

## SEASONAL VARIATION IN WATER RELATIONS OF DESERT SHRUBS FROM KARACHI, PAKISTAN

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### Abstract

Seasonal variation in water potential, stomatal conductance, chlorophyll a and b, and proline content were measured in both young and old leaves of three desert species viz., *Calotropis procera*, *Senna holosericea* and *Aerva javanica*. Water status of young and old leaves showed similar pattern of seasonal fluctuation in all species studied. With an increase in water stress, water potential and xylem pressure potential became more negative. Stomatal conductance was highest in July when humidity was the highest. Overall chlorophyll b concentration of was higher in comparison to chlorophyll a. Proline concentration showed a peak during August and September. Lack of rainfall caused reduction of water potential and water evaporation with a corresponding decrease in proline content.

### Introduction

Desert plants generally follow two main strategies i.e., they tolerate the drought through phenologic and physiological adjustments referred to as tolerance or avoidance of drought through dormancy (Evans *et al.*, 1992). Both tolerance and avoidance mechanisms contribute to the ability of a plant to survive drought but it also depends on the frequency and severity of the drought periods (Alpert, 2000; Otte, 2001). Plants under such conditions regulate their water status using several tactics viz., osmotic adjustment, stomatal aperture, turgor maintenance, root distribution and leaf canopy properties (Rhizopoulou *et al.*, 1997).

Leaves developed under drought conditions generally exhibit small cell size, thick cell walls, small vacuoles and higher concentration of osmotica (Crawford, 1989). The water relation parameters, therefore, do not differ significantly between young and old leaves (Girma & Krieg, 1992). However in other species the ability to tolerate stress is related to leaf age (Ackerson, 1981; Nilsen *et al.*, 1983; Premachandra & Joly, 1992).

Drought in sub-tropical conditions is aggravated by the higher temperature and results into a reduction in stomatal conductance (Hester & Mendelsohn, 1989) associated with change in water content, xylem water potential and water potential (Bradbury, 1990; Shelden & Sinclair, 2000; Gulzar & Khan, 2000). It was also observed that under water stress, chlorophyll formation was markedly depressed and there is no linear relationship between the amount of water and chlorophyll and an inverse relationship was recorded between growth and chlorophyll content (William & Sharon, 1981).

Solutes known to accumulate with water stress and to contribute to osmotic adjustments of non-halophytes include inorganic cations, organic acids, carbohydrates and free amino acids (Munns *et al.*, 1983). Proline is the most common amino acids accumulating with water stress and may increase 100 fold in concentration over the pre-drought levels (Mohammed & Sen, 1987). Proline may be utilized for chlorophyll synthesis and may serve as reserve substance for the synthesis of chlorophyll upon relief of the stress (William & Sharon, 1981) suggesting that proline has some role in drought protection (Treichel *et al.*, 1984) and salt tolerance (Mulholland & Otte, 2002).

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*Calotropis procera*, *Aerva javanica* and *Senna holosericea* are shrubs commonly found growing in extreme xerophytic habitats, sandy areas, flat plains, degraded and disturbed areas, along road sides and hilly areas of Karachi and seem to withstand water stress very well and remain metabolically active through out the year. The aim of this study was to observe how these shrubs regulate their osmotic and water relations during various seasons under natural conditions.

### Materials and Methods

The study was conducted from May 1993 to April 1994, at Karachi University campus. The area is classified as semi-arid (June to September), maritime desert. Rainfall is low and usually occurs during monsoon season averaging about 225 mm per year. Maximum temperature during the study period ranged from 27° C to 36°C and minimum temperature ranges from 12° C to 28° C. From July to September, humidity was higher than the remaining part of the year and no rain was observed during this study period. The common species around the study area were *Prosopis juliflora*, *Calotropis procera*, *Acacia senegal*, *Salvia santolinifolia*, *Senna holosericea*, *Aerva javanica*, and *Iphonia grantioides*. Our study involved three xerophytic shrubs viz., *C. procera*, *S. holosericea*, *A. javanica*.

Five replicates from each species were collected of young and old leaves at 30 days intervals. Leaf samples were randomly collected on the 15<sup>th</sup> of each month. Care was taken to select young and old leaf i.e., the leaves of third node from the top of each plant were considered as young and those from the fifth node as old. Water potential ( $\Psi_w$ ) determination was made on leaf disc (5 mm.) using Wescor C-52 sample chamber and Wescor, HR-33T Dew point microvoltmeter. Leaf stomatal conductance was measured at about 11 am with AP-4 porometer (Delta-T devices) on ad axial surface of fully expanded young and old leaves. Xylem pressure potential was measured with the help of plant water status console (Wagtech International). Chlorophyll was determined by using the modified method of Maclachlam & Zalik, (1963) on a fresh weight basis. Proline was measured by the method of Bates *et al.*, (1973). Results of water relations, chlorophyll and proline content were analyzed by a three-way ANOVA to determine if significant differences were present among means (Anon., 1999).

### Results

Maximum temperature during the study period ranged from 27°C to 36°C with a minimum from 12°C to 28°C. From July to September, humidity was higher than the remaining part of the year and no rainfall occurred during this study period (Fig. 1). A three-way ANOVA of water potential showed highly significant effects of plant species in different months. Monthly variation in water potential of three species was highly significant (Table 1). Seasonal variation in water potential of young and old leaves was not high throughout the year. In *C. procera*, water potential was more negative in old leaves than in young leaves whereas in *S. holosericea* and *A. javanica* no particular pattern was observed for leaf age (Fig. 2). In all the three species water potential became more negative linearly in the end of study period. Minimum value was observed in May and maximum in March for *C. procera* and *S. holosericea* and in December for *A.*

*javanica*, *Senna holosericea* and *A. javanica* had more negative value than *C. procera* (Fig. 2).

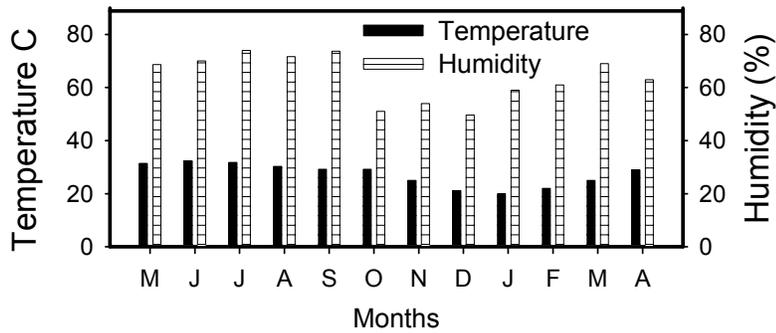


Fig. 1. Seasonal variation in temperature and humidity levels in Karachi desert.

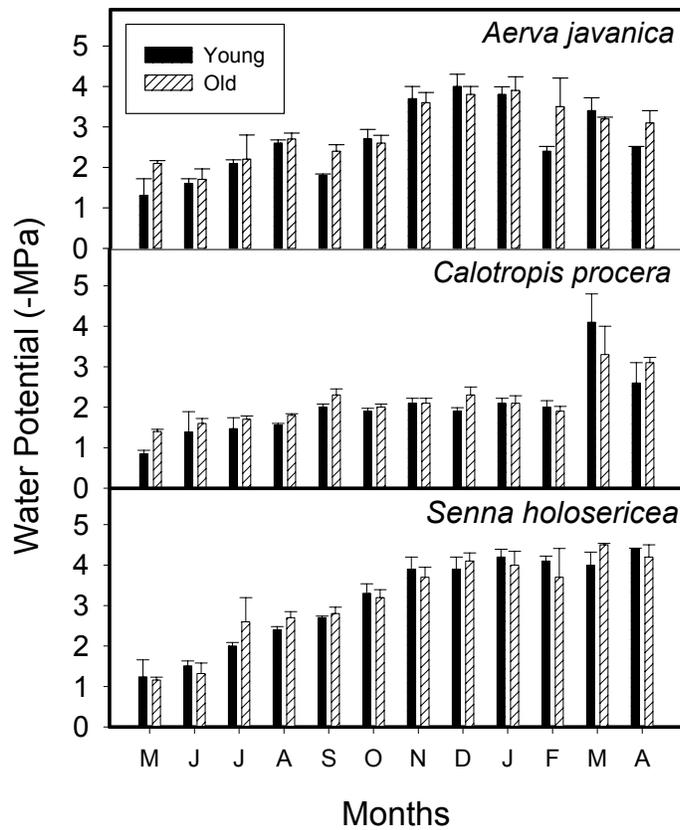


Fig. 2. Seasonal distribution of water potential in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

**Table 1. Results of the three-way analysis of variance of characteristics by month (M), plant species (Sp) and leaf age (La) of treatments**

Dependent Variable	Independent			Variable			
	M	Sp	La	M x Sp	M x La	Sp x La	M x Sp x La
Sc	47.3***	39.9***	24.4***	4.8***	1.3 <sup>n.s</sup>	2.6*	0.5 <sup>n.s</sup>
Wp	35.4***	73.0***	2.9*	4.7***	0.6 <sup>n.s</sup>	0.6 <sup>n.s</sup>	0.8 <sup>n.s</sup>
Pr	60.3***	37.7***	18.8***	2.8***	2.7**	17.3***	1.3 <sup>n.s</sup>
Ch a	26.9***	39.2***	2.2 <sup>n.s</sup>	10.6***	1.2 <sup>n.s</sup>	0.9 <sup>n.s</sup>	0.4 <sup>n.s</sup>
Ch b	1.0 <sup>n.s</sup>						
Ch a/b	12.9***	1.3 <sup>n.s</sup>	0.5 <sup>n.s</sup>	1.1 <sup>n.s</sup>	0.5 <sup>n.s</sup>	2.8*	1.1 <sup>n.s</sup>

Numbers represent F values: NS = not significant; \* =  $p < 0.01$ ; \*\* =  $p < 0.001$ ; \*\*\* =  $p < 0.0001$ .

Sc = stomatal conductance, Wp = water potential, Pr = proline content & Ch = chlorophyll content.

A two-way ANOVA of xylem pressure potential (XPP) showed a significant effect of plant species and their interaction in different months. Stem xylem pressure potential in *S. holosericea* and *A. javanica* was lowest in October. *A. javanica* had more negative XPP than *S. holosericea* (Fig. 3).

A three-way ANOVA of stomatal conductance showed a significant effect of month, plant species and leaf age, and interaction of month and plant species, and plant species and leaf age (Table 1). In all three species stomatal conductance of old leaves was higher than young leaves (Fig. 4). Maximum stomatal conductance was observed in July, decreasing substantially in January and again increasing until the end of study period. In general variation in seasonal pattern of stomatal conductance was similar for all the three species. Stomatal conductance was very high in *C. procera* as compared to *S. holosericea* and *A. javanica*.

A three-way ANOVA of chlorophyll 'a' showed a significant effect of month, plant species and their interaction (Table 1). No major difference was recorded in chlorophyll content of young and old leaves of all 3 species (Fig. 5). In *C. procera* and *S. holosericea*, maximum chlorophyll content was observed in October whereas in *A. javanica* highest values were observed in July. Lowest chlorophyll content was observed in April for *C. procera*, January for *S. holosericea* and August for *A. javanica*. Total chlorophyll content of young and old leaf samples was lower in *C. procera* than in the other two species (Fig. 5). In *C. procera* young leaves had more chlorophyll 'a' and 'b' than old leaves while there was not much difference in *S. holosericea* and *A. javanica*.

A three-way ANOVA of chlorophyll 'b' showed a non-significant effect of month, plant species, leaf age and their interaction (Table 1). Chlorophyll 'b' was higher than Chlorophyll 'a' in all the 3 species (Fig. 6). The ratio of chlorophyll a/b of young and old leaves was almost similar through out the year in all 3 species and a three-way ANOVA indicated no significant difference (Table 1, Fig. 7).

A three-way ANOVA of proline content showed a significant effect of month, plant species, leaf age and their interaction (Table 1). The most significant effect observed was for proline content as compared to any other parameter. The amount of proline was higher in young leaves of *C. procera* and *S. holosericea* than old leaves, while old leaves of *A. javanica* accumulated more proline (Fig. 8). In all the species, maximum proline content was observed in August and September, whereas minimum values were observed in March for *C. procera*, in May for *S. holosericea* and in April for *A. javanica*. The amount of proline was greater in *C. procera* than *S. holosericea* and lowest in *A. javanica*.

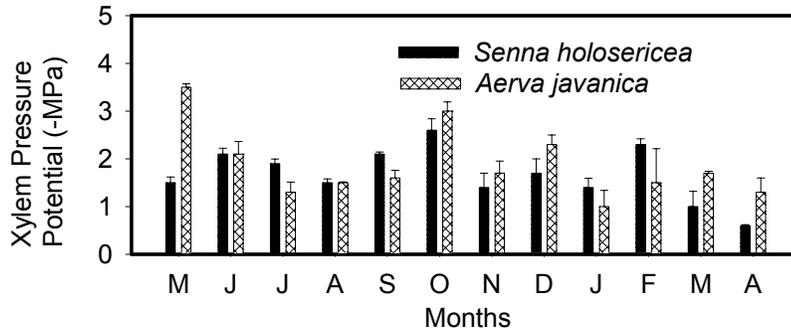


Fig. 3. Seasonal distribution of xylem pressure potential of *Aerva javanica* and *Senna holosericea* from Karachi.

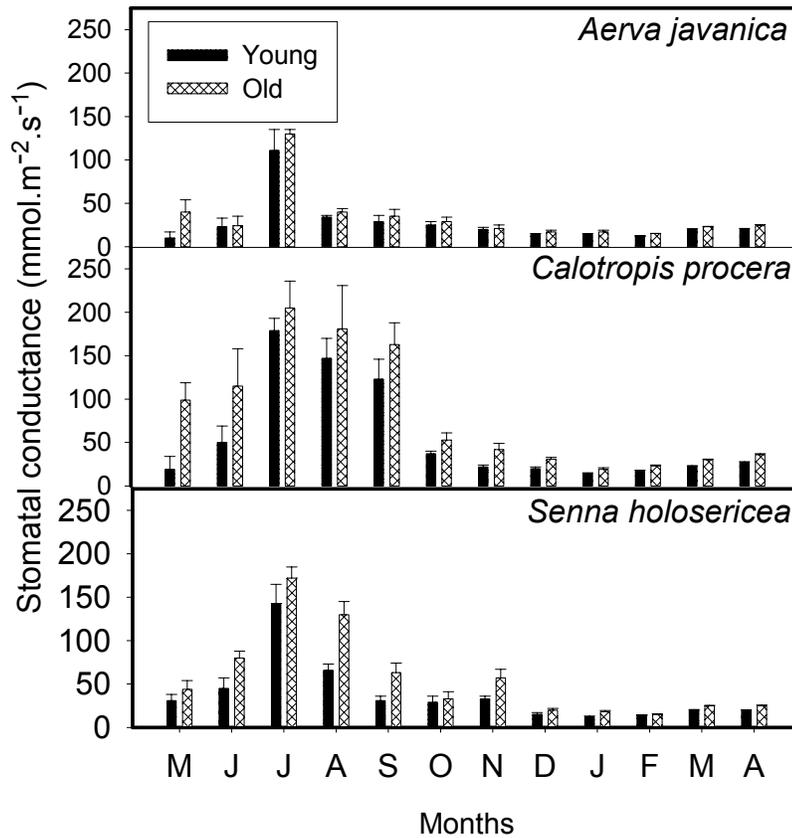


Fig. 4. Seasonal distribution of stomatal conductance in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

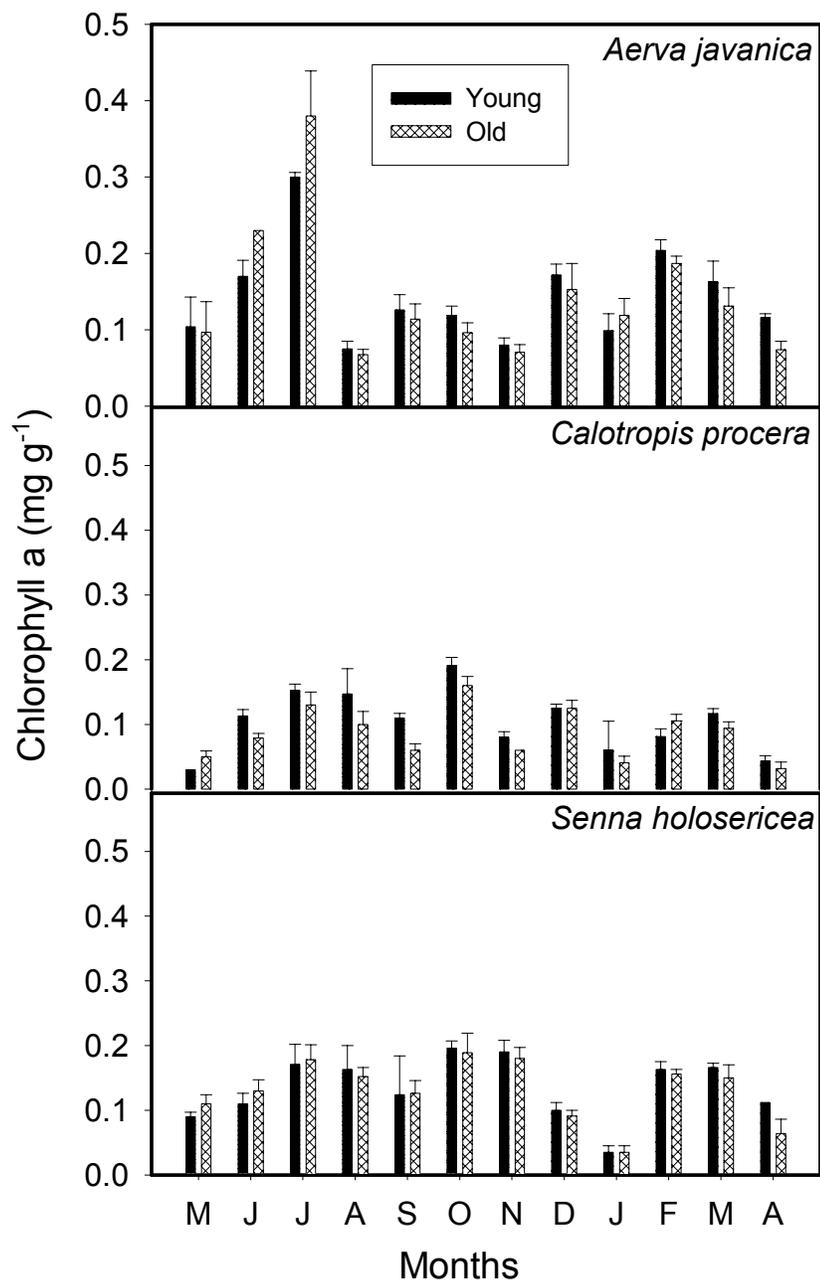


Fig. 5. Seasonal distribution of Chlorophyll a in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

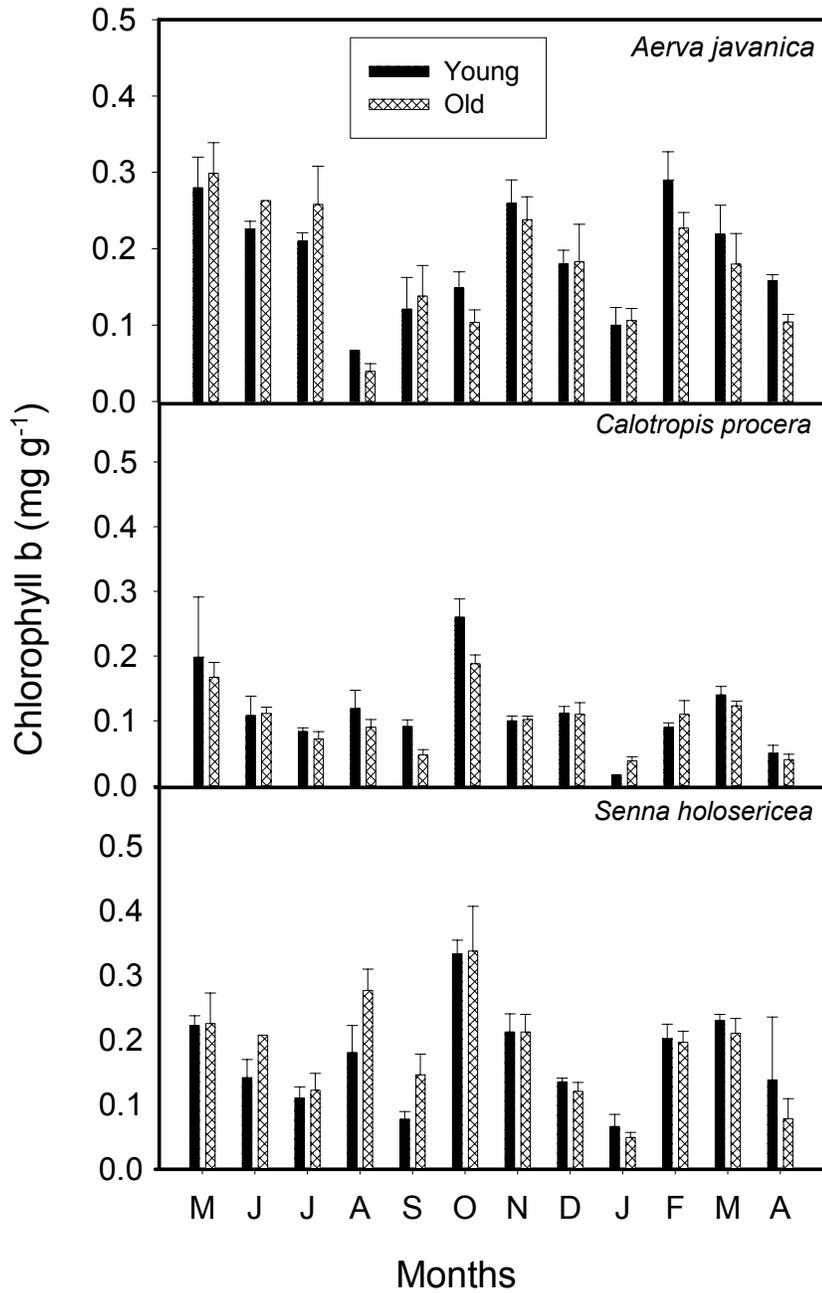


Fig. 6. Seasonal distribution of Chlorophyll b in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

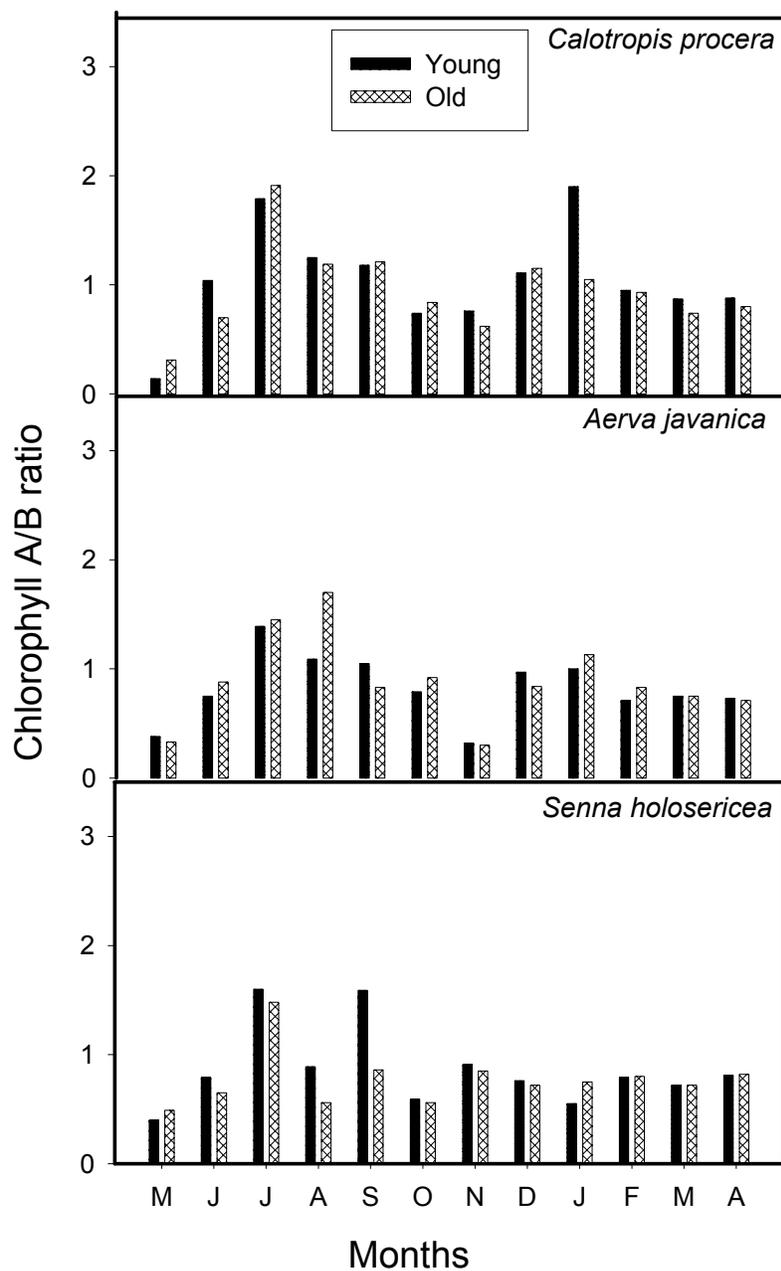


Fig. 7. Seasonal distribution of Chlorophyll a/b ratio in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

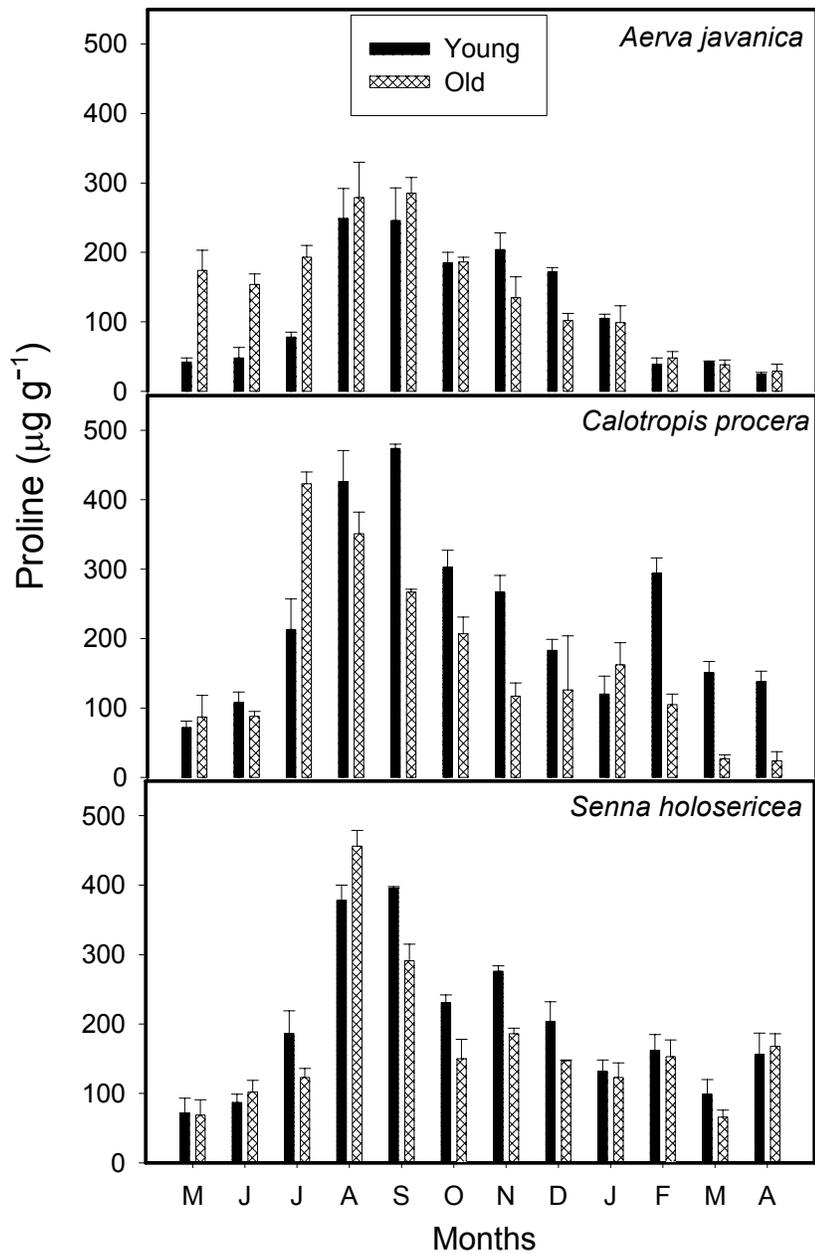


Fig. 8. Seasonal distribution of proline in the young and old leaves of *Aerva javanica*, *Calotropis procera* and *Senna holosericea* from Karachi desert.

## Discussion

Plants either avoid or tolerate periods of drought, often accompanied by high temperatures and excessive irradiance levels (Ehleringer & Cooper, 1992). The survival of plants during drought depends on the maintenance of cell turgor, by decreasing osmotic potentials through osmotic adjustments. Plants under water stress displayed a more rapid reduction in relative water content and leaf water potential than that which occurred during salt stress (Mattioni *et al.*, 1997).

The present work was conducted to study water relations of three desert shrubs with respect to seasonal fluctuation under water and temperature stress under natural conditions. The overall pattern for water relation parameters was similar in three desert species to withstand water stress during the study period. We did not find significant difference in water relations of young and old leaves of three species. Premachandra & Joly (1992) found that leaves of different ages or stages of development exhibit differential sensitivity to plant water deficit.

Water potential of all the three species varied significantly with the seasonal variation in temperature and moisture stress. Farrant (2000) believes that mechanisms to prevent physical damage from mechanical stresses imposed by turgor loss are also important. Less negative values for water potential were recorded in *C. procera*, which is highly succulent plant, than *S. holosericea* and *A. javanica*. Reduced water potential is a common consequence when plants are exposed to both salinity and drought stress, and plants tend to reduce their internal water potential under these stress conditions (Erdei *et al.*, 1990). More negative xylem pressure potentials were observed in October, the driest month of the year. Similarly, water potential also becomes more negative with the increasing drought.

The stomatal conductance of old leaves was significantly higher than young leaves with similar pattern of fluctuation in all species during the study period. The increase was more than twice in both leaf ages in the monsoon season (July) when humidity was higher and temperatures were lower in comparison to other months. Relative humidity increased from May to September and then decreased until December. Stomatal conductance was reduced in all the other months, perhaps as a mechanism to conserve water as stress increased (Gulzar & Khan, 1998). Stomatal conductance is sensitive to higher irradiance, higher temperature and a larger vapor pressure deficit, but only vapor pressure deficit causes a decrease in leaf stomatal conductance (Nilsen *et al.*, 1983; Schulze *et al.*, 1975). High stomatal conductance serves to maintain less negative water potential. In general all three species are minimizing unnecessary plant water loss under water stress.

There is no difference between chlorophyll content of young and old leaves of the three desert species. Total chlorophyll was low in all 3 species. Chlorophyll 'b' was slightly higher than chlorophyll 'a'. Such similar observations have been made by Smith & Morgan (1983) who reported higher concentration of chlorophyll 'b' under water stress. However, this variation was not associated with changes in leaf size as growth is inhibited by drought. Lower chlorophyll content under drought stress could be attributed to the higher rate of chlorophyll degradation in comparison to the rate of synthesis (Rhizopoulou *et al.*, 1991). The mechanism responsible for building up and maintenance of a solute gradient between chloroplasts and extra chloroplastic space are still unknown (Kaiser *et al.*, 1983). It was found that the species with low stomatal conductance and more negative water potential have higher chlorophyll content than the species with higher stomatal conductance and less negative water potential (Kaiser *et al.*, 1983). The mechanisms that may protect drought tolerant plants from light energy damage as they

wilt are curling, rolling, or folding of leaves, anthocyanin synthesis, xanthophyll metabolism, and reversible loss of chlorophyll (Alpert, 2000).

Proline content was significantly different in young and old leaf of three species. Proline accumulation was higher in young leaves of *C. procera* and *S. holosericea* whereas *A. javanica* had high proline in old leaves. In all species the maximum value for proline was observed in August and September. Accumulation of free proline is one of the most pronounced phenomena under water stress conditions (Rhizopoulou *et al.*, 1997; Zhu, 2002). Proline accumulates as a reserve substance for the synthesis of chlorophyll upon relief of the stress (William & Sharon, 1981).

There was no significant difference in the response of leaf age in the three species for any water relation parameter studied. Pattern of seasonal fluctuations in water potential, xylem pressure potential, proline content, stomatal conductance and chlorophyll content were similar for all species studied. Water stress increased with an increase in atmospheric temperature that subsequently decreases water potential, xylem pressure potential (more negative), stomatal conductance and chlorophyll content, whereas proline content increased.

Desert species in the sub-tropical maritime desert of Karachi are subjected to extreme conditions of warm temperatures and persistent drought. The area receives rainfall (220 mm/year) during monsoon season (July-August), however, there are several seasons when no rainfall is received. Perennial shrubs that dominate the area could only complete their life cycle if they have adaptations to survive under these extreme conditions. The local desert species employ various adaptations, such as increase in succulence, thick cuticle with the waxy layer, reduction in leaf size or leaves modified into thorns, etc. However, plant resistance to high temperatures and drought stress is primarily achieved by preventing water loss through decreasing internal water potential and closing stomates. This observation is in agreement with several previous reports on the water relations of desert plants (Alpert, 2000; Otte, 2001).

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