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Short Communication

Variations in Proline Accumulation and Osmotic Potential during Different Seasons and Physiological Stages of Some Arid Plants

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A large number of plants are exposed to water stress due to extreme soil water deficits in arid and semi-arid environments. The survival of plants in arid areas depends on the availability of water and their adaptation under stress, which involves the reduction of cell dehydration by avoidance or tolerance through osmotic adjustment (Morgan, 1984). Osmotic adjustment refers to the lowering of osmotic potential (OP) due to the net accumulation of solutes in response to water deficits or salinity (Munns, 1988). These solutes are potent osmoprotectants that play a role in counteracting the effect of osmotic stress (Yoshiba *et al.*, 1997). Proline is one of the most common compatible osmolytes in water-stressed plants that does not interfere with normal biochemical reactions and make their survival possible under stress (Stewart, 1981). The present study was carried out to understand the changes in osomtic potential and proline levels during different seasons and physiological (growth) stages in *Asparagus racemosus* (Shatavar, Fam., Asparagaceae), *Boerhavia diffusa* (Punarnava, Fam., Nyctaginaceae) and *Sida cordifolia* (Bala, Fam., Malvaceae) growing under natural conditions of the Thar Desert.

Proline and osmotic potential were quantified in selected plants during different seasons, viz., summer (May-June), rainy (July-August) and winter (December-January) as well as at the physiological stages (seedling, vegetative and flowering) during 2004 and 2005. The rainfall (July and August 2004; 9.7 and 139.5 mm, respectively; and July and August 2005; 132.9 and 77.2 mm, respectively) and stress condition (maximum temperature) during summer (May and June 2004: 40.7 and 39.7°C, respectively, and May and June 2005: 40.5 and 41.5°C, respectively) in both the years do not differ significantly. The plant materials of *A. racemosus, B. diffusa* and *S. cordifolia* were collected randomly form the natural habitats, viz., Targhar area (4 km away in north direction), Medical College Campus (5 km in northwest direction) and Mohanpura bridge area (7 km in northeast direction) from the JNV University Campus, Jodhpur, respectively. Proline was estimated in the fresh leaves according to Bates *et al.* (1973) and osmotic potential by Janardhan *et al.* (1975).

Five replicates were used and the experiments were performed twice during

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Table 1. Seasonal and physiological variations in proline and osmotic potential in A. racemosus, B. diffusa and S. cordifolia

Plant species	Seasons				Growth stage			
	Summer	Rainy	Winter	CD	Seedling	Vegetative	Flowering	CD
Proline ($\mu g\ g^{-1}$)								
A. racemosus	5.72	4.21	5.09	0.0418**	2.13	3.95	5.18	0.0205**
B. diffusa	1.34	0.70	0.94	0.0249**	0.57	0.87	1.17	0.0244**
S. cordifolia	4.18	2.22	3.31	0.296**	1.78	3.12	3.56	0.022**
Osmotic potential (MPa)								
A. racemosus	-3.11	-2.30	-2.82	0.142**	-1.02	-2.35	-2.56	0.129**
B. diffusa	-2.18	-1.22	-1.41	0.115**	-2.31	-2.62	-3.25	0.213**
S. cordifolia	-2.91	-2.08	-2.61	0.121**	-1.57	-2.11	-3.02	0.150**

** Significant at (P<0.01) level.

each season and physiological stages of plants for determining proline and osmotic potential during both the years. The data of two years were subjected to analysis of variance in strip-plot design (Gomez and Gomez, 1984), considering plant species as vertical and seasons or physiological stages as horizontal factors.

Asparagus racemosus had maximum proline content during summer (5.72 $\mu g\ g^{-1}$) followed by winter (5.09) and minimum in rainy (4.21) season (Table 1). The trend of proline levels in *B. diffusa* and *S. cordifolia* were also similar. Among the three species, *A. racemosus* accumulated maximum proline through the seasons, while *B. diffusa* showed the least accumulation across all seasons.

Similarly *A. racemosus* displayed maximum OP (-2.30 MPa) during rainy season, whereas the lower (-3.11) was in summer. In *B. diffusa* it ranged from -1.22 to -2.18 MPa, being maximum during rainy season followed by winter and minimum in summer (Table 1). The results were similar in *S. cordifolia*. The data showed a negative correlation between proline accumulation and osmotic potential. Two way ANOVA showed variations in both the parameters and their interactions during different seasons in all plant species were highly significant (P<0.01).

In *A. racemosus*, the proline accumulation in physiological stages ranged from 2.13 to 5.18 $\mu g\ g^{-1}$ and was in the order: flowering>vegetative>seedling. In *B. diffusa*, maximum proline content was at flowering stage and minimum at seedling stage (Table 1). In *S. cordifolia*, maximum proline was recorded at flowering, while minimum at seedling stage. The two way ANOVA showed significant (P<0.01) impact of different physiological stages on proline accumulation in all the plant species.

The osmotic potential in *A. racemosus* ranged from -2.56 to -1.02 MPa and was in the following order: seedling>vegetative> flowering stage (Table 1). The trends were similar in *B. diffusa* and *S. cordifolia*. The two way ANOVA showed significant (P<0.01) impact of different developmental stages on proline and osmotic potential in all plant species.

Proline accumulation is important for osmotic adjustment under abiotic stress conditions (Hyun *et al.*, 203). In addition to acting as an osmoprotectant, several possible physiological roles have also been attributed to supraoptimal level of proline such as stabilization of proteins, prevention of heat denaturation of enzymes and as a hydroxyl radical scavenger (Hsu *et al.*, 2003). Prakash (2001) observed maximum accumulation of proline in *Commiphora wightii* during summer. Saharan *et al.* (2001) observed maximum accumulation of proline during flowering stage in *Evolvulus alsinoides.*

Saharan *et al.* (2001) reported highest OP at seedling stage and lowest at flowering stage in *E. alsinoides*. Kasera and Shukla (2001) reported highest OP during rainy season followed by summer and minimum during winter in *Leptadaenia reticulata.* Prakash (2001) observed maximum and minimum values of OP during rainy and summer seasons, respectively in *Salvadora persica.* Sen and Mohammed (1992), Mohammed *et al.* (1998) and Sen *et al.* (2002) reported that proline accumulation in plants was accompanied by a decrease in osmotic potential.

In the present investigations, proline levels in all the three species substantially increased during the flowering stage and in dry period. Maximum accumulation of proline was observed in summer and minimum in rainy season in all the three plant species. *A. racemosus* accumulated higher proline contents, followed by *S. cordifolia* and lower in *B. diffusa* during the dry period. *A. racemosus* exhibited higher proline content during the dry period. On the contrary, *B. diffusa* showed lower proline content. These results indicate that the survival of *A. racemosus* in arid region depends upon increased proline and osmotic adjustments, whereas in other species it depends upon the availability of moisture during rainy season. In the present investigation, a negative correlation was observed between proline and osmotic potential. The maximum values of OP were observed in rainy season and minimum in summer season in all the three plant species. The lowering of OP in summer season was probably due to increased accumulation of proline.

The analysis of physiological stages revealed increased proline accumulation from seedling to flowering stage (flowering in all species occurred in winter) in all three plants species. There was a significant ($P<0.01$) variation in proline content among all the plant species and between growth stages ($P<0.01$) with maximum accumulation in *A. racemosus* and in minimum in B. diffusa. Maximum OP was observed at seedling and minimum at flowering stage in all the three plants species. Two-way interactions of species x physiological stages on proline and OP were also significant ($P<0.01$). Thus, it could be concluded that significant changes occurred in proline accumulation and osmotic potential during different seasons and physiological stages in all the plant species.

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