

## Morphological and Physiological Responses of *Acacia saligna* (Labill.) to Water Stress

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**Abstract:** This study aimed to investigate some morphological and physiological responses of *Acacia saligna* (Labill) grown under water stress for 14 weeks. In a pot experiments, one year old *Acacia* plants were treated with five irrigation levels i.e., 100% of the field water capacity (FWC) as a control treatment and 80, 60, 40, and 20% of the control. The initial significant reduction of shoot or root fresh weights (threshold level) was recorded at 40% of FWC, meanwhile, leaf area was significantly decreased at 20% irrigation level. Osmotic potential in leaves and roots was gradually decreased by reducing irrigation levels. This reduction of osmotic potential was associated with significant accumulation of K<sup>+</sup> and proline contents either in leaves or roots. Sodium content in leaves and roots was not affected by water stress, while, Ca<sup>++</sup> content significantly declined in leaves but increased in roots. Water deficit caused a significant decrease in stomatal pore area/leaf area and an increase in stomatal closure percentage.

**Key words:** Water stress, K, Na, Ca, Proline, Osmotic potential, Scanning electron microscope, Stomatal closure, Stomatal pore area.

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### INTRODUCTION

In arid and semi arid regions, water is the most important limiting factor for growing plants. Water stress adversely influences growth and metabolism of many plants, the responses depending on severity and duration of the stress, plant genotype, developmental stage and environmental factors (Bray, 1993). Plant have strategies to overcome drought stress normally involve a mixture of tolerance and stress avoidance mechanisms. The tolerance strategies involve immediate physiological and biochemical responses, whereas the avoidance strategies involve long-term developmental and morphological traits (McCue and Hanson, 1990). One potentially important mechanism of drought tolerance is osmotic adjustment, defined as a decrease in osmotic potential of cell sap resulting from more organic and inorganic solutes in cell. A decrease in the osmotic potential of cell sap lead to reducing the cellular water potential below the external water potential, which allows water to move into the plant cells. Under stress conditions this is believed to postpone dehydration and may be to benefit processes dependent on positive turgor, such as cell elongation and stomatal opening (Sánchez *et al.*, 1998).

*Acacia saligna* (Labill.), belongs to family Mimosaceae is known to be drought resistant. Nativ *et al.* (1999) found that osmotic potential of phyllodes of *A. saligna* decreased with reduced availability of water. Also, Tadros (2003) demonstrated that water stress reduced fresh and dry weights of shoots and roots, and increased the accumulation of proline in some forest tree species.

Many investigators studied the effect of water stress on growth and chemical composition of several plants. Li and Wang (2003) stated that total biomass, leaf area and leaf stomatal density of *Eucalyptus microtheca* decreased under water deficit. Whereas, Martinez *et al.* (2003) reported that osmotic potential decreased and Na<sup>+</sup> and K<sup>+</sup> contents increased in leaves of *Atriplex halimus* in response to water stress. While, Aranda *et al.* (2005) declared that water stress decreased leaf osmotic potential of *Quercus suber*. Meanwhile, Inclán *et al.* (2005) indicated that water stress decreased Ca concentration in twigs with a diameter below 3 mm, plant growth and aerial biomass, and increased K concentration in twigs with a diameter above 3 mm in *Pinus halepensis* seedlings. However, Bañon *et al.* (2006) clarified that water stress reduced total leaf area, shoot and root dry weight and leaf osmotic potential at full turgor, and increased leaf stomatal density of *Nerium oleander*. Also, Ren *et al.* (2007) concluded that water stress decreased leaf area of *Populus cathayana* and increased proline concentration.

The aim of this investigation was to study some morphological and physiological responses of *Acacia saligna* (Labill.) grown under water stress.

### MATERIALS AND METHODS

Two pot experiments were carried out to study the effect of water stress on one year seedlings of *Acacia saligna*, during two consecutive seasons of 2006 and 2007, from the first of March to the tenth of July in both seasons. Trials took place in a greenhouse at Faculty of Agriculture, Ain Shams University. Each pot (30×40

cm) was filled with 13 kg of washed sand. Plants were transplanted into the pots (one plants in each pot) and then irrigated with the nutrient solution (Abd-Elmoniem *et al.*, 1997) until the end of the experiment.

Pots containing one plant were subject to one of five soil water levels; 100% (control), 80, 60, 40 and 20% of water field capacity (determined by pre-experiment).

Leaf area and fresh and dry weights of shoots and roots were measured at the end of the experiment. Potassium, sodium and calcium contents in leaves (the third and fourth fully expanded leaves from the top of the plant) and roots were measured using flame photometer (JENWAY model PFP7). Proline in leaves and roots was determined by the colorimetric method according to Magné and Larher (1992). The osmotic potential of leaves and roots was estimated using an osmometer (Osmomat 030). The stomatal pore area/leaf area (%) and stomatal closure (%) for the third leaf (fully expanded mature leaf) from the top of the plant from different treatments were examined and calculated using SemAfore software program linked to a JOEL JSM-T330A Scanning Electron Microscope.

Stomatal pore area/leaf area (%) = stomatal pore area / leaf area  $\times$  100

Stomatal closure (%) = No. of stomata closed / No. of total stomata  $\times$  100

A randomized complete block design with three replicates for each treatment and five plants in each replicate was implemented. Analysis of variance for the obtained data was performed by using computer software MSTAT-C (Freed, 1992) to analyze significant difference among treatments. The means were compared according to Duncan's multiple range test at a probability level of 5%.

### Results:

Results presented in Fig. (1) illustrated that water stress reduced shoots and roots fresh weights. However, the growth was significantly decreased at 40% irrigation level (the threshold). The  $C_{50}$  value was recorded at 33% irrigation level.

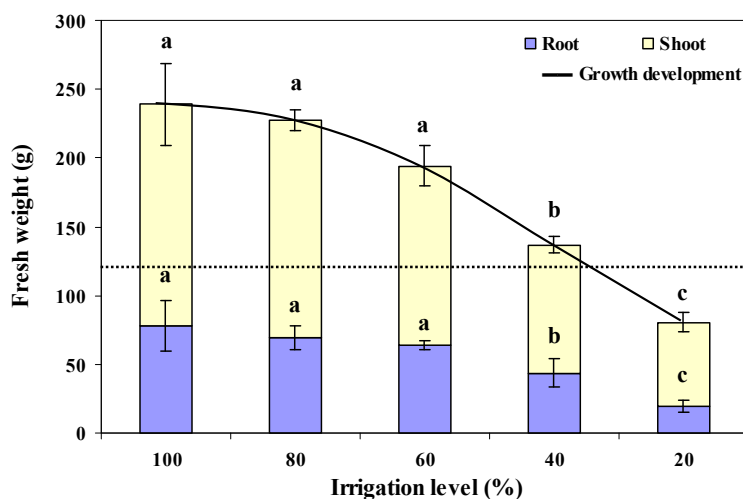
Furthermore, water stress caused a significant decrease in leaf area and shoots and roots dry weights as shown in Fig. (2 a & b).

On the other hand, K content was significantly increased, while Na was not significantly affected in leaves and roots by increasing water stress. K/Na ratio increased at 60 and 20% in leaves and also at all irrigation levels in roots Fig. (3 a, b & c). Concerning Ca content in leaves and roots, it was significantly affected by water stress, where, Ca content decreased in leaves and increased in roots as irrigation level decreased as shown in Fig. (3 d).

Water stress caused a significant increase in proline concentrations in leaves and roots (Fig. 4). However, proline concentrations were higher in roots than in leaves in all treatments.

The osmotic potential in leaves and roots was significantly decreased by reducing irrigation level (Fig. 5). Besides, in leaves a lower osmotic potential (more negative value) was recorded as compared to roots in all treatments.

Water stress caused a significant decrease in stomatal pore area/leaf area and an increase in stomatal closure percentage Fig. (6 a & b). Also, water deficit caused an increase the wax deposition on the leaf surface at the lowest irrigation level (20%) as compared to the control as shown in Fig. (7).



**Fig. 1:** Development of the plant fresh weight at different irrigation levels. The crossover of the dotted and solid black lines reflects the irrigation level where the growth depression falls down to 50% of the control plant ( $C_{50}$  value).

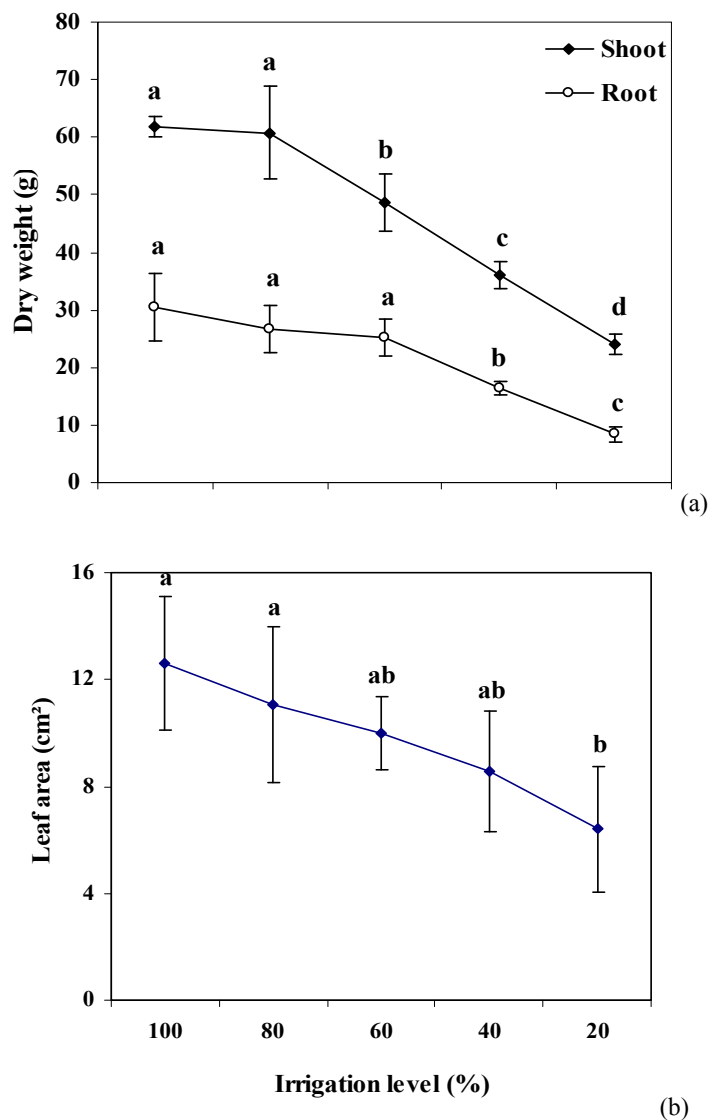


Fig. 2: Effect of water stress on shoots and roots dry weights (a) and leaf area (b) of *Acacia saligna*.

#### Discussion:

Results obtained showed that the growth of *A. saligna* plants fell down under 50% of the control plant (threshold) at 33% irrigation level. Accordingly, *A. saligna* was non economic at 20% irrigation level. Also, increasing water stress caused decreases in leaf area and shoots and roots dry weights. Results of leaf area are in agreement with those reported by Tuomela (1997) and Li and Wang (2003) on *Eucalyptus microtheca*, Ramoliya and Pandey (2003) on *Cordia rothii* and Bañon *et al.* (2006) on *Nerium oleander*. Also findings of shoots and roots fresh weights are in harmony with that reported by Tadros (2003) on *Leucaena leucocephala*, *Prosopis juliflora*, *Acacia saligna* and *Eucalyptus citriodora*. Results of shoots and roots dry weights are in accordance with that obtained by Tadros (2003) on *Leucaena leucocephala*, *Prosopis juliflora*, *Acacia saligna* and *Eucalyptus citriodora* and Bañon *et al.* (2006) on *Nerium oleander*.

The restricted growth observed in this study can be considered as a morphological adaptation of the plant to water stress to reduce the transpiration and induce a lower consumption of water. In this sense, Stanhill and Albers (1974) suggested that plant water consumption is generally related to transpiration. Nevertheless, the reduction in leaf area not only led to a reduction in transpiration but also in the photosynthetic surface area, and consequently reduced growth. Moreover, the growth of plant is usually reduced under the condition of water stress, because the supply of nutrients to a plant is directly related to water movement in roots, and when such movement ceases because of lower soil moisture availability, roots are limited to those nutrient ions within the range of diffusion, this supply must become limiting within a very short time (Bisht, 1993).

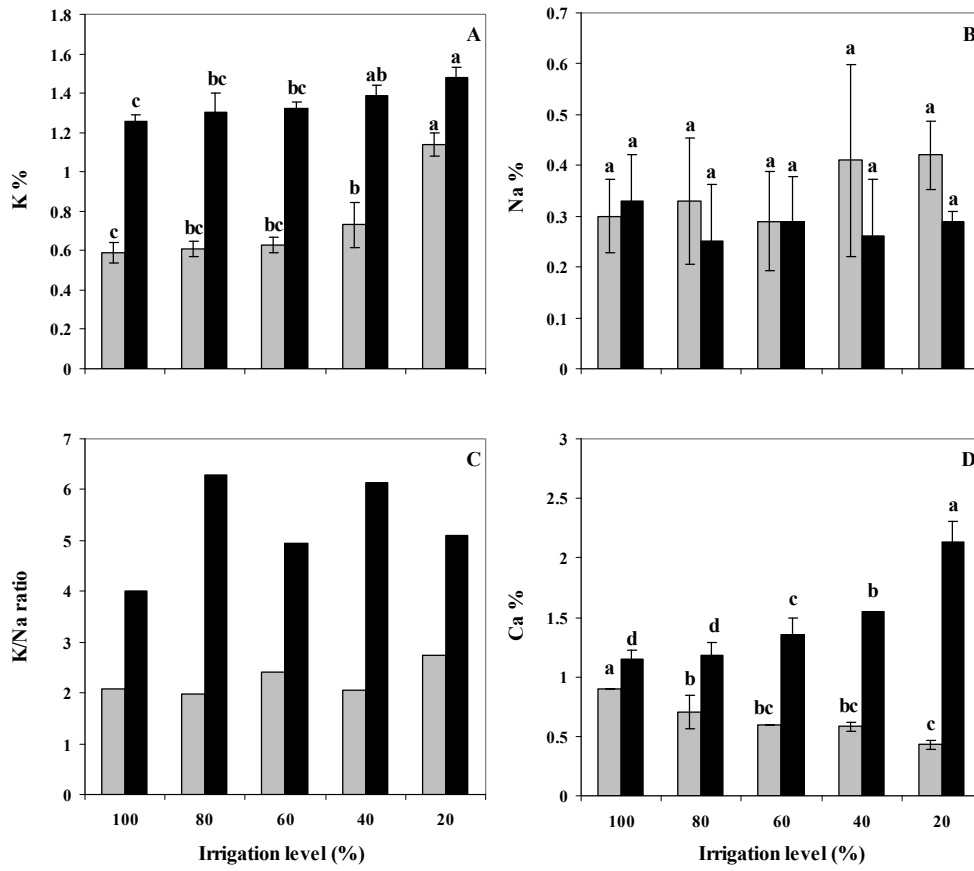


Fig. 3: Effect of water stress on potassium (a) and sodium (b) content, K/Na ratio (c) and calcium (d) content in the leaves (■) and roots (▨) of *Acacia saligna*.

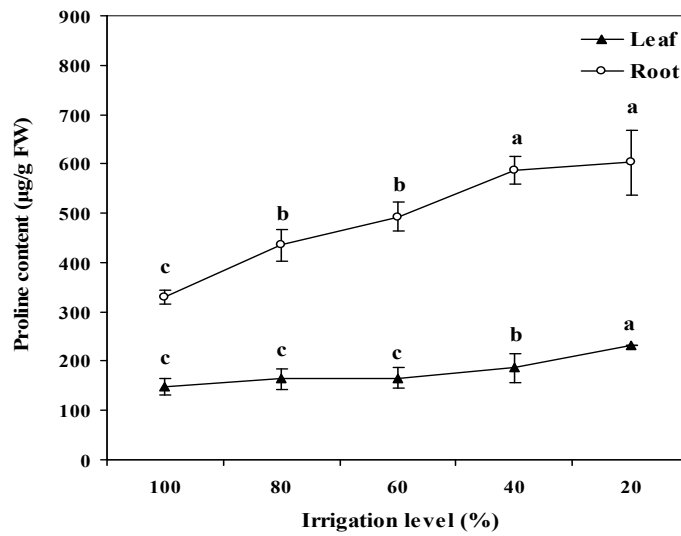


Fig. 4: Effect of water stress on proline content in the leaves and roots of *Acacia saligna*.

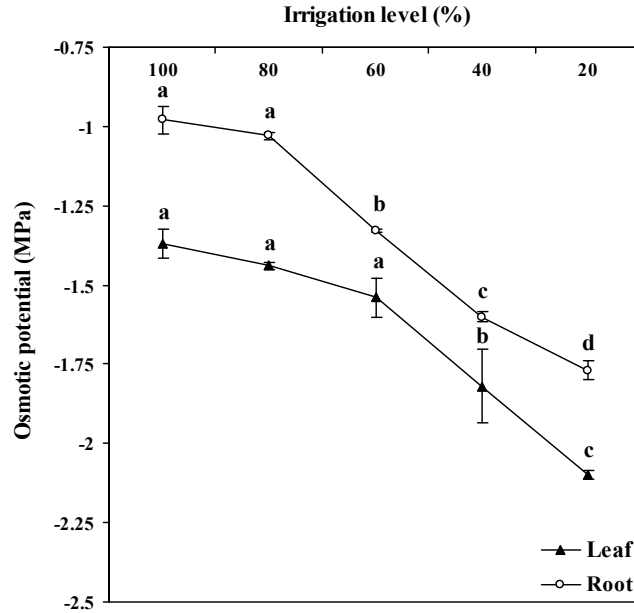


Fig. 5: Effect of water stress on osmotic potential in the leaves and roots of *Acacia saligna*.

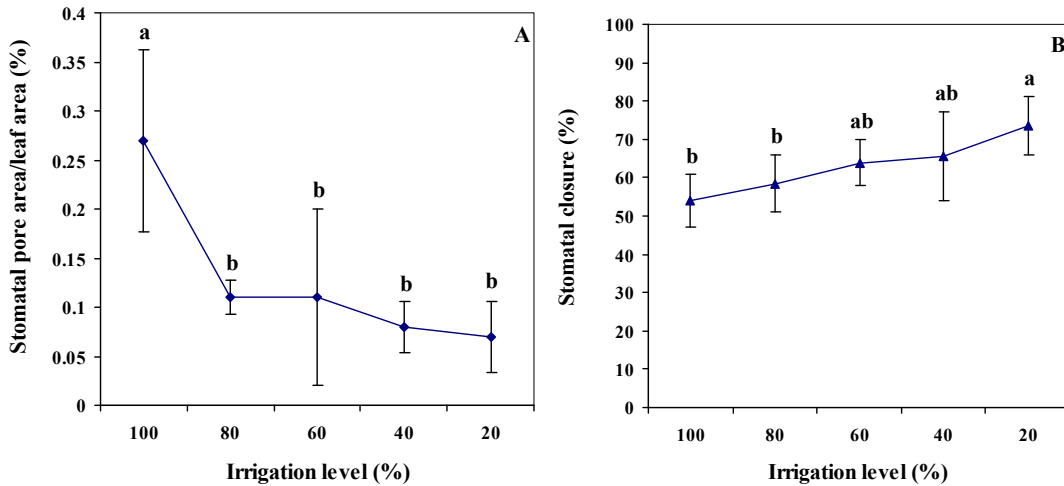
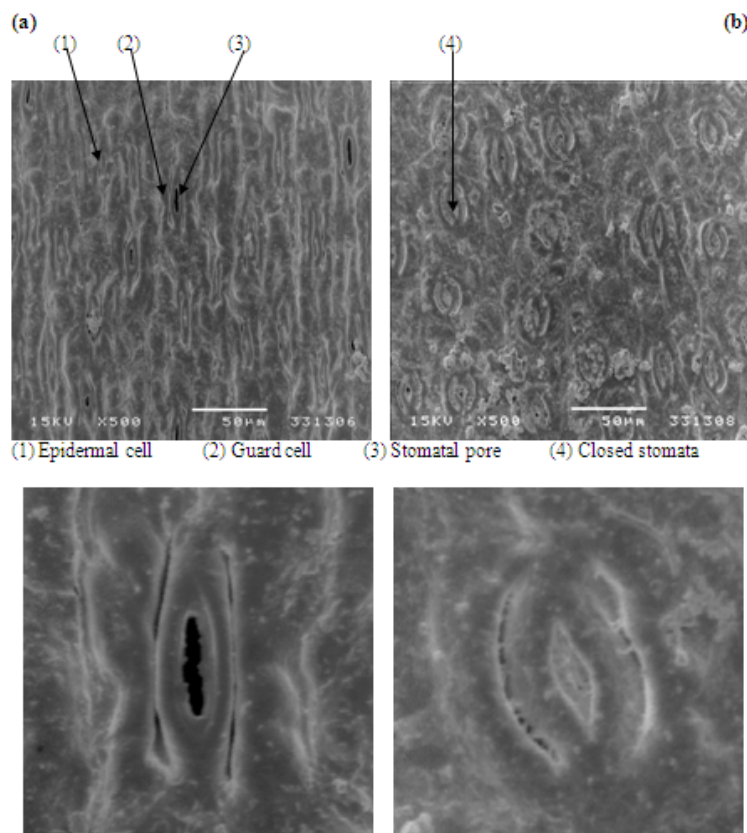


Fig. 6: Effect of water stress on stomatal pore area/leaf area (a) and stomatal closure (b) for lower surface of *Acacia saligna* leaf.

Percentage of K in leaves and roots increased in response to water stress. Findings of K% in leaves are in agreement with that reviewed by Martinez *et al.* (2003) on *Atriplex halimus*. Thiec and Manninen (2003) demonstrated that water stress caused a decrease in the K content of the current needles of *Pinus halepensis*. Meanwhile, Inclán *et al.* (2005) reported that water stress increased K concentration in twigs with a diameter above 3 mm. Moreover, Sardans *et al.* (2008) stated that water deficit decreased K concentration in leaves of *Erica multiflora* and *Globularia alypum* and it did not affect K concentration in roots of *G. alypum*. Na% in leaves and roots were not significantly affected by water stress. Martinez *et al.* (2003) found that water stress increased Na<sup>+</sup> content in leaves of *Atriplex halimus*. K/Na ratio increased at 60 and 20% irrigation levels in leaves and also at all irrigation levels in roots. Ca% decreased in leaves and increased in roots as irrigation level decreased. Inclán *et al.* (2005) on *Pinus halepensis*, clarified that water stress decreased Ca concentration in twigs with a diameter below 3 mm. Water stress caused an increase in proline concentrations in leaves and roots. Results of proline concentrations in leaves are in agreement with those reviewed by Tadros (2003) on *Leucaena leucocephala*, *Prosopis juliflora* and *Acacia saligna*, Yang *et al.* (2005) on *Hippophae rhamnoides*, Ren *et al.* (2007) on *Populus cathayana* and Yanqiong *et al.* (2007) on *Sophora davidiana*, *Bauhinia faberi*

var. *microphylla*, *Convolvulus tragacanthoides*, and *Artemisia gmelinii*. Ashraf and Foolad (2007) suggested that proline is a major organic osmolyte that accumulate in a variety of plant species in response to environmental stresses. Although, its actual role in plant osmotolerance remain controversial, this compound is thought to have positive effects on enzyme and membrane integrity along with adaptive role in mediating osmotic adjustment in plants grown under stress conditions. In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment.



**Fig. 7:** Scann Electromicrograph of stomata on the lower surface of *Acacia saligna* leaf in the control plants (a) and plants treated with 20 % irrigation level (b).

Osmotic potential in leaves and roots was decreased as associated with reducing irrigation level. Findings of osmotic potential in leaves are in harmony with those reported by Tuomela (1997) on *Eucalyptus microtheca*, Nativ *et al.* (1999) on *Acacia saligna*, Martinez *et al.* (2003) on *Atriplex halimus*, Bargali and Tewari (2004) on *Coriaria nepalensis*, Aranda *et al.* (2005) on *Quercus suber* and Bañon *et al.* (2006) on *Nerium oleander*. Stomatal pore area/leaf area decreased and stomatal closure increased as water stress increased. In this respect, increasing percentage of stomatal closure under water deficiency reduce transpiration processes which in turn decrease  $Ca^{2+}$  transport from root to shoot. Besides, water deficit caused an increase the wax deposition on the leaf surface at the lowest irrigation level (20%) as compared to the control. In this regard, Taiz and Zeiger (2002) suggested that water deficit increases wax deposition on the leaf surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present.

Results obtained in the present work revealed a more pronounced accumulation of proline in the roots than in the leaves at increasing water stress. Besides, a higher K% and a lower Na% were observed in roots than in leaves. However, correlation analysis (Fig. 8) identified proline as the main solute for osmotic potential adjustment in leaves of *A. saligna* followed by K then Na under water stress conditions. Moreover, both proline and K are the main solutes for osmotic potential adjustment in roots. On the other hand, correlations presented in Fig. 9 illustrate that osmotic potential in leaf and root was inversely proportional to stomatal closure whereas K%, K/Na ratio and proline content was directly proportional to stomatal closure. It is clear that K and proline

contents in both leaf and root led to a decrease of the osmotic potential which resulted in elevation of stomatal closure under water stress conditions.

In conclusion, it is apparently from the results that *A. saligna* plant used three mechanisms important for drought tolerant: (1) a reduction of growth for reduce transpiration; (2) reducing plant osmotic potential by accumulation K, Na and proline in leaf and K and proline in root, leaf osmotic adjustment, permitting the maintenance of leaf turgor even when water availability in the soil diminished through the absence of irrigation and (3) a more efficient stomatal regulation, avoiding the loss of great quantities of water through transpiration when water levels in the soil were limiting.

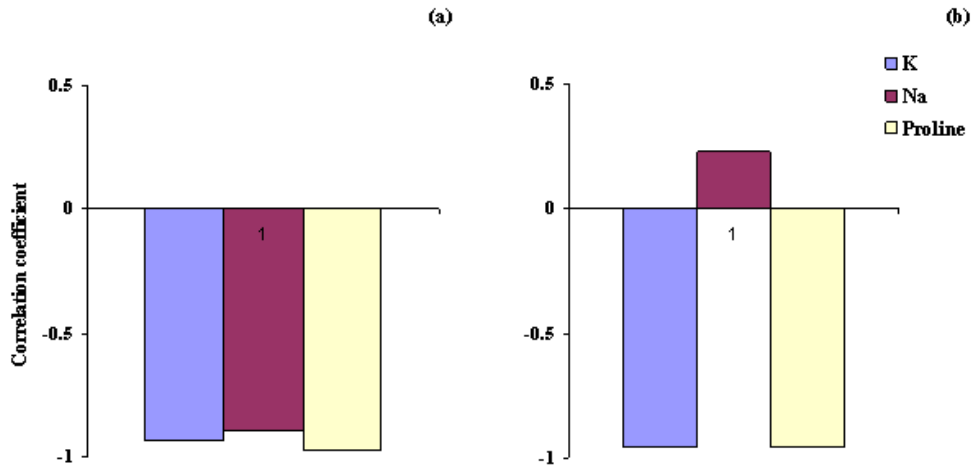


Fig. 8: Correlation coefficient between leaf (a) and root (b) osmotic potential and potassium, sodium and proline of *Acacia saligna* leaf and root under different irrigation levels.

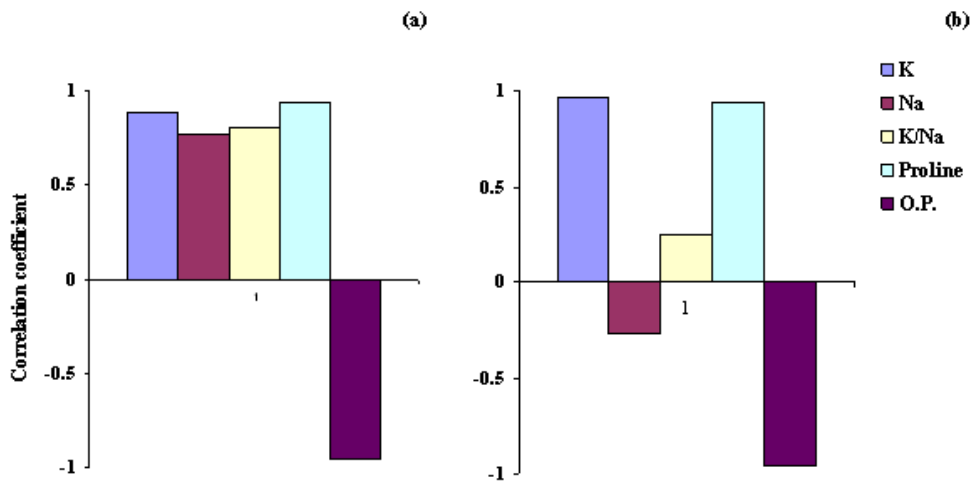


Fig. 9: Correlation coefficient between stomatal closure and potassium, sodium, K/Na ratio and proline of *Acacia saligna* leaf (a) and root (b) under different irrigation levels.

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