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Proline accumulation in *Blepharis sindica* T. Anders: A vulnerable medicinal plant growing in the Indian Thar desert

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ABSTRACT

Blepharis sindica is an important medicinal plant which used as an invigorating tonic, given to cattle to increase milk production and its roots are used for urinary discharge & dysmenorrhoea. In the present study, an attempt has been made to establish a correlation between proline accumulation and osmotic potential (OP) in Blepharis sindica, a vulnerable medicinal plant of the Indian arid zone. Results revealed that proline accumulation is associated with the increase in osmotic potential in this plant. The maximum amount of proline was recorded during late season (November-December) when the OP values were also more, whereas minimum proline was observed during onset of monsoon with initial phase of vegetative growth, i.e. in July- August.

Keywords: *Blepharis sindica*, proline, osmotic potential, stress, vulnerable, Indian desert

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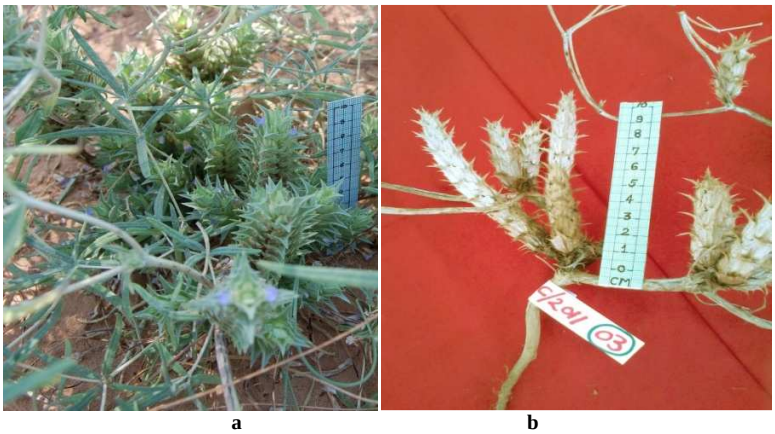
INTRODUCTION

The Indian Thar desert suffers from paucity of water, erratic rainfall, extremes of temperature and hence reduced plant productivity. In deserts, water demand by vegetation and crops is high but availability is restricted [1]. The uncongenial conditions impose heavy constraints on plants and make their survival difficult. Adaptations to adverse climatic conditions are the ways by which these plants maintain themselves despite the existing inhospitable conditions. These adaptations appear in form of morphological and physiological modifications, change in certain biochemical reactions/pathways and expression of certain genes [2]. As a result desert plants show an extra ordinary individual flexibility of structural and metabolic processes, which reflect the extremes of environment [3, 4, 5].

Plants during stress undergo osmotic adjustment by accumulating one or more low molecular weight organic solutes known as compatible osmolytes. These potent osmoprotectants play a vital role in counteracting the effect of osmotic stress [6, 7]. Proline is the most common compatible osmolytes in water stressed plants that do not interfere with normal biochemical reactions and make their survival possible under stress [8]. It is accumulated in various plants that are subjected to different stresses such as water, osmotic salts, low temperature, nitrogen deficiency, etc. [9, 10]. Proline acts as an osmotic mediator, maintaining proteic and membrane balance, inducer for osmotic stress linked genes, carbon and nitrate available sources, involved in cell dehydration, source reduction (proline catabolism) to support oxidative phosphorylation and ATP generation during recovery period after stress. The proline accumulation is not always governed by environmental factors; rather some innate factors are also responsible for it [11]. Proline related osmoregulation enables plants to maintain growth as plant water potential decreases [12].

There are more than 35,000 plants species being used in various human cultures around the world for medicinal purpose. Biologically active compounds present in medicinal plants have always been of great interest to scientist working in this field [13]. Medicinally important plant species, *Blepharis sindica* T. Anders (Billi Khojio/Bhangari/Unt-kantalo: Family: Acanthaceae) is with an about 45 cm height, dichotomously branched, woody annual plant. It is a typical species with its spikes coming from near the base; make it quite a distinct plant (Plate1). The large spike of previous year dries on the plant itself and turned blackened. In dense associations the plant becomes erect and rather attractive. Cream coloured seeds are of medicinal importance and boiled in milk and used as an invigorating tonic. It is also given to cattle to increase milk production. Its roots are used for urinary discharge and dysmenorrhoea. Powdered plant is applied locally on infections of the genitals and on burns [14]. UNDP [15] have published Red List Categories for 39 medicinal plants of Rajasthan State, out of which 19 are listed as Vulnerable, 12 as Endangered, 6 as Critically Endangered and one each of Near Threatened and Data Deficient, respectively. Out of these, *B. sindica* is considered as "Vulnerable". Thus, it is very important to conserve and know the adaptability of this plant. Hence, in the present study, an attempt has been made towards the proline accumulation and its correlation with osmotic potential during different months collected from various localities of the Churu district, a part of Indian Thar desert.



a **b**

Plate1. a, maturing plant with green spikes attached on basal nodes. b, ripened spikes.

MATERIALS AND METHODS

The leaf samples of *B. sindica* were collected from healthy plants at three different sites, *viz*. Khasoli (site-I; 10 km away in east direction from College Campus), Shyampura (site-II; 12 km in west-south) and Bootiyan (site-III; 10 km away in north from College Campus) during July to December 2010 & 2011. From December onwards plants starts dried up in natural habitats. The climatic conditions of all experimental sites are arid and more or less similar precipitations. The leaf samples were collected from similar nodes of different plants as far as possible to minimize the variability in sampling selection. Fully mature fresh sunny leaves were collected in the morning hours and estimation of proline & osmotic potential were carried out after randomly mixing the leaf samples in triplicate.

(i) **Proline** values were estimated using Sulphosalycylic acid (SSA) and special Ninhydrin (SN) method as suggested by Bates *et al*. [16]. Leaves (0.5 g) were crushed in 10 ml of 3% SSA to extract out the accumulated proline. After reacted with SN, the proline in supernatant was absorbed in Toluene, which is measured as OD at 700 nm against Toluene as blank.

(ii) **Osmotic potential (OP)** was evaluated by crushing 1.0 g fresh leaves in 50 ml of double distilled water. The suspension was sieved by muslin cloth and electric conductivity & suspension temperature were measured. Final values for OP were carried out as per method suggested by Janardhan *et al*. [17].

Experimental results obtained during both years were subjected to analysis of variance (ANOVA) as suggested by Gomez and Gomez [18]. The mean values of both years are presented in graphical forms. The CD values were calculated at 5% ($p > 0.05$) and 1% ($p > 0.01$) and compared with the observed differences. The results showed that

different parameters, seasons and their interactions produces significant effects on values at 99% ($p > 0.01$) probability levels.

RESULTS AND DISCUSSION

(i) **Proline:** The estimated values of proline accumulation in *B. sindica* leaves at different sites are given in Fig.1-3. At sites-I, II & III the proline content was analysed minimum (0.01µg g^{-1}) in July, which increased gradually till the end of growing season. On or after November, it reached to its maximum levels, when plants were in late senescence stages, open to face low temperature of winter and struggling for soil-water. Highest (13.77µg g^{-1}) value of accumulated proline was estimated in November at site-I, which is followed by (13.08µg g^{-1}) in December at site-III. *B. sindica* has a deep tap root system extending approximately 2-3 times more than of plant height, *i.e.* about 90 to 135 cm. As the tap root grows, the older parts get lignified. Thus, the plants rely on young root parts for its water requirements. Till September and October (during vegetative and flowering stages) the proline accumulation is mainly affected by moisture availability to the plant but in November and December, significant increase was noted that may be due to decreasing growth efficiencies of plant as well as cold stress that also support to accumulate more proline.

(ii) **Osmotic Potential:** The estimated values of OP in *B. sindica* leaves at different sites are given in Fig. 1-3. Minimum level of OP values was observed in July, with an increasing trend till September at all sites. After a fall in October, it again showed rise till December. Maximum (3.67 -MPa) value of OP was analysed in December at site-II, followed by 3.48 –MPa at site-III. Whereas, at site-I, OP was analysed maximum (2.19 –MPa) in November.

Inorganic ions such as Na^+, K^+ and Cl^-, accounted for most of osmotic potential in several species, but sugars and amino acids, especially proline are major osmoregulator in some vascular plants. The reason is probably the convenience of osmolytes storage in large, osmotically inactive molecules such as starch or protein, which may serve several functions and from which they can be retrieved under conditions of stress [19]. Boscaiu *et al*. [20] also investigated that the role of proline is as an osmoprotectants against the stress in plants. The present findings revealed that *B. sindica* growing at three different sites accumulated highest proline and OP values at the end of season, *i.e.* December. It appears that cold stress is also effective in proline accumulation as compared to water and high temperature. Low temperature dose not result into the less absorption of water, however, it causes more rapid biosynthesis of proline, which results in lowering of OP as suggested by Dashek and Erickson [8] & Mohammed and Sen [2]. On the contrary, in the present findings the values of OP are also increasing along with proline. It shows the positive relationship between proline and OP.

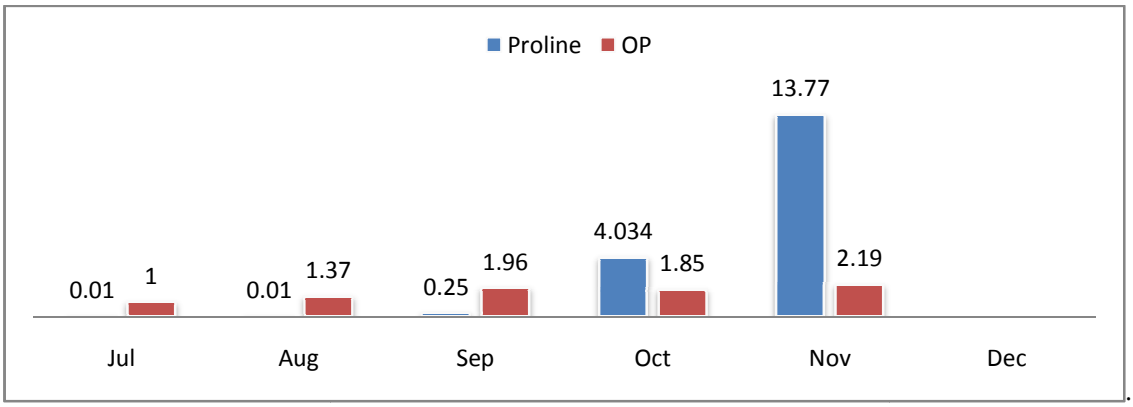


Fig.1. Monthly variations in proline (µg g^{-1} f. wt.) and OP (-MPa f. wt.) in leaves of *B. sindica* collected from site-I.

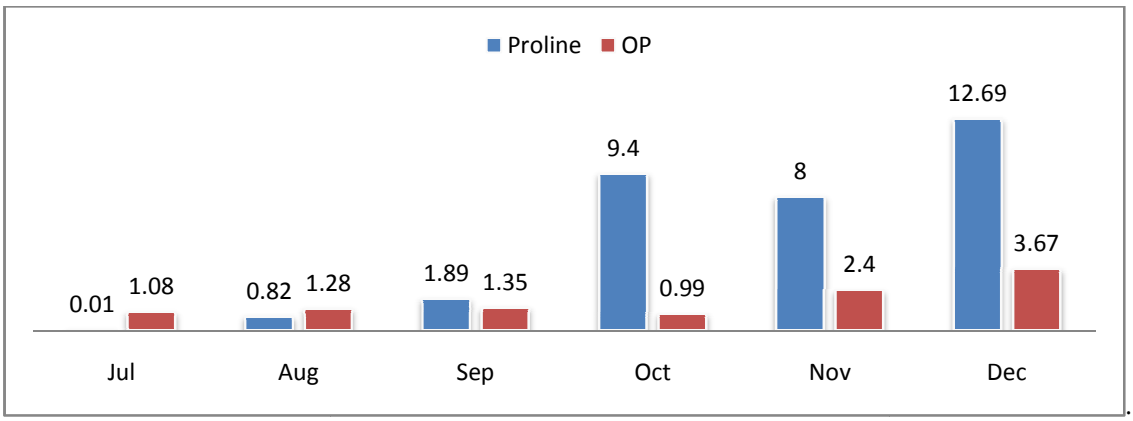


Fig.2. Monthly variations in proline (µg g^{-1} f. wt.) and OP (-MPa f. wt.) in leaves of *B. sindica* collected from site-II.

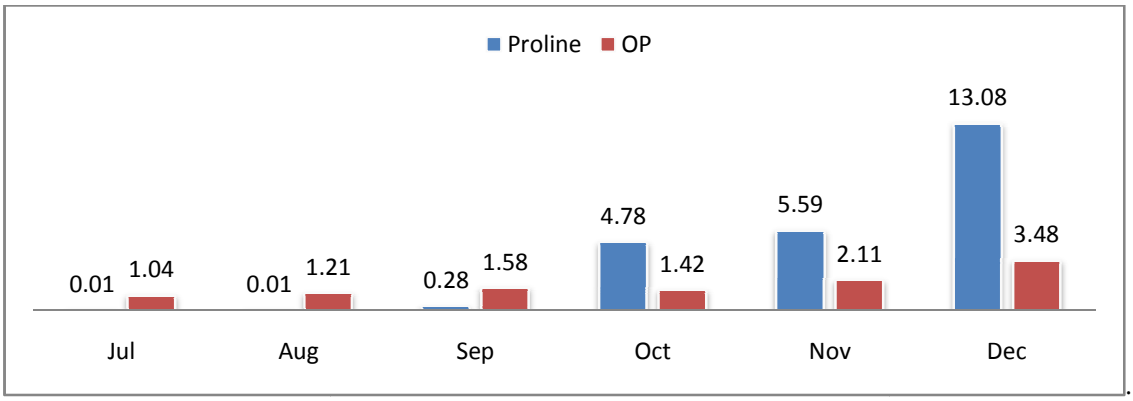


Fig.3. Monthly variations in proline (µg g^{-1} f. wt.) and OP (-MPa f. wt.) in leaves of *B. sindica* collected from site-III.

CONCLUSION

B. sindica growing at three different localities accumulates maximum proline and OP during December, which indicates positive relationship between both parameters. After monsoonal showers in July and August, the water availability for the species shrinks continuously. Before the metabolic efficiencies slow down, the plant shows adjustment toward stress. In late season, photosynthetic capabilities, water availability reduce and cold stress grows which push the plant to face extreme stress in December. Observed values of highest proline accumulation in December strongly support this. Thus, it is assumed that proline accumulation was associated with osmotic potential in plants, but some other factor(s) are also responsible for establishing correlation between proline and OP.

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