Effect of Water Stress on Relative Water and Chlorophyll Contents of *Juniperus Procera* Hochst. Ex Endlicher in Saudi Arabia

Ibrahim Aref, Hashim El Atta*, Pervaiz Khan, Mohamed Iqbal, Mudawi El Obeid, and Abdalla Ahmed

***Abstract*—**Water stress effect on RWC (needle relative water content) and chlorophyll content of *Juniperus procera* Hochst. ex Endlicher was studied in three locations in South Western Saudi Arabia. The species was subdivided into seedlings, saplings, mature and over-mature. Water stress had significantly reduced the RWC of needles and the effect was more pronounced in older trees compared to seedlings and saplings. Similarly, both chlorophyll *a* and *b* were significantly reduced and correlated with tree age.

Keywords*—Juniperus procera*; water stress, RWC, Chlorophyll content.

I. Introduction

THE effect of water stress on RWC (relative water content) has been investigated by several researchers. McCutchan and Shackel (1992) compared the relative sensitivity of plant- and soil-based measures of water availability for prune (*Prunus domestica* L. cv. french) tress by applying a range of irrigation regimes under field conditions. They found that leaf and stem water potentials (Ψ) were different between frequently irrigated trees and unirrigated trees, which were growing on stored soil moisture. Siddique et al. (2000) found that drought had decreased RWC in *Triticum aestivum* L. cultivars. Alexieva et al. (2001) stated that relative water content was the main factor which caused growth reduction in response. Earlier studies (Kaiser 1987) reported that photosynthesis was rather in-sensitive to dehydration down to 50 – 70% relative water content, and different plant species have a very similar response. Atteya (2003) investigated the impact of drought stress on the internal water status in three Egyptian corn (*Zea mays* L.) genotypes at different developmental stages. It was found that drought stress decreased the leaf water potential, relative water content and osmotic potential. It was also observed that in stressed plants the reductions in both leaf water potential and relative water content were associated with lower stomatal conductance and photosynthetic rate. In a field study, Tsuji et al. (2003) evaluated the drought tolerance of three sorghum (*Sorghum bicolor*) cultivars; Gadambalia, Arous elRimal and Tabat. It was observed that the leaf water potentials and relative water contents of Ghadambalia under wet and dry treatments were similar, while those of Tabat were significantly decreased by water stress. Under greenhouse conditions, Klamkowski and Treder (2006) examined the effect of water deficit on growth and plant physiological response of strawberry (*Fragaria* x *ananassa* Duch. Cv. Salut). It was observed that water-stressed plants displayed the lowest values of water potential coupled with a strong decrease in photosynthetic rates. Early drought responses were investigated in needles of 1-year-old seedlings of Norway spruce (*Picea abies*) when subjected to gradual desiccation for six weeks (Bloedner *et al*. 2007). It was found that drought exposure caused significant reduction in shoot water potential without any effect on needle relative water content. Rahimi et al. (2010) evaluated the effect of gradual drought stress and stress recovery in two plantago species (*Plantago ovata* and *P. psyllium*). The relative water content, RWC of both species was 90% up to two days after irrigation stop without significant difference from the control, but with intensification of water stress the relative water content was significantly reduced. The decrease in RWC coupled with further decrease in leaf water potential resulted 17 and 37% leaf chlorophyll content in *P. ovata* and *P. psyllium* at a RWC of 60% and/or leaf water potential of -1.5 MPa, respectively. Recently, Alvarez et al. (2011) evaluated the physiological and whole plant response of Callistemon (*Callistemon laevis*) plants to water deficit conditions under controlled conditions. It was found that the greater hydraulic resistance in water stressed plants caused decreases in leaf and stem water potentials with a consequent lowering in stomatal conductance indicating that water flow through the roots strongly influences shoot water relations..

II. Materials and Methods

A. The study area

The study was conducted in locations in south western Saudi Arabia. The region was dominated with *Juniperus procera* Hochst. ex Endlicher as pure woodlots and/or forests at high altitudes (> 2000 m.a.s.l.) or in association with *Acacia origena* Hunde (< 2000 m.a.s.l.). Table 1. shows

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locations in the study area. The Juniper ecosystem has been showing progressive decline due to biotic and abiotic factors. The common symptom of decline is the tip mortality where a greater proportion of Juniper is dying from the tips (El Atta and Aref, 2010).

B. Meteorological data

Meteorological data were collected using a Vantage Pro 2 solar operated weather station installed approximately in the middle of the study area. Data were collected for one year.

C. Measurement of RWC

Leaf RWC was calculated according to Morgan (1984) as follows:

$$RWC = [(Mf – M d)(Mt - M d)-1] \times 100$$

Where, Mf is the leaf fresh weight; Mt is the turgid weight and M d is the dry weight.

In each location the relative water content was determined in trees of different developmental stages wherever applicable (seedlings, saplings, young, mature and over-mature). To avoid water loss from the plants, sampling was performed during the early the morning. At each age a twig with several needles was detached from the leading shoot at the tips of the trees in healthy trees as well as from unhealthy ones (showing death of the tip branches. The detached plant materials were immediately placed inside well closed polythene tubes to avoid moisture loss through evaporation. From each twig, five needle samples were immediately weighed to determine the fresh weight. To estimate the saturated (turgid) weight all samples were soaked in distilled water for 24 hours at room temperature. Oven dry weight was obtained by placing the samples in an oven for 24 hours at 70 ± 1 °C.

D. Measurement of chlorophyll

After determination of the relative water content, the remaining leaf materials were stored in a fridge for chlorophyll content analysis (chlorophyll *a* and *b*). For chlorophyll extraction from the leaf tissues, N, N-Dimethylformamide, extra pure was used. Chlorophyll content was spectrophotometrically determined using Thermo Scientific GENESYS™ 10 Scanning UV/Visible Spectrophotometer with one cuvette position (Genesis 10-S, Thermo Fisher Scientific, Madison, USA). Chlorophyll *a* and *b* content was determined in three replicates each.

III. Results Discussion

A. Meteorological data

Metrological data recoded are summarized in fig. 1-3. Fig. 1. summarizes the rainfall in the study area (March2011-March 2012). No rains were recorded in the period March-June 2011. However, the rain onset started in July 2011 and increased gradually through August, September, Oct. and Nov. 2011. The period Dec.-March 2012 was characterized by very little to no rains.

No considerable fluctuations in temperature were recorded. Maximum temperature recorded was approximately 25 °C and can go down to about 10 °C at night (Fig. 2). Generally, temperature was mild over the study period.

The results of humidity and solar radiation were summarized in table 2. Humidity % ranged from 19.8 to 60.4 % and solar radiation 131.18-264.7.

B. RWC

1. Ain Al Ghalab

RWC recorded was 73.5-84.4% in healthy trees regardless of tree age and this was significantly more than in unhealthy trees (65.4-73.2%) (Table 3). The reductions in RWC were as follows: Saplings= 3.5%; Mature trees= 2.9%; - Over-mature trees= 19.6%.

2. Al Souda

The results were summarized in table 4. The recorded leaf RWC ranged from 75.6 to 84.4% in healthy trees and this was significantly more than in unhealthy trees (61.4-65.9%). The reduction of RWC was: Saplings= 21.9%; Mature trees= 18.8%.

3. Tor Al Yazeed

The trend was not different in this location where the maximum RWC occurred in healthy trees (74.6-78.6%) and much less in unhealthy trees (65.4-65.6%) (Table 5). RWC was reduced as follows: Saplings= 12.1%; Mature trees= 14.2%; - Over-mature trees= 11.2%.

C. Chlorophyll analysis

Generally, both chlorophyll a and b were highly significantly reduced in unhealthy as compared to healthy trees (tables 6-8). These reductions were as follows:

1. Ain Al Ghalab

Chlorophyll a: 90 % in s saplings; 42 % in mature trees; Chlorophyll b: 85.7 % in saplings and 45.5 % in mature trees.

2. Al Souda

Chlorophyll a: 30 % in saplings; 50 % in mature trees; Chlorophyll b: 57.1% in saplings and 50% in mature trees.

3. Tor Al Yazeed

Chlorophyll a: 44.8 % in saplings; 52.6 % in mature trees and 76.9 % in over-mature trees; Chlorophyll b: 56.3 % in saplings; 42.5 % in mature trees; 80 % in over-mature trees.

IV. Discussion

The results clearly indicated that unhealthy juniper trees were characterized by significant reductions in RWC and chlorophyll content. It may be concluded that all unhealthy and even some healthy *J. procera* trees encountered water deficit and hence the consequent adverse effects that followed. These effects were well established by several investigators. Alexieva et al. (2001) stated that relative water content was the main factor which caused growth reduction in response. Earlier studies (Kaiser 1987) reported that photosynthesis was rather in-sensitive to dehydration down to 50 – 70% relative water content, and different plant species have a very similar response. Early drought responses were investigated in needles of 1-year-old seedlings of Norway spruce (*Picea abies*) when subjected to gradual desiccation for six weeks

(Bloedner et al. 2007). It was found that drought exposure caused significant reduction in shoot water potential without any effect on needle relative water content. It was also observed that in stressed plants the reductions in both leaf water potential and relative water content were associated with lower stomatal conductance and photosynthetic rate. In a field study, Tsuji et al. (2003) evaluated the drought tolerance of three sorghum (*Sorghum bicolor*) cultivars; Gadambalia, Arous elRimal and Tabat. It was observed that the leaf water potentials and relative water contents of Ghadambalia under wet and dry treatments were similar, while those of Tabat were significantly decreased by water stress. Rahimi et al. (2010) evaluated the effect of gradual drought stress and stress recovery in *Plantago ovata* and *P. psyllium*. The relative water content, RWC of both species was 90% up to two days after irrigation stop without significant difference from the control, but with intensification of water stress the relative water content was significantly reduced. The decrease in RWC coupled with further decrease in leaf water potential resulted 17 and 37% leaf chlorophyll content in *P. ovata* and *P. psyllium* at a RWC of 60% and/or leaf water potential of -1.5 MPa, respectively. Drought stress significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content of three cultivars of chickpea (Mafakheri et al., 2010). Gholamin and Khatnezhad (2011) investigated the effect of drought stress on chlorophyll fluorescence and chlorophyll content of leaf in five maize genotypes. The authors showed that drought had reduced chlorophyll content and fluorescence as well as grain yield. Chlorophyll content was reduced by varying degrees in *Avena* species cultivars as a result of moisture stress at vegetative and flowering stages (Pandey et al., 2012). Water stress also significantly reduced chlorophyll a, chlorophyll b, total chlorophyll and net photosynthesis of oriental lily plants (Zhang et al. 2012). In Maize, chlorophyll loss due to water stress has been attributed to reduction in the lamellar content of chlorophyll a/b-protein (Randall et al., 1979). Water deficit was reported to reduce stomatal conductance and net photosynthetic rate of common bean (Santos et al., 2009).

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