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ORIGINAL RESEARCH

Proline accumulation patterns with relation to surface soil moisture in *Mollugo cerviana* (linn.) Ser. - a small-sized medicinal herb from the Indian arid zone

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ABSTRACT:

The present work deals with the proline accumulation in *Mollugo cerviana*, a drought escaping xerophytes from two different sites during July to October 2012-14 in arid conditions of Churu region. The Indian arid zone primarily suffers from moisture deficiencies all over the year excepting the precipitation days. During the present study, the lowest proline values were observed during July at both sites when monsoon showers moistened the upper-most soil layers as well as the plants were in juvenile stage. As the season proceeds, the proline accumulation showed an increasing trend till September, being highest (4.9165 µg g^{-1} f. wt.) from site-I. Interestingly, the values decreased during October when the plants were in nearly to dry indicating the enhanced protoplasmic respiration in senescence plants. It is obvious from the study that plants accumulate more proline against moisture scarcity in upper soil layers (0-5 cm) during July to September as compared to end of the season, *i.e.* October.

Keywords:
Mollugo cerviana, proline, water scarcity, soil moisture, medicinal, Indian arid zone

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INTRODUCTION:

The Indian arid zone ecosystem is a peculiar indication of climatic compel on its structural and functional entities. Uncongenial climatic conditions impose manifold constrains on arid plants of the Thar desert and make their survival complicated (Krishnan, 1977). In arid zones, water scarcity, temperature extremes, intense solar radiations, high wind velocity and rainfall limitations strictly tune the plant metabolism (Bartels, 2005, Srivastava, 2006). Small-sized drought escaping plants exhibit a high degree of developmental plasticity, being able to complete their life-cycle before physiological water deficit occurs (Manuela *et al*., 2003). Escape strategies depend on successful reproduction before the onset of severe stress.

This trend is important in arid regions, where native annuals may combine short life cycles with high rates of growth and gas exchange, using maximum available resources while moisture in the soil lasts (Maroco *et al*., 2000). Moisture stress induces the appearance of certain chemical compounds in plants, which may either be synthesized *de novo* or may result from the disintegration of the existing ones which lead adaptive responses in them through changes in the endogenous level of growth regulators and metabolic changes (Yadav *et al*., 2003).

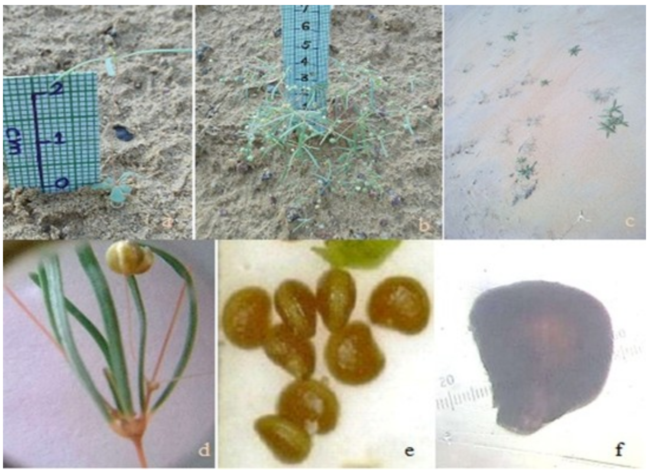

Figure 1: *Mollugo cerviana*: young seedling with dimorphic leaves (a), plants in vegetative growth with simultaneous flowering (b), association with *Gisekia pharnacioides* on sandy soils (C), nodal appendages at apex (d), fresh seeds (e), and close-up view of seed (f).

To counter diverse environmental stresses, many plants increase the accumulation of compatible osmolytes such as proline and glycine betaine (McCue and Hanson, 1990). The role of proline biosynthesis confers some adaptive advantage under water stress or triggers a consequence of stress-induced changes in metabolism (Cushman *et al*., 1990). Free proline [L-pyrrolidine 2-carboxy acid ($C_5H_9NO_2$)] accumulation in plants has been considered as one of the most widespread stress induced response, so its measurement would be an excellent stress detector. However, there are many controversial issues with proline accumulation and stress resistance (Mohammed and Sen, 1990; Sen and Mohammed, 1992; Ober and Sharp, 1994).

Mollugo cerviana (Linn.) Ser. (Chiria ka Bajara/ Chiria ka Khet; Fam: Molluginaceae) is a many-stemmed, erect, slender, annual herb (5-18 cm height) with fill form thin rigid branches growing in poor associations with other weeds (Fig. 1; a-c) bearing linear leaves in whorls at thickened nodes (Fig. 1; d). Perianth lobes have greenish white and membranous margins. Brownish compressed seeds are more or less triangular in outline (Fig. 1; e & f). It is a multifunctional annual herb useful in fever (Khare, 2007), gonorrhea (Anon., 2005) and to treat infected wounds (Valarmathi *et al*.,

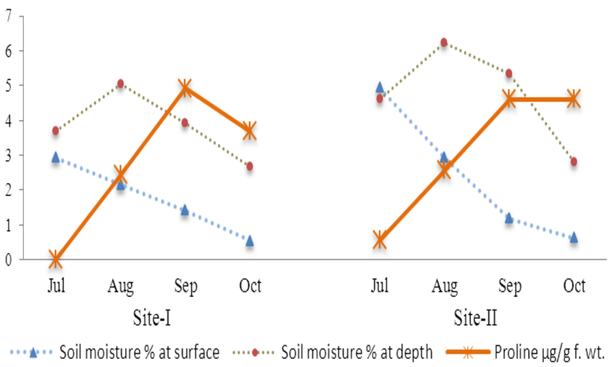

Fig. 2: Monthly variations in moisture % at surface (0-5 cm) and depth (20-25 cm) soil layers in comparison to accumulated proline (µg g^{-1} f. wt.) in leaves of *M. cerviana* collected from sites-I & II.

2012). Traditionally, dried plant decoction is orally given to cure typhoid fever among local tribes.

A complete understanding of stress-induced changes in the proline biosynthesis may reveal how plants adapt to environmental stresses. Hence in the present study, an attempt has been made to estimate the proline accumulation patterns in *M. cerviana* with relation to the soil moisture conditions at two different sites of Churu region, a part of the Indian arid region.

MATERIALS AND METHODS:

The leaf samples of *M. cerviana* were collected from healthy plants at two different sites, *viz*. Shyampura (Site-I; 12 km towards west-south) and Buntia (Site-II; 10 km towards north from the College Campus) during July to October 2012-14. The leaf samples were collected from similar nodes of different plants to minimize the variability in sample selection. Fully-matured fresh leaves were collected in the morning hours and estimation of proline was carried out after randomly mixing the leaf samples in triplicate. The estimation of proline was carried out according to Bates *et al*. (1973). The climatic conditions of experimental sites are arid with no significant differences. Soil moisture on surface (0-5 cm) and depth (20-25 cm) levels underneath *M. cerviana* plants were calculated as per Pandeya *et al.* (1968). To better reflect the relationship between proline and the moisture in surface and depth soil layers, the percentage changes in their values were calculated at successive monthly intervals, *viz.* Jul.-Aug., Aug.-Sep. and Sep.-Oct. The data were analyzed statistically as per the methods of Gomez and Gomez (1984). The mean values of two successive years are presented in tabular and graphical forms.

RESULTS:

The data on accumulated proline in *M. cerviana* leaves and the soil moisture (%) are presented in Fig. 2 which reveals that proline values invariably increased from July to September, with a steep fall in October at both sites. Among sites, the lowest (0.012 μg g^{-1} f. wt.) and the highest (4.9165 μg g^{-1} f. wt.) values were reported during July and September, respectively at site-I.

The values for soil moisture percentage at surface (0-5 cm) and depth (20-25 cm) levels exhibited negative changes during entire season, excepting depth layers during Jul.-Aug. at both sites. The changes in soil moisture at surface levels were more as compared to deeper ones, indicating the role of aridity to deprive moisture from soil (Table 1). The proline values changed most positively (+19333.33%) during Jul.-Aug. at site-I. The negative changes in proline values were highest for Sep.-Oct., being highest (-24.85%) at site-I. Proline data from both sites and surface & deeper layers soil moisture from site-II and site-I, respectively were significant at $p<0.05$, while others were non-significant.

So, the results showed that *M. cerviana* leaves

Table 1. Percentage increase (+) /decrease (-) during successive months in the values of moisture % at surface (0-5 cm) and depth (20-25 cm) soil levels in comparison to accumulated proline (μg g^{-1} f. wt.) in leaves of M. *cerviana* collected from sites-I & II.

Successive months	Site-I			Site-II		
	Soil moisture (surface)	Soil moisture (depth)	Proline (μg g^{-1} f. wt.)	Soil moisture (surface)	Soil moisture (depth)	Proline (μg g^{-1} f. wt.)
Jul.-Aug.	-26.37	+35.95	+19333.33	-40.77	+35.06	+366.12
Aug.-Sep.	-33.95	-22.27	+101.64	-59.25	-14.42	+80.08
Sep.-Oct.	-61.97	-31.97	-24.85	-47.90	-47.75	0.00

accumulated maximum amount of free proline during September. At the commencement of vegetative growth in July, it was lowest at both sites. During October, its values exhibited a steep fall. Invariably, the soil moisture amounts revealed a regular decreasing trend in addition to a high moisture loosing tendency from surface layers as compared to deeper ones at both sites with a clear exception for deeper layers from site-I during August.

DISCUSSION:

In a large part of their growth period, the arid plants are open to face moisture regulated constrains. Sum of all abiotic factors inflict dryness in arid habitat, which consequently lead to accumulate free proline in plants to compete their physiochemical slow down in spite of hampered habitat features. Life span extents (time amount) as well as plant body sizes (surface area) are the crucial ways in arid plants to minimize aridity interactions.

M. cerviana is a small-sized annual herb with a shallow root system. Interestingly, the plants grow in open sandy areas with a poor association with other weeds. Such conditions keep it restricted to thrive only on surface soil moisture contents. As soon as the moisture lasts in upper soil layers, the plants culminate their life within 2-3 weeks. Hence, this species is well known as moisture indicator in arid areas (Sen, 1982).

Proline accumulation is a very common aridity induced physiochemical stress indication in desert plants (Sen *et al.*, 2001). This amino acid acts a major osmoregulator in plants, probably due to 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 (Pugnaire *et al*., 1994). Boscaiu *et al*., 2011 also envisaged the role of proline as an osmoprotectant against the stress in plants. During studying ecophysiological changes in 23 different plant species from the Indian Thar desert in rainy (non-stressed), post-monsoon (water stressed and non-stressed conditions) and winter season (cold stress), Mohammed and Sen (1990) observed a clear positive correlation between dryness and accumulated proline amounts excepting a few stress resistant plants species. Lal *et al.* (2012, 2014) also reported dryness coupled proline accumulation with an increasing trend along the plant ageing in *Blepharis sindica* from Churu region.

The present findings reveal that *M. cerviana* growing at two different sites accumulated highest proline during September (post-monsoon), when the area experienced higher moisture losses from upper soil layers in comparison to July & August (monsoon). The surface soil moisture values were lowest in October, but its effects were minimal on proline accumulation indicating the retroactive proline metabolism in senescence or plants reaches to dry conditions in contrast to physiologically struggling during July to September. Moisture contents in deeper soil layers were not found in a way to control proline metabolism suggesting about the surface feeding mode of the species. Proline degradation in late season reflects the use of nitrogenous compounds as respiration substrates. In the present studies, it is assumed that proline accumulation in *M. cerviana* is associated with surface soil moisture deficiencies and plant growth tendencies till September, afterwards the plants trigger exceeded catabolism on cost of pre-accumulated osmoprotectants like the proline with its steep fall during October.

CONCLUSIONS:

M. cerviana is a small-sized shallow rooted herb, growing in poor association with wild grasses at most preferred open sandy areas. Its characteristic body plan, plant height and root extensions make it highly tuned species with that of surface soil moisture contents. In turn, the surface soil moisture amounts in arid areas of

Churu region are quite erratic because of low and interrupted precipitations coupled with accelerated evaporation from both soil and plants surfaces. In a very short life-cycle (July-October) the plants accumulated highest proline during September, *i.e.* matured plants were in physiologically most struggling stage against the prevailed moisture deficits, especially in surface (0-5 cm) soil layers. The steep fall in proline after September onwards indicated the slowed physiology when the senescence plants turned to sustain on pre synthesized phyto products including proline. Present study revealed that proline accumulation in *M. cerviana* is a function of surface moisture deficits and plant ageing determined by its shallow root system.

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