Pak. J. Bot., 34(2): 129-134, 2002.

FREE PROLINE CONTENT IN *ASTRAGALUS* SPECIES

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Abstract

Free proline content was determined in roots and leaflets of 23 species of *Astragalus* collected from different regions of Iran. The content of free proline in roots and leaflets varied from 0.09-1.23 and 0.04-0.95 % dry weight, respectively. In a number of species proline content was higher in leaflets than in roots, whereas in some other species proline content in roots was higher than in leaflets. The accumulation of free proline in different organs of different species can be attributed to the osmotic conditions of the plants which could represent differences in their mode of adjustment to the environmental condition. The habitat of the species can also affect the organ of proline accumulation. In the present paper, the proline content in roots and leaflets of various species of *Astragalus* is reported for the first time.

Introduction

Plants are exposed to many types of environmental stresses. Among these stresses, osmotic stress and in particular that due to drought and salinity is the most serious problem that limit plants growth and crop productivity (Boyer, 1982). Many plants, including halophytes, accumulate compatible osmolytes such as proline, glycine betaine and sugar alcohols, when they are exposed to drought or salinity stress (Hellebust, 1976; Csonka, 1989; Delauney & Verma, 1993). It has been suggested that compatible solutes do not interfere with normal biochemical reactions and act as osmoprotectants during osmotic stress. Among known compatible solutes, proline is probably the most widely distributed osmolyte. The accumulation of proline has been observed not only in plants but also in eubacteria, marine invertebrate, protozoa and algae (McCue & Hanson, 1990; Delauney & Verma, 1993).

High concentration of proline are characteristic of many water- or salt stressed plants (Stewart & Larher, 1981). Compatible solutes such as proline and glycine betaine accumulate in stressed tissues and take part in osmotic adjustment and have also protective properties (Wyn Jones & Storey, 1981; Paleg *et al.*, 1981, 1984). Membrane and metabolic dysfunction which occurs in the transition from dry state to full dehydration is ameliorated by the production of protectants which interact with membranes and proteins to preserve their integrity during rapid rehydration. Many of the solutes which accumulate in stressed plants and which have protective properties are also reported to reduce free radical activity. Implicit in the theory of free radical induced damage under conditions of dehydration is notion that the cell's defensive mechanisms breakdown or are overloaded. Proline is also able to detoxify free radicals by forming long-lived adducts with them. Oxidative damage to membranes and proteins as the results of free radical attacks is reduced by increased free radical scavengers (Stewart, 1989).

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Iran is the classical country of great salines and Kavirs (Zohary, 1973). Saline and alkaline soils are expanding in arid and semiarid regions of Iran and cover 12.5% (204800 km^2) of the total area of the country. A comparison of the annual precipitation with the distribution of saline soils shows that a majority of the area has total annual precipitation of less than 250mm/y (Akhani & Ghorbanli, 1993).

The genus *Astragalus* is generally considered the largest genus of vascular plants with an estimated 2500-3000 species (Podlech, 1986; Lock & Simpson, 1991). The greatest number of *Astragalus* species are found in arid, continental regions of Western North America (400 species) and Central Asia (2000-2500 species). *Astragalus* species are considered among the species distributed in dry and arid environments (Badri & Hamed, 2000). The present report describes the variation in free proline content in roots and leaflets of different species of *Astragalus* in their natural habitats which does not appear to have been reported before.

Materials and Methods

Plant material: Samples were collected from different regions of Iran and air dried in the shade. The nomencalture of *Astragalus* species and section classification used herein is based on Maassoumi (1998). Taxonomy and locality data of the materials used for extraction of free proline are listed in Table 1.

Sample preparation and extraction of free proline: Dried roots and leaflets were powdered in a grinder and 0.1 g of the powder of plant samples were homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenates filtered through Whatman no. 2 filter paper (Bates, 1973).

Reagents: Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid with agitation, until dissolved. The reagent remains stable for 24 h at 4°C.

Spectrophotometric determination of free proline: Shimadzue UV-visible recording spectrophotometer (UV-160) with 10 mm matched glass cells was used for the absorbance measurements.

Two ml of filtrates were reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in test tubes for 1 h at 100°C and the reactions terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The upper toluene phase was aspirated from the lower aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as the blank. The proline concentration was determined by the standard curve using L-proline as the standard between 0 to 40 μmol $liter^{-1}$ (Bates, 1973).

Results and Discussion

Free proline content in roots and leaflets of 23 species of *Astragalus* from different regions of Iran showed that the content of free proline in roots and leaflets varied from 0.09 to 1.23 and 0.04 to 0.95% dry weight (DW), respectively (Table 1). In some of the species, proline content in roots was higher than in leaflets whereas, in some other species proline content in leaflets was higher than in roots.

Table 1. Free proline content (%DW) in roots and leaflets of *Astragalus* species from Iran. The values are the mean of three determinations.

Section	Species	Distribution	Location and date of collection	Roots	leaflets
Anthylloidei	*ebenoides* ssp. *ebenoides*	Iran: Endemic	Zanjan: 16.6.1997	0.45	-[a]
Anthylloidei	*submitis* ssp. *submitis*	Iran: Endemic	Tehran: 28.5.1997	0.67	0.08
Anthylloidei	*tortuosus* DC.	Iran, Iraq, Turkey, Russia	Sanandaj: 18.6.1997	0.41	0.22
Astragalus	*caragana* Fisher and C.A. Meyer	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.21	0.20
Caraganella	*parvistipulus* Rech. f.	Iran, Afghanistan, Pakistan	Zanjan: 15.6.1997	0.38	0.05
Caprini	*aegobromus* Boiss. and Hohen.	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.33	0.12
Caprini	*basilicus* Podlech and Maassoumi	Iran: Endemic	Loshan: 28.5.1997	0.10	0.42
Caprini	*semilunatus* Podlech	Iran, Russia	Zanjan: 15.6.1997	-[a]	0.16
Hololeuce	*alyssoides* Lam.	Iran, Iraq, Russia	Zanjan, Bijar Road 16.6.1997	0.25	0.21
Hololeuce	*alyssoides* Lam.	Iran, Iraq, Russia	Zanjan, Soltaniieh 15.6.1997	0.07	0.18
Hymenostegis	*chrysostachys* Boiss.	Iran, Iraq, Turkey, Russia	Sanandaj: 18.6.1997	-[a]	0.05
Hymenostegis	*glumaceous* Boiss.	Iran: Endemic	Zanjan: 16.6.1997	0.13	0.07
Hymenostegis	*paralurges* Bunge	Iran: Endemic	Zanjan: 15.6.1997	0.54	0.08
Hymenostegis	*pauxillis* Maassoumi and Ghahremani	Iran: Endemic	Zanjan: 15.6.1997	-[a]	0.07
Hymenostegis	*sciureus* Boiss. and Hohen.	Iran: Endemic	Tehran: 28.5.1997	-[a]	0.07
Incani	*monspessulanus* ssp. *monspessulanus*	Iran, Turkey, Russia	Sanandaj: 17.6.1997	0.18	0.28

Table 1. Contd.

Section	Species	Distribution	Location and date of collection	Roots	leaflets
Incani	*monspessulanus* ssp. *monspessulanus*	Iran, Turkey, Russia	Zanjan to Dandi: 18.6.1997	0.28	0.53
Incani	*monspessulanus* ssp. *monspessulanus*	Iran, Turkey, Russia	Gheidar: 15.6.1997	0.07	0.04
Malacothrix	*eugenii* Grossh.	Iran, Russia	Sanandaj: 17.6.1997	1.23	0.25
Malacothrix	*iranicus* Bunge	Iran, Iraq, Russia	Hamadan: 16.6.1997	0.20	-[a]
Malacothrix	*iranicus* Bunge	Iran, Iraq, Russia	Zanjan: 15.6.1997	0.67	0.12
Malacothrix	*mollis* Bunge	Iran, Iraq, Turkey, Russia, India, Libya	Zanjan: 15.6.1997	0.64	0.14
Melanocercis	*angustifolius* ssp. *angustifolius*	Iran, Turkey, Libya	Sanandaj: 16.6.1997	0.35	0.37
Onobrychoidei	*strictipes* Bornm.	Iran: Endemic	Tehran: 28.5.1997	0.20	0.36
Ornithopodium	*glochideus* Boiss.	Iran, Russia	Loshan: 28.5.1997	0.33	0.23
Ornithopodium	*schistosus* Boiss. and Hohen.	Iran: Endemic	Tehran: 28.5.1997	0.14	0.26
Theiochrus	*siliquosus* ssp. *siliquosus*	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.58	0.95
Theiochrus	*siliquosus* ssp. *siliquosus*	Iran, Iraq, Turkey, Russia	Sanandaj: 17.6.1997	0.09	0.26

[a] Proline content in some species was not determined for lack of root or leaflet samples.

According to the study of proline accumulation in *Tephrosia purpurea* Pers, proline content was higher in shoots, especially in leaves than in roots (Erakar & Murumkar, 1995). Higher content of proline in leaves than in roots under water stress has also been reported by Tyagi *et al.* (1999) in *Lathyrus sativus*. In *Atriplex halimus* proline accumulation in roots was less affected by salt stress than in leaves (Bajji *et al.*, 1998). It is generally assumed that when soil water supply is limited, shoot growth is more inhibited than root growth because of exposure to the dehydrating affects of the atmosphere (Sharp & Davies, 1989). A study by Westgate & Boyer (1985) showed that the growth of maize nodal roots is intrinsically less sensitive than that of the aerial parts of the plant at low water potentials in the growing regions.

The content of proline in both roots and leaflets of *Astragalus* species varied both inter- and intraspecifically. Very large differences in proline content were found in different species. These differences were also found in a single species collected from different regions (Table 1). Highest and lowest content of proline in roots was determined in *A. eugenii* Grossh. (1.29% DW) and *A. alyssoides* Lam. (0.07% DW) or *A. monspessulanus* ssp. *monspessulanus* (0.07% DW), respectively. According to the analysis of proline content in leaflets, highest and lowest content was observed in *A. siliquosus* ssp. *siliquosus* (0.95% DW) and *A. monspessulanus* ssp. *monspessulanus* (0.04% DW), respectively.

Regarding the proline content variation in various species and in a single species in different regions (Table 1), it has been reported that high concentration of proline are characteristic of water- or salt- stressed plants (Tymms & Gaff, 1979). Accumulation of compatible solutes occurs in many drought stessed plants and they act as cytoplasmic osmotica for osmotic adjustment (Smirnoff & Stewart, 1985). A 10- to 100- fold increase of free proline content occurs in leaf tissues in many plants during moderate water stress (Hanson & Hitz, 1982). Proline accumulation in growing cells act as cytoplasmic osmotica for osmotic adjustment as their water potential falls (Morgan, 1984). It has been suggested that because of the resulting maintenance of turgor, such adjustment allowed plants to maintain growth at low water potential (Sharp & Davies, 1989). Osmotic adjustment in mature leaves may also help to sustain photosynthesis by maintaining leaf water content at reduced water potentials (Morgan, 1983). However, proline may have other function, which include enhancing the stability of macromolecules and membranes (Smirnoff & Stewart, 1985).

It would suggest that proline which increase proportionally faster than other organic osmotica in plants under osmotic stress can be used as a water stress indicator, for selection of drought-resistant *Astragalus* species and varieties and to evaluate the osmotic status of natural habitats of the species.

Acknowledgements

The financial support of this research was provided by the Research Council of University of Tehran (project no. 513/3/558). The authors thank Dr. Ali Asghar Maassoumi from Research Institute of Forests and Rangelands, Tehran, Iran for determination of the *Astragalus* species. We are also thankful to Mr. Masoud Mir Maassoumi, Director of Plant Physiology Lab., for his help during the study and to Mr. Behzad Heidari, student of Biology, for his assistance in the analyses.

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(Received for publication 6 September 2001)