

## SEASONAL FLUX IN WATER POTENTIAL, CHLOROPHYLL AND PROLINE CONTENT IN PLANTS AT ZIARAT VALLEY BALOCHISTAN, PAKISTAN

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### Abstract

Seasonal changes in water relations and accumulation of proline during drought stress were assessed in different plant species viz., *Artemisia scopria*, *Juniperus excelsata*, *Onobrychis cornuta* and *Sophora alopecuroides* growing under arid region of Ziarat valley. Water status of the plants was evaluated by measuring pre-dawn water potential and water use efficiency of plants. All the plant species showed higher values for both parameters during the wet period of April when soil moisture and organic matter was abundant. In general, *Onobrychis cornuta* and *Artemisia scopria* had more negative water potential than *Juniperus excelsata* and *Sophora alopecuroides* over all seasons. A substantial decrease in water potential and water use efficiency was observed during dry period (October and January). Proline concentration peaked with a decrease in chlorophyll content during January in all species. The maximum proline concentration was observed in *O. cornuta* followed by *A. scopria*. As soon as drought stress was released following the melting of snow and spring showers in April, an increase in chlorophyll content with corresponding decrease in proline was observed.

### Introduction

The juniper forest of Ziarat has evolved in an unusual combination of soil and climate, highly calcareous, stony and shallow soil, receiving very low precipitation (200-350 mm per annum) and mostly comprising of xerophytic vegetation (Hussain, 1989). Snow is the dominant form of precipitation in these forests (Anon., 1988). Although spring showers are common from February to April, this forest type is beyond the reach of Southwest monsoon (Champion *et al.*, 1965). It is situated 133 km away from Quetta valley at an altitude of 2449 meters above sea level on the northern edge of Balochistan plateau (Lat. 30° 23' N; Long. 67° 44'E). The survival of land plants in such areas relies on the availability of water and their adaptation to stress (Kramer, 1984). Along with tree form *Juniperus excelsata*, most of the short stature shrubs are co-dominant in these areas including *Sophora alopecuroides*, *Artemisia scopria*, *Onobrychis cornuta* and *Thymus linearis* in four stands (Aziz, 2007; Unpublished data). The presence of such arid communities depends upon soil moisture depletion which strongly correlates with plant cover (Branson *et al.*, 1976). Plants grow sparsely leaving a wide area of bare soil (Hussain, 1989) that is the characteristic feature of arid environments (Burke & Mennheimer, 2003).

Plants during physiological drought vary in their response to water and salinity stress (Munns, 2002) and they lower their osmotic potential due to net accumulation of osmotically active solutes (Aziz & Khan, 2001; 2003; Mullholland & Otte, 2002). These osmolytes include proline and glycinebetaine in high concentration (Hasegawa *et al.*, 2000) and low molecular weight carbohydrates in stressed plants (Naidu *et al.*, 1992). Proline is one of the most common compatible osmolyte in water stressed and arid plants (Aziz & Khan, 2003) that does not interfere with normal biochemical reactions and make

their survival possible under stress (Stewart, 1981). It may increase 100 folds in concentration over the pre-drought levels (Mohammed & Sen, 1987) and also be utilized for chlorophyll synthesis during drought suggesting its role in drought protection (Treichel *et al.*, 1984). The tolerance of arid plant communities also depends upon the severity of drought (Alpert, 2000; Otte, 2001; Zhu, 2002) during which they not only accumulate compatible osmolytes but also regulate their water status (Rhizopoulou *et al.*, 1997) and increase water use efficiency by stomatal closure (Robinson *et al.*, 1997). Higher values for pre-dawn water potential with seasonal amplitude indicates the moisture availability for the plants (De Lucia *et al.*, 1988) and to minimize unnecessary water loss (Carrol *et al.*, 2001).

The presence of unique shrub species along with Juniper trees in arid conditions in Ziarat valley raises some important questions. First, the question of maintenance, how do these species persist in a desert like climate? Secondly, the question of drought tolerance, how these plant species behave during water stress? To answer these questions, present study was designed in Ziarat valley to understand the seasonal variation in water potential, water use efficiency and accumulation of proline under natural conditions.

### Materials and Methods

The experiments on different co-dominant plant species (*Artemisia scoparia*, *Juniperus excelsata*, *Onobrychis cornuta* and *Sophora alopecuroides*) were conducted at different timings of the year to observe seasonal variations. Different parameters were analyzed during the months of January, April, July and October (classified as winter, spring, summer and fall respectively). Water potential was measured with a plant water status console (Pressure bomb) on five shoots of each plant. For proline measurements, 0.5 g of fresh leaves was boiled in 10 ml of water for two hours at 100°C using a dry heat bath. This hot water extract was cooled and filtered using Whatmann no. 42 filter paper and then used directly to measure proline according to Bates *et al.*, (1973) and chlorophyll was determined by using modified method of Maclachlan & Zalik (1963) on a fresh weight basis.

Water use efficiency of primary productivity (WUEp) was calculated by using the formula:

$$\text{WUE} = \frac{\text{Organic dry matter production}}{\text{Water consumption.}}$$

For this purpose, 10 leaf samples were removed from each plant, collected in pre-weighed plastic bags, quickly stoppered, weighed and then removed. The leaves were then wrapped in papers, placed in an oven at 80°C and dried to constant weight. Organic content was then calculated after dry oxidation of leaf samples in blast furnace at 600°C. These weights were then converted into DM Kg<sup>-1</sup> H<sub>2</sub>O (Gram dry organic matter per kilogram water). For soil analysis, 20 samples were randomly collected from the area with the help of a corer. Titration method (acid neutralization) was used to calculate carbonates and bicarbonates pH was measured by the help of pH meter and organic content was determined by dry oxidation of soil in blast furnace at 600°C. Statistical analysis of the data was done using SPSS version 11.0 (2002).

**Table 1. Soil chemical properties in the Juniper forest.**

Months	pH	CO <sub>3</sub> (m.eq. / L)	HCO <sub>3</sub> (m.eq. / L)	Organic matter (%)	Soil moisture (%)
January	6.8 ± 0.2	2.5 ± 0.1	7.8 ± 1.1	5.2 ± 0.3	11 ± 1.3
April	5.5 ± 0.7	3.1 ± 0.4	9.4 ± 0.7	9.6 ± 1.3	19 ± 3.2
July	6.0 ± 0.2	3.4 ± 0.8	9.1 ± 1.2	9.1 ± 1.8	17 ± 2.5
October	6.8 ± 0.5	1.8 ± 0.2	6.9 ± 0.8	6.2.8 ± 1.2	13 ± 2.3
ANOVA for main effects					
Months	n.s	*	*	**	*
Soil properties	*	*	*	*	*

All data are means with S.E. ANOVA was used to differentiate soil characters in different seasons.

n.s = Non-significant; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$  and \*\*\*  $p < 0.001$

## Results

A one way ANOVA of soil analysis showed significant decrease ( $p < 0.05$ ) in soil moisture, organic content, carbonates and bicarbonates during the drier months (October and January), whereas pH of the soil remained more or less acidic throughout the study period and did not vary significantly (Table 1). The percentage of soil moisture and accumulation of organic matter in the soil was more pronounced during the wet period of April when snow started to melt in the area followed by spring showers indicating that biodegradation in the soil maximized during the humid conditions.

Pre-dawn water potential in all plant species was higher in April which was correlated with the availability of moisture (Figs. 1 & 2). A Bonferroni test indicated that water potential decreased significantly ( $p < 0.05$ ) from July to January when soil moisture was also less (Table 1). *Onobrychis cornuta* and *A. scoparia* had more negative values (ca -3 to -3.5 MPa) for water potential in comparison with *J. excelsata* and *S. alopecuroides* (ca -2.5 to -2.7 MPa) (Figs. 1 & 2). A significant increase in water use efficiency (WUE<sub>p</sub>) of plants ( $p < 0.05$ ) was observed with an increase in soil moisture and organic matter. It was maximum in *A. scoparia* (Fig. 2), followed by *J. excelsata* and *S. alopecuroides* (Fig. 1) and minimum in *O. cornuta* (Fig. 2).

The synthesis of chlorophyll in plants was also associated with the availability of moisture in the soil. It was maximum during the wet period of April in all plants (Figs. 3 & 4) when organic matter accumulation was also higher (Table 1). A significant decrease in chlorophyll production was also observed during the dry months of October and January. Values for chlorophyll "a" were higher than chlorophyll "b" in all seasons (Figs. 3 & 4) and were maximum in *J. excelsata*. The accumulation of proline was more pronounced during the dry months in comparison to the wet period of April (Figs. 3 & 4). *Onobrychis cornuta* had maximum proline content as compared to the other species. A substantial decline ( $p < 0.05$ ) in proline synthesis was observed in *A. scoparia* and *J. excelsata* with an increase in soil moisture, whereas *O. cornuta* maintained abundant amount of proline even during the wet period (Fig. 4).

## Discussion

Soil moisture depletion in arid plant communities occur due to low precipitation, causing drought stress (Larcher, 2003) hence only those plants are able to survive which can either avoid or tolerate drought periods (Ehleringer & Cooper, 1992). Plants under such stress follow osmoconformer strategy by lowering their tissue water potential through net accumulation of solutes (Aziz & Khan, 2003; Munns, 1988).

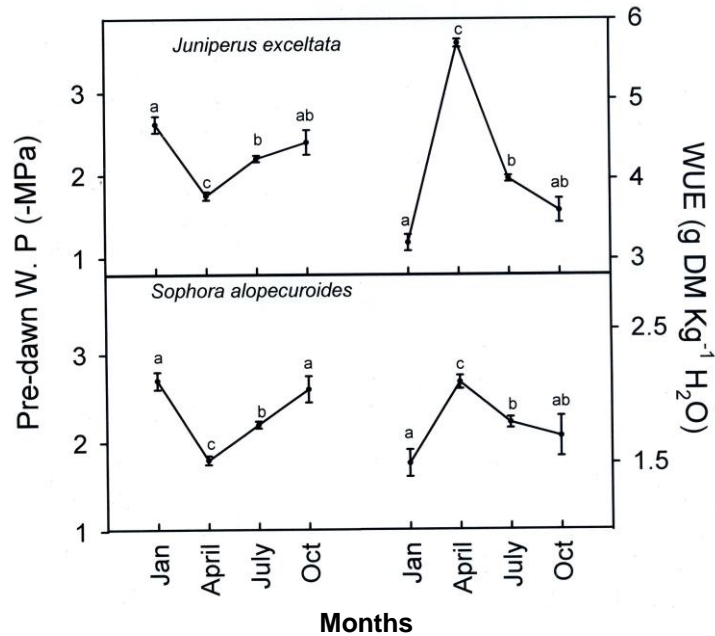


Fig. 1. Pre-dawn water potential and water use efficiency in leaves of different plant species. Different letters on error bars represent significant differences at  $p < 0.05$  (Bonferroni test).

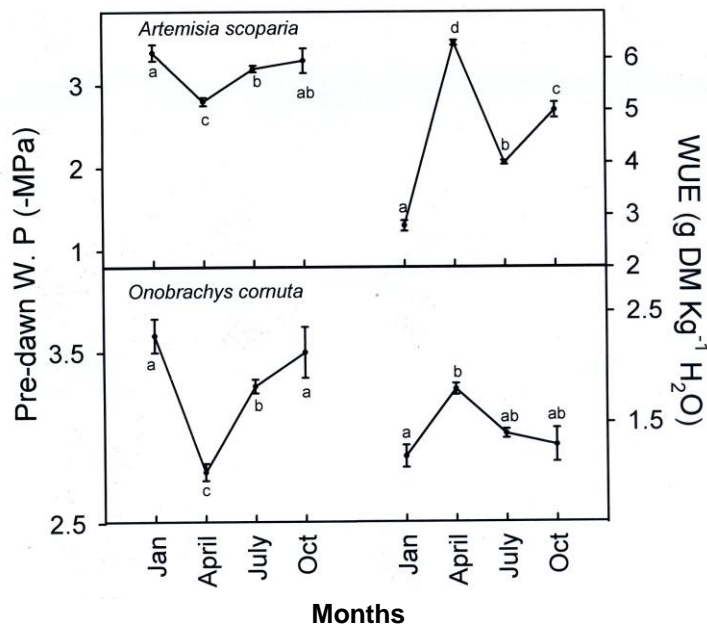


Fig. 2. Pre-dawn water potential and water use efficiency in leaves of different plant species. Different letters on error bars represent significant differences at  $p < 0.05$  (Bonferroni test).

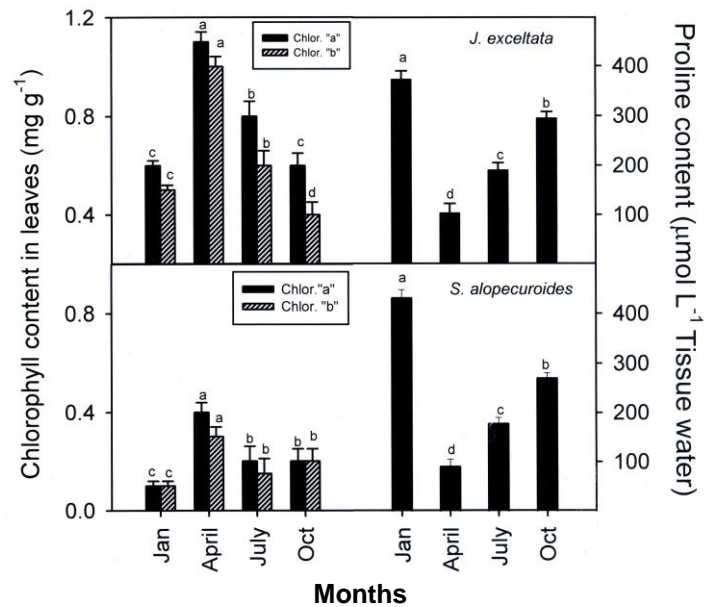


Fig. 3. Seasonal variation in chlorophyll and proline content in leaves of *Juniperus excelsata* and *Sophora alopecuroides*. Different letters on error bars represent significant differences at  $p < 0.05$  (Bonferroni test).

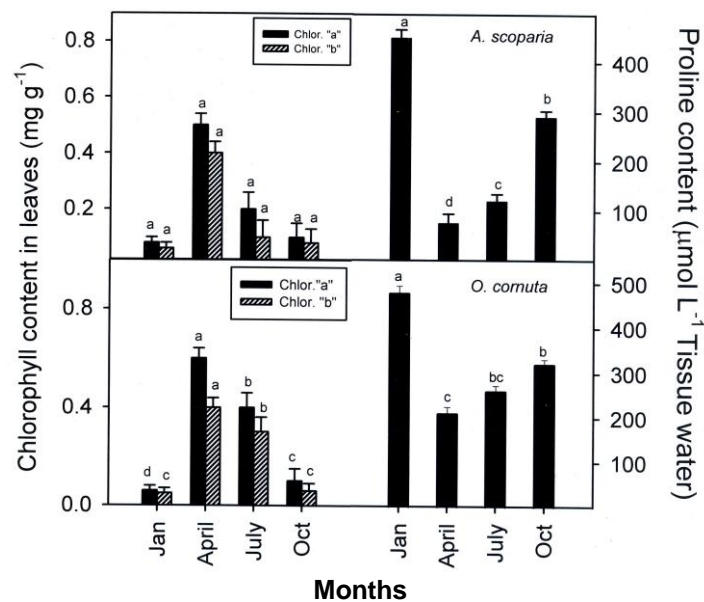


Fig. 4. Seasonal variation in chlorophyll and proline content in leaves of *Artemisia scoparia* and *Onobrachys cornuta*. Different letters on error bars represent significant differences at  $p < 0.05$  (Bonferroni test).

The overall pattern for pre-dawn water potential was similar in all species to withstand water stress during the study period. Maintenance of higher pre-dawn water potential in April indicated abundant moisture availability for all plant species although it was significantly different due to a difference in water use efficiency of the species studied. *Juniperus excelsata* showed little seasonal variation in water potential which maintained more positive values whereas, shrubs of short stature such as *A. scoparia* and *O. cornuta* had substantially lower pre-dawn water potential perhaps due to shallow rooting system than deeply rooted junipers. According to De-Lucia *et al.*, (1988), maintenance of higher water potential even during drought stress is the characteristic feature of the conifers as they are deeply rooted than short stature shrubs and a sudden drop in pre-dawn water potential in dry period occurs due to shallow rooting system. Plants respond in different ways to water and salinity stress (Munns, 2002) and reduced water potential in drier months prevents physical damage from mechanical stresses imposed by turgor loss (Farrant, 2000). Water use efficiency (WUE) is attributed to the extent of drought and salinity tolerance in plants (Larcher, 2003). Plants subjected to these stresses not only reduce their stomatal conductance to conserve water (Beena & Khan, 2002; Aziz & Khan, 2001) but also maintain higher water potential to minimize unnecessary water loss (Carrol *et al.*, 2001). Higher water use efficiency in plants correlates with higher water potential in some drought tolerant species especially during drier months (Campbell & Harris, 1977). Our results indicate that water use efficiency of plants increased substantially during April when soil moisture and organic matter were abundant and plants maintained higher water potential. In dry months, higher water use efficiency in *A. scoparia* and *J. excelsata* showed relatively small magnitude of seasonal change in water potential suggesting that these species were free of competition for water with other shrubs (*O. cornuta* and *S. alopecuroides*). Similar results were observed for water use patterns in *Artemisia tridentata* during extended dry periods (Campbell & Harris, 1977).

Solutes known to accumulate with water and salinity stress and to contribute to osmotic adjustments include inorganic cations, carbohydrates and free amino acids (Munns, 2002) and osmotically active solutes such as proline (Aziz & Khan, 2003) and glycinebetaine (Allakhverdiv *et al.*, 2003). These solutes not only reduce water potential (Aziz *et al.*, 2005) but also provide protection to biochemical substances in plants against desiccation (Mullholand, & Otte, 2002). Further, they can also be utilized as reserve substances for the synthesis of chlorophyll upon relief of stress (William & Sharon, 1981). Proline is the most common amino acid (Aziz & Khan, 2003) and may increase hundred folds in concentration over the period of drought (Mohammed & Sen, 1987). Our results are in agreement to this statement showing a substantial increase of proline in dry months. The maximum accumulation of proline in *O. cornuta* and its greater concentration even after the relief of stress suggested that it had greater role in drought protection and chlorophyll synthesis than in any other species. Lower chlorophyll content under drought stress could be attributed to the rate of chlorophyll degradation in comparison to the rate of synthesis (Rhizopoulo *et al.*, 1991).

Although the levels of proline were higher in *O. cornuta* on a persistent basis, almost all other plants possessed abundant proline during drier months of January and October. On the other hand, synthesis of chlorophyll was higher in *J. excelsata* perhaps due to increased water use efficiency and maintenance of higher water potential. This feature provides a possible explanation to *J. excelsata* as a dominant species in such an arid climate, whereas, shrubs of short stature adapt themselves by employing an osmoconformer strategy, reducing their growth and conserve water for longer periods of drought and found co-existing with junipers.

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