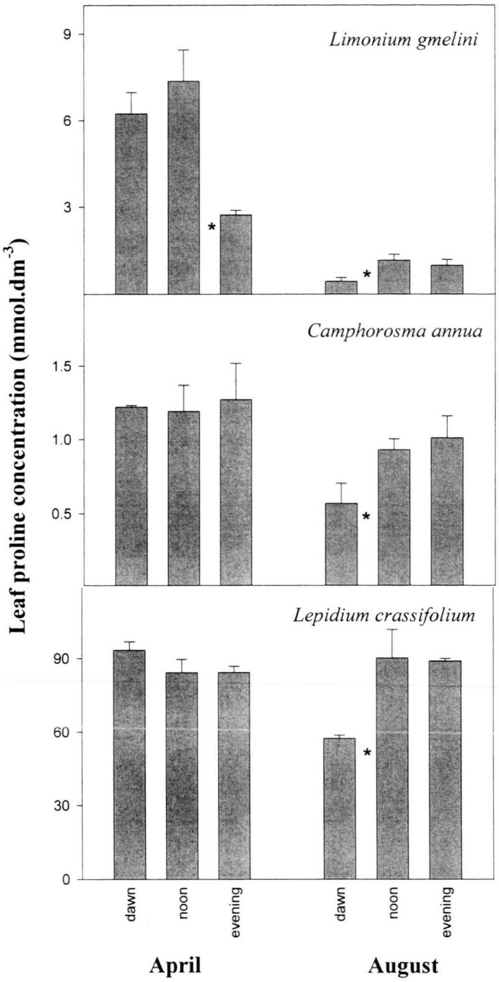

Fig. 2. Daily changes in the leaf proline content (mmol.dm^{-3}) of *Limonium gmelini*, *Camphorosma annua* and *Lepidium crassifolium* in mid-April and in August. Significant difference ($P \leq 0.01$) is indicated by asterisk.

Table II. Standardized regression coeffiecients for temperature (°C), sodium concentration (mEq.dm^{-3} water) and potassium to sodium ratios. Multiple linear regression was carried out on the data with proline as a dependent variable, and temperature (T; given in °C), sodium concentration (Na^+_w; given in mEq.dm^{-3} water) and potassium to sodium ratio (K/Na) as independent variables.

Species	**T**	**Na^+_w**	K/Na
Limonium gmelini	−1.304	−0.144	−0.360
Camphorosma annua	−1.436	−1.267	−1.968
Lepidium crassifolium	−5.698	−0.143	−2.918

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Adams P., Thomas J. C., Vernon D. M., Bohnert H. J. and Jensen R. G. (1992), Distinct cellular and organismic responses to salt stress. Plant Cell Physiol. **33**, 1215–1223.

Bates L. S. Waldren R. P. and Teare I. D. (1973), Rapid determination of free proline for water-stress studies. Plant Soil **39**, 205–207.

Biebl R. and Kinzel H. (1965), Blattbau und Salzgehalt von *Laguncularia racemosa* (L.) Gaertn. f. und anderer Mangrovebäume auf Puerto Rico. Öst. bot. Z. **112**, 56–93.

Bodrogközy Gy. (1962), Die standortökologischen Verhältnisse der halophilen Pflanzengesellschaften des Pannonicum I. Untersuchungen an den Solonchak-Szikböden des südlichen Kiskunság. Acta Bot. Acad. Sci. Hung. **8**, 1–37.

Boyer J. S. (1982), Plant productivity and environment. Science, **218**, 443–448.

Borhidi A. (1993), Characteristics of the climate of the Danube-Tisza Mid-Region. The Flora of the Kiskunság National Park vol. I. (J. Szujkó-Lacza and D. Kováts, eds.). Magyar Természettudományi Múzeum, Budapest, 9–20.

Csonka L. N. (1989), Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. **53**, 121–147.

Delauney A. J. and Verma D. P. S (1993), Proline biosynthesis and and osmoregulation in plants. Plant J. **4**, 215–223.

Duncan D. R. and Widholm J. M. (1987), Proline accumulation and its implication in cold tolerance of regenerable maize callus. Plant Physiol. **83**, 703–708.

Elthon T. E. and Stewart C. R. (1981), Submitochondrial location and electron transport characteristics of enzymes involved in proline oxidation. Plant Physiol. **67**, 780–784.

Larher F., Leport L., Petrivalsky M. and Chappart M. (1993), Effectors for the osmoinduced proline response in higher plants. Plant Physiol. Biochem. **31**, 911–922.

Magné C. and Larher F. (1992), High sugar content of extracts interferes with colorimetric determination of amino acids and free proline. Anal. Biochem. **200**, 115–118.

Popp M. and Albert R. (1995), The role of organic solutes in salinity adaptation of mangroves and herbaceous halophytes. In: Biology of Salt Tolerant Plants (M. Ajman Khan and I. A. Ungar, eds.). Department of Botany, University of Karachi, Pakistan, 139–149.

Radin J. W. (1993), Water relations of cotton in controlled environments and the field. In: Plant responses to cellular dehydration during envionmental stress (T. J. Close and E. A. Bray, eds.) Vol. **10**. American Soc. Plant Physiol., 67–78.

Rozema J. (1995), Biology of Halophytes. In: Halophytes and Biosaline Agriculture, (C. V. Choukr-allah and M. A.Hamdy, eds.). Dekker, New York, 17–30.

Samaras Y., Bressan R. A., Csonka L. N., García-Ríos M. G., Paino D'Urzo M. and Rhodes D. (1995), Proline accumulation during drought and salinity. In: Environment and Plant Metabolism (N. Smirnoff, ed.). BIOS Scientific Publishers Ltd., 161–187.

Santarius K. A. (1992), Freezing of isolated thylakoid membranes in complex media. VIII. Differential cryoprotection by sucrose, proline and glycerol. Physiol. Plant. **84**, 87–93.

Smirnoff N. and Cumbes Q. J. (1989), Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry **28**, 1057–1060.

Songstad D. D., Duncan D. R. and Widholm J. M. (1990), Proline and polyamine involvement in chilling tolerance of maize suspension cultures. J. Exp. Bot. **41**, 289–294.

Stewart G. R. and Larher F. (1980), Accumulation of amino acids and related compounds in relation to environmental stress. In: The Biochemistry of Plants, Vol. **5** (B. J. Miflin, ed.). Academic Press, New York, 609–635.

Taylor C. B. (1996), Proline and water deficit: ups, downs, ins and outs. The Plant Cell **8**, 1221–1223.

Troll W. and Lindsey J. (1955), A photometric method for determination of proline. J. Biol. Chem. **215**, 655–660.

Tuba Z. and Csintalan Zs. (1993), Bioindication of road motor traffic caused heavy metal pollution by lichen transplants In: Plants as Biomonitors for Heavy Metal Pollution of Terrestrial Environments (B. Markert, ed.). VCH – Publisher, Inc. Weinheim, New York, 329–342.

Yancey P. H. (1994), Compatible and counteracting solutes. In: Cellular and Molecular Physiology of Cell Volume Regulation (K. Strange, ed.). CRC Press, Boca Raton, FL, 81–109.

Várallyai Gy. (1994), Climate change, soil salinity and alkalinity. In: Soil responses to climate change (M. D. A. Rounsevell and P. J. Loveland, eds.). NATO ASI Series, Springer-Verlag, Berlin, 39–54.

Wyn Jones R. G. and Gorham J. (1986), The potential for enhancing the salt tolerance of wheat and other important crop plants. Outlook Agric. **15**, 33–39.