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SHORT COMMUNICATION

PRIMARY AND SECONDARY METABOLITES PRODUCED DURING DIFFERENT SEASONS AND PHYSIOLOGICAL STAGES IN SHANKHAPUSHPI (*CONVOLVULUS MICROPHYLLUS)*

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The present study was undertaken to examine the synthesis of various metabolites such as leaf pigments, proline, osmotic potential (OP), crude protein, phosphorus, alkaloids and phenols during different seasons and physiological (growth) stages in *Convolvulus microphyllus*. Leaf pigments, proline, total alkaloids and phenols were maximum in summer season during flowering stage. Winter season was most favourable for maximum phosphorus and crude protein during seedling and flowering stages, respectively. Maximum total sugars were observed in rainy season during seedling stage.

Key words: **Alkaloids, *Convolvulus microphyllus*, crude protein, growth stages, leaf pigments, osmotic potential, phenols, phosphorus, proline.**

The climate plays a dominant role in structuring its physical as well as biotic environment. For successful survival, desert plants must adapt to soil moisture stress, air dryness, high atmospheric temperature associated with intense solar radiations, etc. Water is one of the most dominant factors in determining the vegetation pattern of arid region (Sen 1982). Shankhapushpi (*Convolvulus microphyllus* Sieb. ex Spreng., Family: Convolvulaceae) is a prostrate perennial herb found all over India and very common throughout the sandy areas. The plant is an important source of drug in Ayurveda known as Shankhpushpi, which is used as brain tonic and laxative. The plant and leaves were found to be intellect promoting and efficacious in nervine affections, epilepsy, insanity, internal haemorrhages and bowel complaints (Khare 2004).

Chlorophyll contents are known to vary with species, age of the plant and growing seasons. Free proline accumulation in plants has been considered as one of the most widespread stress induced response, so its measurement would be an excellent stress detector. The accumulation of free proline depends on the type and intensity of stress (Tamayo and Bonjoch 2001). Carbohydrates in plants are considered as the energy reserve to be used under stress conditions and have been widely used as a physiological measure of stress tolerance. Environmental factors such as temperature and plant nutrition can modulate storage protein accumulation during development. Water stress causes both reductions in the role of protein synthesis as well as changes in the type of proteins produced. Phosphorus is also vital in the energy transfer compounds needed by both plants and animals to carry on their life activities. In general, seed crops contain the largest concentrations of phosphorus and forage crops contain moderate percentages (Troch and Thompson 1993). Environmental conditions affect the plant growth as well as the formation of alkaloids, because most alkaloids are formed in young and actively growing tissues (Waller and

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Nowacki 1978). Phenols are present in all plant parts, which are highly influenced by changes in environmental conditions.

Thus, different developmental stages of growth and seasons have profound effect on various ecophysiological parameters. Therefore, the present study was undertaken to obtain a better understanding of plant metabolic processes and their products such as leaf pigments, protein, sugar, proline, osmotic potential, phosphorus (primary metabolites), total alkaloids and phenols (secondary metabolites) under the influence of various seasons and physiological (growth) stages.

Leaf samples of *Convolvulus microphyllus* were collected from Medical College Campus, Jodhpur (4 km away from the J.N. Vyas University Campus, Jodhpur in northwest direction) during 2004-2006. Fully mature leaves facing to direct sunlight were collected randomly during rainy (July-September), winter (December-February) and summer (April-June) seasons, which were used for plant analyses. The leaf samples were collected at different developmental (physiological) stages such as seedling, vegetative and flowering in nursery raised plants. In nursery, plants were raised from freshly collected seeds pretreated with conc. H_2SO_4 for 45 minutes. The plants survived upto five months of seedlings emergence under nursery conditions, thereafter their growth parameters as well as biomass reduced significantly. Fresh leaves were analyzed for leaf pigments, proline and osmotic potential (OP), while remaining parameters were assessed from dry leaf samples. Leaf pigments were estimated as per Arnon (1949). Proline and OP were assessed according to Bates *et al*. (1973) and Janardhan *et al*. (1975), respectively. Total sugars (Plummer 1971), crude protein (Peach and Tracey 1955) and phosphorus (Allen *et al.* 1976) were estimated. Total alkaloids and phenols were quantified according to Higuchi and Bodin (1961) and Sadasivam and Manickam (1992), respectively. Experimental results were subjected to analysis of variance (ANOVA) and mean values of two years are presented here.

The data on various parameters such as leaf pigments, proline, OP, total sugars, crude protein, phosphorus, total alkaloids and phenols during different seasons (Table 1) indicate that total chlorophylls were maximum during summer. It is evident from Table 2 that total chorophylls were maximum at flowering stage, while lowest at seedling stage. According to Naidu and Swami (1996) the increase in chlorophylls during summer might be due to the flushing of new leaves. In the present investigations, *C. microphyllus* showed maximum total chlorophylls in summer season at flowering stage. The results suggest that increase in chlorophylls is indicative of high efficiency of photosynthetic apparatus under extreme desert conditions.

Maximum free proline accumulation was observed during summer followed by winter and minimum in rainy season (Table 1). Proline and OP values varied from 0.602 to 1.119 µg g^{-1} and –1.464 to –0.667 MPa during different stages, respectively (Table 2). Proline was maximum during flowering stage, while OP during seedling stage. Maribona *et al*. (1992) reported a decrease in OP under stress, which may be due to decreased availability/absorption and translocation of water from soil and roots and ultimately to leaves. *C. microphyllus* accumulated maximum proline during

Table 1. Seasonal variations in ecophysiological parameters of *C. microphyllus* (values are the mean of six replicates).

Parameters	Seasons			CD
	Rainy (July-Sept.)	Winter (Dec.-Feb.)	Summer (Apr.-June)	
Total chlorophylls (mg g^{-1} fw)	1.197	1.293	1.497	0.068**
Proline (µg g^{-1} fw)	0.605	1.686	1.690	0.477**
Osmotic potential (-MPa)	0.796	1.127	1.752	0.498**
Total sugars (mg g^{-1} dw)	96.82	75.40	32.84	2.53**
Crude protein (% dw)	17.76	27.34	11.35	0.68**
Phosphorus (% dw)	1.322	1.755	1.468	0.381**
Total alkaloids (% dw)	1.03	2.00	2.70	0.199**
Total phenols (µg g^{-1} dw)	401.70	438.59	618.23	5.580*

* & ** = Significant at P = 5 & 1%, respectively.

Table 2. Ecophysiological parameters of *C. microphyllus* during growth stages (values are the mean of six replicates).

Parameters	Stages			CD
	Seedling (20 DAS)	Vegetative (60 DAS)	Flowering (120 DAS)	
Total chlorophylls (mg g^{-1} fw)	0.419	0.605	0.950	0.029**
Proline (µg g^{-1} fw)	0.876	0.602	1.119	0.231**
Osmotic potential (-MPa)	0.667	1.464	0.704	0.08**
Total sugars (mg g^{-1} dw)	96.68	91.84	92.90	2.99**
Crude protein (% dw)	15.57	6.78	21.95	5.25**
Phosphorus (% dw)	1.854	1.48	1.541	0.0198**
Total alkaloids (% dw)	0.866	1.56	1.80	NS
Total phenols (µg g^{-1} dw)	28.64	253.48	416.73	17.954**

NS = Non-significant, ** = Significant at P = 1% and DAS = Days after seedling emergence

summer season with minimum osmotic potential, which showed direct correlations between these two parameters. The highest amount of proline during summer season can be related to tolerance towards water stress. Increased accumulation of proline under water stress is known to be affected by *de novo* synthesis of proline under induction of acute water stress to maintain normal osmoregulation (Chen and Kao 1993).

The highest values of total sugars were recorded during rainy season and lowest in summer season. These values were maximum and minimum at seedling and vegetative stages, respectively. The sugar content in leaves increased with the age and growth of plants (Mishra and Bhatt 2004). Xu and Huang (2003) reported that carbohydrate decreased during summer season in *Agrostis stolonifera*. Mohammed *et al.* (2000) reported maximum total sugars in *Trianthema triquetra* during rainy season.

In the present studies, the values of crude protein ranged from 6.781 to 27.343% of dry weight, being maximum in winter season. It was maximum during flowering stage. The soluble nitrogenous compounds play an essential role in plant metabolism, being the primary products of inorganic nitrogen assimilation and precursors of protein and nucleic acids. Mishra and Bhatt (2004) reported maximum protein content in winter season in *Leucaena leucocephala*. Mohammed and Sen (1988) observed highest protein content at flowering stage in *Trianthema portulacastrum.*

The phosphorus values ranged form 1.322 to 1.854% of dry weight, being maximum during winter and minimum in rainy season. During growth stages, it was highest at seedling, while lowest at vegetative stage. Naidu and Swami (1994) reported maximum phosphorus contents in *Gmelina arborea* during winter season. In the present studies, significant differences were observed in phosphorus content between seasons and developmental stages.

The total alkaloids content ranged from 1.03 to 2.70% and phenols from 401.70 to 618.23 µg g^{-1} dry weight during different seasons. These were maximum during summer followed by winter and minimum in rainy season (Table 1). Data presented in Table 2 clearly revealed that they were maximum at flowering followed by vegetative and minimum at seedling stage. Selvaraj and Chander (1978) reported maximum sennoside alkaloids during flowering stage in *Cassia angustifolia*. There is still considerable uncertainty as to whether phenolic compounds have a physiological role in plant growth and metabolism. Many phenols are clearly able to exert significant effect on growth processes when applied to plant tissues at physiological concentrations, but this does not necessarily imply that they have an endogenous role. According to Sen and Sharma (1996) phenolic content increased under water stress by hydrolyzing the glycosides. Verma and Kasera (2007) observed maximum total alkaloids and phenols in summer season during flowering stage in *Sida cordifolia*.

Thus, it is concluded from the present studies that in *C. microphyllus* summer season was found to be the most favourable for maximum production of leaf pigments, proline, total alkaloids and phenols during flowering stage. Maximum accumulation of phosphorus and crude protein were found during winter season at

seedling and vegetative stages, respectively. However, maximum accumulation of total sugars took place during rainy season at seedling stage.

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REFERENCES

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C. and Roberts, J.D. (1976). Chemical analysis. In: S.B. Chapman (ed.), Methods in Plant Ecology, pp. 412-466. Blackwell Scientific Publications, Oxford.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. I. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24:** 1-15.

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.

Chen, C.T. and Kao, C.H. (1993). Osmotic stress and water stress have opposite effects on putrescine and proline production in excised rice leaves. *Plant Growth Regul.* **13:** 197-202.

Higuchi, T. and Bodin, J.I. (1961). Alkaloids and other basic nitrogenous compounds. In: T. Higuchi and E.B. Hansen (eds.), Pharmaceutical Analysis, pp. 313-345. Interscience, New York, USA.

Janardhan, K.V., Murthy, A.S.P., Giriraj, K. and Panchaksharaih, S. (1975). A rapid method for determination of osmotic potential of plant cell sap. *Curr. Sci.* **44:** 390-391.

Khare, C.P. (2004). Encyclopedia of Indian Medicinal Plants: Rational Western Therapy, Ayurvedic and other Traditional Usage: Botany. Springer-Verlag, Berlin.

Maribona, B.R., Tenorio, J.L., Conde, J.R. and Ayerbe, L. (1992). Correlation between yield and osmotic adjustment of peas (*Pisum sativum* L.) under drought stress. *Field Crop Res.* **29:** 15-22.

Mishra, S. and Bhatt, R.K. (2004). Varietal differences in photosynthetic pigments and biochemical constituents in *Leucaena leucocephala*. *Indian J. Plant Physiol.* **9:** 86-89.

Mohammed, S. and Sen, D.N. (1988). Variations in two forms of *Trianthema portulacastrum* of the Indian desert. *Sci. & Cult.* **54:** 309-310.

Mohammed, S., Kasera, P.K. and Sen, D.N. (2000). Eco-physiology of *Trianthema triquetra* Rottler ex. Willd, a halophyte of Indian desert. *Ecoprint* **7:** 57-62.

Naidu, C.V. and Swami, P.M. (1994). Seasonal variation in N, P and K contents of different plant parts in tropical deciduous tree species. *Indian J. Plant Physiol.* **37:** 224-228.

Naidu, C.V. and Swami, P.M. (1996). Seasonal patterns of carotenoids and chlorophyll contents in some selected tropical deciduous tree species under natural conditions. *Ann. For.* **4:** 89-93.

Peach, K. and Tracey, M.V. (1955). Modern Methods of Plant Analysis. Springer-Verlag, Berlin.

Plummer, D.T. (1971). An Introduction to Practical Biochemistry. Tata McGraw Hill Publishing Co. Ltd., New Delhi.

Sadasivam, S. and Manickam, A. (1992). Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi.

Selvaraj, Y. and Chander, M.S. (1978). Senna: its chemistry, distribution and pharmaceutical value. *J. Indian Inst. Sci. Sect.* **60:** 179-196.

Sen, D.N. (1982). Environment and Plant Life in Indian Desert. Geobios International, Jodhpur, India.

Sen, S. and Sharma, V. (1996). Properties and interaction with phytohormones and phenolics: tonoplast ATPase of peanut seedlings. *Plant Physiol. Biochem.* **23:** 1-8.

Tamayo, P.R. and Bonjoch, P. (2001). Free proline quantification. In: M.J.R. Roger, (ed.), Handbook of Plant Ecophysiology Techniques, pp. 365-382. Kluwer Academic Publishers, The Netherlands.

Troch, F.R. and Thompson, L.M. (1993). Soil and Soil Fertility. Oxford University Press, New York.

Verma, V. and Kasera, P.K. (2007). Variations in secondary metabolites in some arid zone medicinal plants in relation to season and plant growth. *Indian J. Plant Physiol.* **12:** 203-206.

Waller, G.R. and Nowacki, E.K. (1978). Alkaloid Biology and Metabolism in Plant. Plenum Press, New York.

Xu, Q. and Huang, B. (2003). Seasonal changes in carbohydrate accumulation for two creeping bentgrass cultivars. *Crop Sci.* **43:** 266-271.