Middle-East Journal of Scientific Research 28 (5): 448-456, 2020
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2020.448.456

The Response of *Juniperus procera* Seedlings to Irrigation Water Deficit

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Abstract: *Juniperus procera* is a tree species that dominates the natural forests of southwestern Saudi Arabia. It is a very important species for its multiple uses and benefits for the locals for a long time. This species is suffering from the low capacity of natural regeneration so it is necessary to replant the deforested areas with seedlings grown from local seeds. Due to the sacristy of water, growing seedlings in the nurseries may face a shortage of water. Therefore, a pot experiment was carried out to examine the response of *J. procera* seedlings to water deficit. RCD was used including two watering treatments; every 3 days (well-water) and every 9 days (water deficit). After 180 days, water deficit caused decreases in stem height, total leaf area, branch number. Dry matter production reduced and by 68, 10, 40 and 56% for leaves, stem roots and whole plant, respectively. The relative growth rate, net assimilation rate, specific leaf area, leaf area ratio all reduced in water deficit. Relative water content and structural and non-structural carbon compounds in the leaves all reduced in water deficit and by 46, 12 and 24%. These results suggest that *J. procera* seedlings tolerated water deficit without marked injury, as they survived without losing much growth and looked shiny up to the end of the experiment.

Key words: *Juniperus procera* · Water deficit · Growth analysis · Relative water content · Structural and non-structural carbon compounds

INTRODUCTION

Water deficit is one of the main environmental problems that affect the growth of plants. According to Kramer [1], about one-third of the world's potentially arable land suffers from an inadequate supply of water and on most of the remaining crop yields are periodically reduced by drought. The worldwide losses in yield from water stress probably exceed the losses from all other causes combined. Water deficit reduces plant growth and crop yield more than all the other stresses combined, because of its ubiquitous nature [2]. This is not surprising as water deficit affects almost every aspect of plant physiology and morphology [3].

The natural forest area in southwest Saudi Arabia is considered a unique ecosystem in the West Asia region. *Juniperus procera* represents approximately 95 percent of the tree species grown in these forests [4]. *J. procera* has been a very important source of wood for timber and fuel. Its wood is fragrant, fine-textured and strait-grained. It is hardy and resistant to termites and fungal diseases [5]. Because of these distinctive qualities, it is highly valued for the construction of houses. The juniper forests in Saudi Arabia are severely damaged by the excessive harvesting of wood and/or non-wood forest products, poor management, repeated fire, grazing, or other disturbances [6]. El-Juhany [7] asserted that the features of the deterioration of the juniper forests can be seen in large areas lost their trees and other areas stricken by die-back where many of their trees are partly or completely dead. Also, these forests suffer from the low capacity of natural regeneration [8]. *J. procera* is listed in the IUCN red list of the threatened species due to overexploitation and the global decline in its populations [9].

Understanding the growth nature of juniper trees and their regeneration is of great importance when thinking about remedying and improving these forests. To overcome the problem of deterioration and ensure natural regeneration of juniper trees, it is necessary to replant the deteriorated areas with trees grown from local

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seeds to produce seedlings and planting them in the natural forest in southwest Saudi Arabia and evaluate their growth [8]. Producing juniper seedlings need proper silvicultural practices in the nurse. The most important role of these practices is producing seedlings with good quality and desired characteristics. Dutra *et al*. [10] described the quantification of the hydric requirement during the production of quality seedlings as is of extreme importance. In this regard, irrigating the seedlings in the nursery must be done in a way with which seedling drying and its negative consequences can be avoided. Nurseries in Saudi Arabia are usually established outside the cities so that they probably not benefit from the municipal water network. Therefore they use groundwater either from wells dug in the sites or by bringing water from wells outside the sits on tank trucks. In such a case, the supply of water may be subjected to a decrease.

The effects of water deficit on the growth of tree seedlings have been extensively studied. However, the research on the response of *J. procera* seedlings to irrigation water deficit is few, if any. The present research be aims toevaluate the response of *Juniperus procera* seedlings to irrigation under water deficit conditions.

MATERIALS AND METHODS

Plant Materials: One-year-old seedlings of African pencil-cedar (*Juniperus procera* Hochst.ex Endl) were obtained from the nursery of Raidah Reserve, affiliated to the Saudi Wildlife Authority in Abha, Asir region. The seedlings were transferred to the experiment site at the College of Food and Agricultural Sciences at King Saud University, Riyadh with taking due precautions to preserve them during transportation. The seedlings were placed on a table in a greenhouse with a fan cooling system and have heat and lighting controls, with the temperature was 24/18 Celsius day/night.

The seedlings were transferred to pots of 25 cm size, using soil consisting of sand and peat moss mixture in a ratio of 1: 2 v/v. Watering of the pots continued for 50 days until the plant became firm before imposing the treatments.

Preliminary Measurements: Preliminary measurements were carried out before the experiment on 7 seedlings. They were plant height and diameter, total leaf area and leaf and total plant fresh weights. These parts were then dried by placing them in an oven with 72°C for 72 hours and then the dry weight of each was estimated.

Experimental Design: A randomized complete design with five replications was used to examine the response of the *J. procera* seedlings to water deficit. 30 seedlings were distributed into two groups represented well-water and water-stressed treatments. The well-watered seedlings were receiving irrigation every 3 days while the water-stressed ones were receiving irrigation every 9 days. Each replication had 3 seedlings. Tap water containing 500 ppm ofTDSat a rate of 350 ml was added to each pot.

Harvesting and Measurements: The seedlings were harvested after 180 days since the starting of the treatment. The stem height was measured using a metric ruler, while the stem diameter was measured by a caliper. Total leaf areas of all seedlings were scaled immediately after harvesting using CI-202 Laser Leaf Area Meter - CID Bio-Science. The number of branches for each seedling was counted and their root length was measured using a ruler after extracting from the soil.

Partitioning of Dry Weight and Plant Growth Analysis: Partitioning of dry weight to leaves, stem and roots of each seedling was calculated as a proportion of total plant dry weight. Relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA) and net assimilation rate (NAR) all were calculated according to Evans [11]. This was carried out using the following equations:

$$RGR = (\text{Log } W_2 - \text{Log } W_1) / t_2 - t_1$$
$$LAR = TLA/TW$$
$$SLA = TLA/LWt$$
$$NAR = (W_2 - W_1 / L_2 - L_1) \times (\text{Log } L_2 - \text{Log } L_1 / t_2 - t_1)$$

where: Log = natural logarithm, W_2 and W_1 = total plant dry weight at the beginning of the experiment and at the time of harvest, $t_2 - t_1$ = the length of the period between the two measurements (weeks), TLA = total leaf area (cm^2), TW = total plant dry weight, LWt = leaf dry weight and L_1 and L_2 are the total leaf areas at the beginning of the experiment and at the time of harvest.

Determining Leaf Relative Water Content (RWC): Determining leaf relative water content was done for each seedling in the experiment according to Barrs [12], through taking three leaves and quantifying their fresh weight then placed them in distilled water for 24 hours to saturation. Thereafter, saturated leaves were weighed and placed in the oven at 70°C for 48 hours then their dry weight was measured. Leaf relative water content was calculated as follows:

RWC = (FW – DW) / (SW – DW) × 100

where: RWC = leaf relative water content, FW = leaf fresh weight, SW = leaf saturated weight and, DW = leaf oven-dry weight.

Quantification of Structural and Non-structural Carbon Compounds: Structural and non-structural carbon compounds in the leaves, stems and roots were quantified using an extraction procedure previously described by Browning [13] and modified by Ibrahim [14]. Non-structural carbon compounds included both starch and non-structural carbohydrates.

Preparation of Samples: Oven-dried samples of leaves, stem and roots of the seedlings grown in both the irrigation treatments were ground in an electric grinder. The ground material was then sieved through a set of laboratory sieves. The particles that passed the 40-mesh sieve and remained on the 60-mesh sieve were dried in an oven at 60-70°C until weight constancy was achieved Three grams of the dried ground material was transferred into a white winceyette sack that was 2 × 5 cm in size. Empty sacks were tested for leachability by being subjected to the extraction procedure.

Extraction Procedure: A sample of 2-5 g with 40-60-mesh particle size was weighed in a glass thimble and placed into a 100 cm Soxhlet extractor. Then, 125 mL of 95% ethanol was placed in a 250 mL round bottom distillation flask. Extraction was carried out for a period of 4-8 h. After extraction, the thimble was removed from the extractor and placed upright on an absorbent tissue for approximately three days to air dry the sample at room temperature. After the drying period, the thimble and its contents were weighed and the ethanol-soluble extractives were calculated.

The ethanol-extracted contents of the thimble were transferred to a 250 mL Pyrex glass beaker. After the addition of 100 mL of distilled water, the beaker was placed in a boiling water bath for 3 h. After the hot water extraction, the contents of the beaker were filtered through a medium-fast filter paper (Whatman No. 1) and washed with small amounts of hot water. The filter paper and the precipitate were then placed in an aluminum can and dried at 105±1°C to constant weight.

Calculations: The weight of structural carbon compounds (SCC) was obtained by subtracting the can weight from the oven-dry weight of the can and its contents. The weight of the non-structural carbon compounds (NSCC) is equal to the difference between the dry weight and SCC weight. SCC can also be obtained from the dry weight minus the sum of both ethanol and water extractives, which represent the NSCC weight [14].

Statistical Analysis: Data were analyzed with analysis of variance (ANOVA) using SAS software [15]. The differences between the means were determined using Fisher's least significant difference (LSD) test at $P< 0.05$. Data were log or arcsine transformed when necessary.

RESULTS

Plant Growth: The results of the analysis of variance showed that the increase in the irrigation period from 3 to 9 days led to a significant decrease in the growth of the mean height of *J. procera* seedlings ($P<0.05$). The mean height of the seedlings grown under irrigation every 9 days had 33.20 cm compared to 42.53 cm for those received irrigation every 3 days while diameter growth was not affected (Table 1).

The mean total plant leaf area decreased significantly ($P<0.05$) and by 20% in the seedlings irrigated every 9 days compared with those irrigated every 3 days (Table 1). The *J. procera* seedlings that received irrigation every 9 days produced 20 branches comparing with 24 branches for those received irrigation every 3 days ($P<0.05$).

Dry Weight Production: The analysis of variance showed a significant decrease ($P<0.0001$) in the mean total plant dry weight of *J. procera* seedlings due to significant decreases in mean leaf, stem and root dry weights ($P<0.05$), ($P<0.0001$) and ($P<0.0001$) respectively as a result of increasing the irrigation interval from 3 to 9 days (Table 2).

Dry Matter Partitioning: Increasing the irrigation interval from 3 to 9 days resulted in changing dry matter partitioning between the parts of *J. procera* seedlings. Stem weight ratio increased significantly in water deficit treatment from 30 to 39.87% ($P<0.0001$) at the expense of both leaf and root dry weight ratios which decreased significantly from 49 to 40% ($P = 0.0012$) and from 21 to 20% ($P<0.0001$), respectively (Fig. 1).

Table 1: Mean stem height (cm plant^{-1}), diameter (cm plant^{-1}), total leaf area (cm^2 plant^{-1}), number of branches (branch plant^{-1}) and root length (cm plant^{-1}) of *Juniperus procera* seedlings grown under two irrigation; every 3 days and every 9 daysafter 180 days in the greenhouse

	Irrigation treatments	
Trait	Every 3 days	Every 9 days
Seedling height (cm plant^{-1})	42.50^{a*}	33.20^{b}
Seedling diameter (cm plant^{-1})	00.73^{a}	0.66^{a}
Total leaf area (cm^2 plant^{-1})	402.20^{a}	319.80^{b}
Number of branches (branch plant^{-1})	24.10^{a}	20.10^{b}
Root length (cm plant^{-1})	59.00^{a}	57.00^{a}

Table 2: Mean dry matter production of leaf, stem, root and total (g plant^{-1}) of *J. procera* seedlings grown under two irrigation treatment; every 3 days and every 9 days after 180 days in the greenhouse

	Irrigation treatments	
Trait	Every 3 days	Every 9 days
Leaf dry weight (g plant^{-1})	45.69^{a*}	14.71^{b}
Stem dry weight (g plant^{-1})	15.07^{a}	13.52^{b}
Root dry weight (g plant^{-1})	24.12^{a}	14.46^{b}
Total dry weight (g plant^{-1})	74.94^{a}	32.70^{b}

Table 3: Means of specific leaf area (SLA), leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (RGR) of *Juniperus procera* seedlings grown under irrigation every 3 and 9 days for 180 days in the greenhouse

	Irrigation treatments	
Trait	Every 3 days	Every 9 days
Specific leaf area (cm^2 g^{-1} leaf dry weight)	29.83^{a}	13.42^{b}
Leaf area ratio (cm^2 g^{-1} total dry weight)	13.44^{a}	04.50^{b}
Net assimilation rate (cm^2 g^{-1} week^{-1})	00.19^{a}	00.15^{b}
Relative growth rate (g g^{-1} week^{-1})	00.17^{a}	00.13^{b}

*The same letters in each of two consecutive horizontal boxes mean that they do not significantly difference at the P<0.05 level

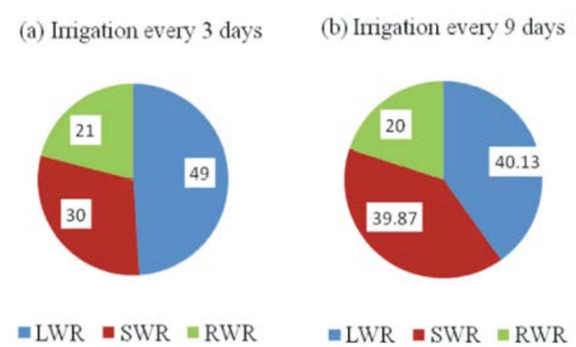

Fig. 1: Dry matter partitioning of *J. procera* seedlings into leaves, stem and roots as affected by increasing the irrigation interval from 3 (a) to 9 (b) days after 180 days in the greenhouse. Leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) represented by the blue, red and green color, respectively

Growth Analysis: The Analysis of variance revealed significant decreases (P<0.0001),) P<0.0001), (P<0.05) and (P<0.05) in the means of specific leaf area (SLA), leaf area ratio (LAR), relative growth rate (RGR) and net assimilation rate (NAR) of *J. procera* seedlings grown under irrigation treatment every 9 days compared with those received irrigation every 3 days (Table 3).

Relative Water Content: Increasing the irrigation interval from 3 to 9 days caused a significant decrease (P<0.005) in relative water content of the leaves of *J. procera* seedlings where they had less than half RWC of those grown in well-watered treatment (Fig. 2).

Structural and Non-Structural Carbon Compounds: The Analysis of variance procedure revealed that the mean of the amounts of structural carbon compounds in the leaves of *J. procera* seedlings grown in 9 days irrigation interval decreased by 12% (P = 0.013) while that of non-structural carbon compounds decreased by 24% (P<0.005). The amount of structural and non-structural carbon compounds in stem, roots and the whole plant did not change due to water deficit (Table 4).

Table 4: Means of the amount of structural (SCC) and non-structural carbon compounds (NSCC) in the leaves, stem, roots and whole plant and the ratio non-structural/ structural carbon compounds (NSCC/ SCC) in the leaves of *Juniperus procera* seedlings that grown under irrigation every 3 and 9 days for 180 days in the greenhouse

	Irrigation treatments	
Trait	Every 3 days	Every 9 days
Leaf SCC (g plant^{-1})	1.00^{a}*	0.88^{b}
Stem SCC (g plant^{-1})	1.07^{a}	1.06^{a}
Root SCC (g plant^{-1})	1.35^{a}	1.22^{a}
Whole plant SCC (g plant^{-1})	3.32^{a}	3.28^{a}
Leaf NSCC (g plant^{-1})	0.72^{a}	0.55^{b}
Stem NSCC (g plant^{-1})	0.91^{a}	0.88^{a}
Root NSCC (g plant^{-1})	1.13^{a}	1.01^{a}
Whole plant NSCC (g plant^{-1})	2.64^{a}	2.57^{a}
NSCC/ SCC ratio in the leaves	27.00^{a}	22.89^{b}

*The same letters in each of two consecutive horizontal boxes mean that they do not significantly different at the P<0.05 level

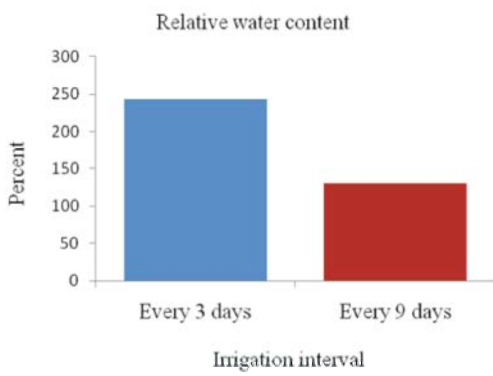

Fig. 2: Relative water content (RWC) of the leaves of *Juniperus procera* seedlings as affected by increasing the irrigation interval from 3 to 9 after 180 days in the greenhouse

On the other hand, only the ratio of non-structural/ structural carbon compounds in the leaves of *J. procera* seedlings that were changed due to water deficit where it decreased significantly (P<0.0001) (Table 4).

DISCUSSION

Growth of the Seedlings: Decreasing the growth of trees due to water deficit has been well documented (*e.g.* [16]). Water stress has been known to reduce the growth of many plant species. However, the level of stress that results in a reduction in growth varies with the condition under which the plants are grown [17] and the ability of plants to cope with such conditions.

The water deficit in the present study caused a reduction in the height growth of *Juniperus procera* seedlings by almost 22%. This result concurs with previous reports (*e.g.* [18-22]).

Decreasing the growth of *J. procera* seedlings due to water deficit included a reduction in the number of branches by 17%. Similar results were obtained by El-Juhany*et al*. [21], EL-Juhany*et al*. [22], Munns and Termaat [23], El-Juhany and Aref [24].

One of the most marked effects of irrigation water deficit on plant growth is the reduction of leaf area. Linder *et al*. [25] mentioned that it is well known that trees respond to a period of water stress by reducing their leaf area. Kozlowski *et al*. [3] asserted that reduction in leaf area is one of the damaging effects of water stress, which reduces the loss of water, but unfortunately also reduces the surface that carries on photosynthesis, thus decreasing the amount of photosynthate available for growth.

The reduction of total leaf area of *J. procera* seedlings in the present work as a result to water deficit and by 20% accords with many other reports on decreasing total leaf area due to water deficit (*e. g.* [19], [21], [22], [24], [26], [27]).

The reduction of the total leafy area in this study may suggest that the seedlings were able to resist water deficit conditions and reduce water loss as one of the adaptations and mechanisms by which plants, in general, can cope with drought conditions.

Dry Matter Production: The reduction of the total dry weight of *J. procera* seedlings and its components (i. e. leaves, stem and roots) in water deficit treatment was inevitable due to the decrease of their height growth and their number of branches and total leaf area.

In the present study, leaf dry weight of *J. procera* seedlings decreased in water deficit and by 68%. This was consistent with studies on forest trees that showed decreasing the dry weight of the leaves due to the water deficit (*e. g*. El-Juhany and Aref [19] on *Leucaena leucocephala*, El-Juhany*et al*. [22] on *Hibiscus tiliaceus* trees, El-Juhany and Aref [24] on *Conocarpus erectus*, Mohiuddin [28] on two poplar species, Ibrahim *et al*. [29] on *Populous* 'Balsam Spire' hybrid,). The reduction in the root dry weight of *J. procera* seedlings due to water deficit accounted for 40%. Mohiuddin [28] mentioned that the dry weight of poplar roots decreased by the lack of irrigation water and El-Juhany and Aref [19] found a significant decrease in the dry weight of roots of different acacias species as a result of exposure to irrigation water shortage. Stem dry weight of *J. procera* seedlings decreased in water deficit and by 10% which is considered a lower extent comparing with either leaf or root dry weight. Decreasing stem dry weight due to a lack of irrigation water weight has been reported in several studies (*e.g*. [18, 19, 22]). The total dry weight of *J. procera* seedlings as is the product of its components (Leaves, stem and roots) therefore it also decreased in water deficit and by 56%. This result corresponds to what El-Juhany and Aref [24] found for*Conocarpus erectus* seedlings and also to what El-Juhany and Aref [19] mentioned that the total dry weight of acacia trees was significantly affected by irrigation water deficiency. Aref and El-Juhany [30] also found similar results with seedlings of 8 exotic acacia species.

Dry Matter Partitioning: Dry matter partitioning refers to the apportionment of growth to tree components [31-33]. Partitioning of dry matter to different plant parts is subjected to change as a response to lack or excess in resources such as water, nutrients, carbon dioxide and sunlight or over time.

Water stress not only decreases the total dry matter production but also alters the partition of dry matter between the different plant organs [14]. In the present study, water deficit increased the fraction of dry weight partitioned to the stem (SWR) of *J. procera* seedlings at the expense of that partitioned to the leaves (LWR) while that partitioned to the roots (RWR) was almost unchanged. This result concurs with other findings (*e. g*. [26], [14], [24]). Contradictory, some results showed that there was no effect of water deficit on dry matter partitioning of woody species (*e.g*. [24]).

Growth Analysis: Growth analysis represents the first step in the analysis of primary production, being a link between merely recording plant production and analyzing it employing physiological methods [34]. Relative growth rate (RGR) and its components (*i.e.* net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR)) were examined in this study.

The relative growth rate (RGR) at a given instance of time (t) is "the increase in plant material per unit of material present per unit of time." RGR is a prominent indicator of plant strategy in terms of productivity and its relationship to environmental stress. This criterion refers to the rate of growth per unit of dry matter and expressed in grams of dry matter produced by the existing dry matter before the increase in the unit of time (day/ week/ month/ year).

Calculating relative growth rate (RGR) of *J. procera* seedlings in the present study showed that water deficit caused a decrease in their RGR of about 24%. El-Juhany and Aref [24] found that the RGR of *Conocarpus erectus* seedlings decreased significantly in the low water supply. Other researchers reported decreased RGR of tree species under water deficit conditions (*e.g*. [14], [19], [22], [27], [35]). The components of RGR; Net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) of *J. procera* seedlings all decreased in water deficit treatment and by 17, 66.5, 55 and 9%, respectively. Similar results for woody species were found to prove the reduction of these components in water deficit (*e.g*. [27], [35], [37] for NAR; [19], [36] for LAR and [19], [21], [22], [37] for SLA.

Relative Water Content: Water deficit reduced relative water content in the leaves of *J. procera* seedlings. This finding concurs with other resulted (*e.g*. [21], [22], [24], [38]).

Structural and Non-Structural Carbon Compounds: The concept of dry matter partitioning into structural and non-structural compounds may be a more appropriate approach to carbon partitioning within plants concerning the physiological activity of the organ as well as its morphological characteristics [14]. All of the dry matter in a tree, whether in the form of major constituents such as cellulose and lignin or as minor extractives, is derived from the carbon and energy which is made available by photosynthesis [29]. Both starch and non-structural carbohydrates have been included as non-structural carbon compounds which can be easily quantified [14]. Thus, quantification of the structural and non-structural carbon compounds in the whole tree or in its different organs will provide a better understanding of any modifications of the physiological processes in the whole tree or specific organs as a result of internal or environmental factors [14].

The significant decrease in the structural and non-structural carbon compounds in the leaves of *J. procera* seedlings under water deficit in the current study is consistent with similar results, where decreases in the concentration of sucrose in the leaves of oak shrubs grown under water stress [40] and a decrease in sucrose was also observed in Apple trees or grapevines for the same reason [41, 42]. Silva *et al*. [43] also found declines in dissolved carbohydrates in the leaves of four genotypes of the Umea tree (Brazilian palm) after 31 days of water stress.

El-Juhany [44] asserted that changing the concentrations of structural and non-structural carbon compounds is among the adaptive mechanisms that the plant uses to adapt to the conditions of irrigation water shortage.

Calculating the ratio of non-structural/structural carbon compounds in the leaves of *J. procera* seedlings showed that it decreased significantly due to water deficit. The ratio of non-structural to structural carbon compounds (NSCC/ SCC) in an organ reflects the function of this organ and its physiological performance. The ability of plants to tolerate water deficits has been frequently attributed to their capacity for osmotic adjustment through the accumulation of organic compounds such as amino acids or soluble carbohydrates [45, 46].

Our results indicate that the movements of non-structural carbon compounds are an important physiological feature of plant adaptation to resist drought. Yang *et al*. [47] reported a similar conclusion. Liu *et al*. [48] also pointed out that dehydration generally resulted in increased concentrations of non-structural carbon compounds.

Observations of changes in the concentrations of non-structural carbohydrates in trees during drought are somewhat inconclusive, as they may increase, decrease or not change at all (*e. g*. [49-54]). This response can even vary across plant members of individual trees [55].

CONCLUSION

Although the water deficit level imposed in this study caused decreases in most growth traits measured, however, these decreases were not acutefor some main growth characters. This suggests that *J. procera* seedlings tolerated the conditions of irrigation water deficit without losing much growth, survived, looked shiny and did not show any apparent damage. As the research work on the effects of water deficit on *J. procera* is few, if any, we recommend carrying out experiments on the effect of low water supply on seed germination and on the growth of trees of this species in forests itself.

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