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Seasonal Variation in Free Proline Content in *Christella dentata* (Forssk) Brownsey & Jermy

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Abstract

Pteridophytes are shade loving plants which generally require low temperature and high humidity for their optimum growth. But in spite of xeric and hostile climate, a good number of pteridophytes grow in various parts of Rajasthan. In the present investigation, study on stress physiology of the fern Christella dentata has been attempted, by estimating free proline content in the leaves of this fern. C. dentata is an orophilous fern i.e. not specific to any particular altitude, habitat or exposure to sun. The species is also variable with regard to nature of rhizome, height of fronds and soil moisture. It often forms large dense colonies. It prefers well exposed fringes of forest and similar localities. Plants growing in shaded areas often have larger pinnae, greenish stipe and rachis. Water stress plays an important role in the functioning of various metabolic processes in a plant body. The influence of proline on the mechanism of drought resistance is well known in angiosperms but its role in pteridophytes is less studied.

INTRODUCTION

Ferns are the most conspicuous spore-bearing land plants. They have evolved remarkable adaptations to extreme environments, from tropical to cold temperate regions, from lowland to alpine zones and from xeric to aquatic conditions. Ferns are the oldest vascular plants in existence. To understand successful survival of ferns on land, efforts have been focused on the physiological ability and stress tolerance of both the sporophyte and the gametophyte generations.

Eco-physiological studies of the ferns of Rajasthan have always drawn much attention. This is perhaps because of the fact that the desert state has nearly 40 fern species growing luxuriously. These ferns have developed drought tolerance. Sharma and Bohra[15] were the first to study the eco-physiology of the pteridophytic flora of Rajasthan. They also suggested possible modes of perennation during severe periodic drought conditions of this region. Stress physiology of pteridophytes in general has largely been ignored as compared to morphology, anatomy and cytology. Low water deficit influences the pigment forming mechanisms in the chloroplast.
It is well described that the under stress conditions many species accumulate proline as an adaptive response to adverse conditions. Sharma and Rathore[16] investigated proline contents in 11 species of ferns of Rajasthan during stress and non-stress conditions. They confirmed presence of more amount of proline in drought resistant ferns than in aquatic or moisture loving plants. They further concluded

the influence of proline on stress tolerance in ferns is probably because of its effect on chlorophyll degradation and carotenoid accumulation.

Gena[7], Kaur[11], Yadav[17], Bhardwaj[2], D'Souza[5], Kumar[13], Rollan[14] and Gena[8] in their investigations of Rajasthan pteridophytes have concluded that higher accumulation of free proline provides endurance against drought conditions.

Plants reduce or prevent osmotic stress by making use of various phenomena relating plant anatomy and physiology with cellular mechanisms (Bray[3]; Evelin et al.[6]). Proline has been considered as a unique low molecular weight osmolyte which response to stresses related to osmosis in wide plant varieties (Delauney and Verma[4]; Hasegawa et al.[9]). The significant co-relation between proline accumulation and osmotic stress tolerance has been extensively reported earlier (Delauney and Verma[4]; Hong et al.[10]; Kishor et al.[12]). However, proline accumulation alone cannot be correlated with osmotic stress tolerance in plants.

MATERIAL AND METHOD

Pteridophytes are mostly found in and restricted to Aravalli range and south-eastern parts of Rajasthan. The present investigation is based on fresh plants collected from 3 sites during the months of December, June and August for two consecutive seasons. The three sites are-
Site I- Nakki Lake area, Mt. Abu
Site II- Sitabari, Kelwara, Baran
Site III- Sitamata Forest, Pratapgarh

All the three sites are situated in the southern and south-eastern part of the state. Site I lies in the valley of Aravalli ranges while the Site II is present in the Vindhyan scarpland in south east of the state. Site III is the confluence point of the two types of ranges.

Proline was estimated in the fresh leaves according to Bates et al[1]. It was extracted using 3% sulphosalicylic acid. The clear extractant was reacted with glacial acetic acid and ninhydrin in water bath and his reaction was terminated in ice bath. The chromophore thus developed was transferred in toluene for measuring its optical density at 520 nm. It was estimated as follows-

$$\text{Proline } (\mu g\ g^{-1}) = \frac{\mu g \text{ proline} \times \text{toluene (ml)} \times 5}{\text{Aliquot (ml)} \times 115.5 \times \text{sample weight (g)}}$$

Where,
μg proline = standard graph reading
115.5 = molecular weight of proline

RESULT

The amount of free proline was recorded to be variable at all the sites studied. The maximum amount recorded was at site III during the summers and minimum during rainy season at site I. Thus, it may be inferred that the free proline increases during stress and is directly proportional to the drought resistant capacity of the plant.

The values of proline are presented in Table 1 and Text Fig. 1. The values ranged from 3.08 to 10.56, 5.12 to 11.21 and 3.12 to 14.66 μg g^{-1}f.wt. at sites I, II and III respectively. The highest values were observed in summer and lowest in rainy season in all the three populations studied.

Table 1: Mean seasonal variation in proline (µg g^{-1} f.wt.) in the leaves of *Christella dentate*

Sites	Months		
	December	June	August
I	5.02	10.56	3.08
II	6.71	11.21	5.12
III	5.28	14.66	3.12

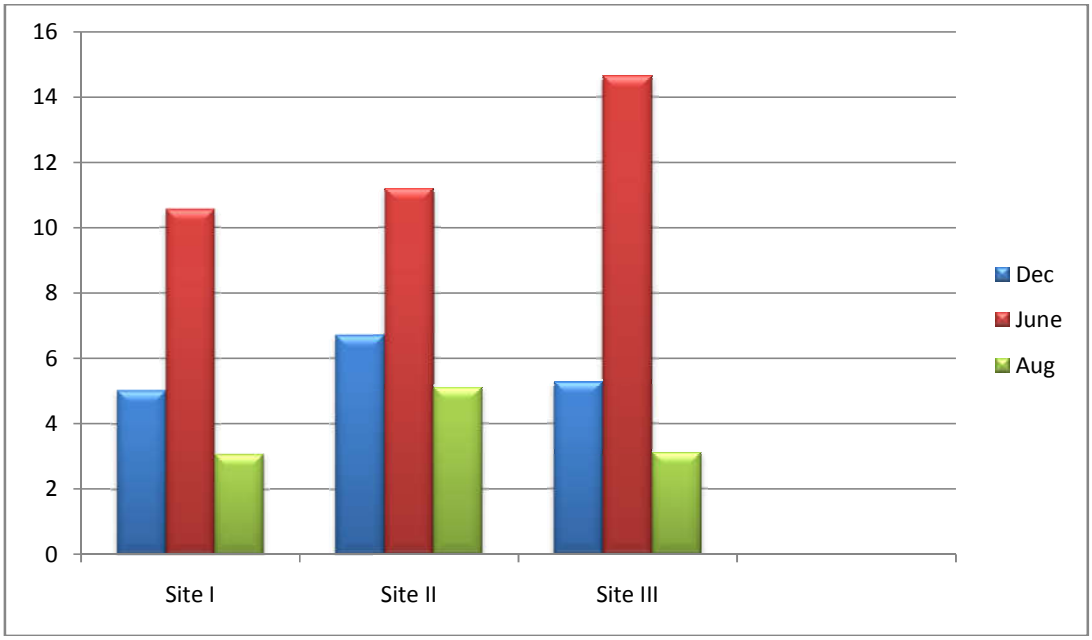

Figure 1: Mean seasonal variation in proline (µg g^{-1} f.wt.) in the leaves of *Christella dentata*

CONCLUSION

When plants are subjected to water stress, several metabolic changes take place. One of the important changes is the accumulation of proline. Proline is an amino acid and its accumulation is noticed within a few minutes of stress. During stress, protein is hydrolysed and results in accumulation of soluble nitrogen containing compounds such as urea and ammonium ions, which are potentially toxic to cells. Synthesis of proline may be involved in detoxification of ammonia. The higher value of proline may help plants to tolerate dehydration by maintaining cell turgidity.

In the present study *C. dentata* showed the highest values of proline in summer and lowest in rainy season at all the three sites. It has also been observed that total free proline is maximum in exposed ferns and minimum in shaded ferns. This indicates that the Vindhyan scarplands provide comparatively stressful habitat for the moisture loving ferns as compared to the soil of Aravalli ranges. Increase of proline has some correlation with stress metabolism.

It may be inferred that the free proline increases during stress and is directly proportional to the drought resistant capacity of the plants. This observation is in accordance with studies made by earlier workers.

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