Changes in vapour pressure deficit and air-to-leaf temperature difference due to the effects of watering frequency and seasonal variation-induced adaptive responses in \textit{Balanites aegyptiaca} in Saudi Arabia

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This study examines intraspecific variability among three sources (KSA, SD5.1 and SD6.2) of \textit{Balanites aegyptiaca} in Saudi Arabia in their response to different watering frequencies and seasonal changes in vapour pressure deficit (VPD) and air-to-leaf temperature difference ($\Delta T$) under field condition. Irrigation was done once a week, once every two weeks or once every three weeks. Traits measured include: tree height, diameter (DM), relative monthly height (RMHI) and diameter (RMDI) increments, stomatal resistance (Rs), specific leaf weight (SLW). VPD and $\Delta T$ were measured during the same time of Rs and SLW measurements. Both Rs and SLW directly responded to irrigation treatment and seasonal variation in $\Delta T$ and VPD. Interactive effects of hot weather and water stress increased leaf temperature, resulting in less $\Delta T$ and more VPD that induced higher Rs and SLW values. SD5.1 accounted for better responses under water stress, due to its higher Rs and SLW in the same time maintained better growth. DM and RMDI were more responsive to watering stress and varied among the sources. Early seedlings root-to-shoot ratio was associated with better growth performance later in the field. The results highlight the role of hot weather and water stress in producing large changes in $\Delta T$ and VPD that have a major impact on Rs and SLW. In addition, there is large intraspecific variation in field growth and adaptive responses among seeds brought from different provenances.

\textbf{Keywords:} Air-to-leaf temperature difference, \textit{Balanites aegyptiaca}, intraspecific variability, vapour pressure deficit, watering frequency.

\textit{Balanites} is a true dryland species with a wide range of geographical distribution\textsuperscript{1}. The tree is reported to have wide intraspecific variability among variable geographical zones in seeds and fruits\textsuperscript{2}, seedling response to imposed drought\textsuperscript{3} and adaptive mechanisms for salinity and water stresses\textsuperscript{4}. In addition to its survival under harsh environments, \textit{Balanites} has many products and values\textsuperscript{5}. It is a good dry season shade and fodder tree\textsuperscript{6,7} and potential shelterbelt and agroforestry species\textsuperscript{8,9}. The seed oil is highly stable and rich in saturated fatty acids\textsuperscript{10}, that can be used as food by humans\textsuperscript{11}, in biodiesel production\textsuperscript{12,13} and pharmaceutical purposes\textsuperscript{14}. The cake remaining after oil extraction is good for animal feed supplement\textsuperscript{15}. The other valuable product is saponin, which contains wide industrial and pharmaceutical applications\textsuperscript{16-18}. Domestication of this multi-product tree in the drylands is important. To ensure good survival and performance in these drylands with little or no rainfall, it is necessary to determine the best watering requirements for the species. In turn, the high genealogical variation in phenotypic traits\textsuperscript{19}, calls for testing many different sources in the field to determine the extent and magnitude of variability among different geographical sources.

Almost all lands in Saudi Arabia are either hyper arid or arid with little or no rainfall\textsuperscript{20} and scarce forest cover\textsuperscript{21}. The newly formulated forest policy and strategy focuses on enriching forest plantations to increase the forest area of the country from its present levels\textsuperscript{22}. This requires proper selection of best species and geographical sources within species that are able to match planting sites. Intraspecific (provenance) variation in many trees is important for species survival in changing environments\textsuperscript{23}.

Many physiological parameters in trees serve as adaptive responses to environmental stress. For example, stomatal resistance, which is the resistance of stomata to the transport of carbon dioxide and water vapour. Water stress tends to increase stomatal resistance\textsuperscript{24} as part of the plant strategy to withstand low water availability\textsuperscript{25}. Leaf and air temperature and vapour pressure deficit (VPD) have a strong influence in regulating stomatal functions.

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and hence affect net photosynthesis. VPD varies according to the season of the year, leading to seasonal variation in stomatal opening and closure. It induces stomatal closure under drought stress conditions. Specific leaf weight (SLW; leaf mass per unit area) plays an important role in the production and allocation of carbohydrates in plants. Higher levels of SLW indicate more cell-wall material in the leaves to increase leaf thickness as part of the survival strategy.

The main objective of this study is to examine the effect of watering frequency on growth and physiological responses among three different intraspecific sources of *B. aegyptiaca* established under field conditions of western Kingdom of Saudi Arabia (KSA).

**Materials and methods**

**Study area**

The study site is located in dry arid land with saline soil in the experimental research farm of King Abdulaziz University, Saudi Arabia. The experiment was set-up under drip irrigation system.

**Seed sources**

Seeds of *B. aegyptiaca* used in this study were collected from three different geographical zones. Two sources were selected using Sudan tree seed zonemap: zone 5.1 in Blue Nile and zone 6.2 in Nuba Mountains, while seeds of the third source were collected from the University experimental research farm plots previously established using seeds obtained from local sources. The three sources have been coded SD5.1, SD6.2 and KSA respectively, throughout this study.

**Seedlings establishment**

During 2012, seeds from the three sources were germinated in the nursery at the university experimental farm. The seedlings were tended in the nursery for six months. Before transplanting the seedlings into the field, destructive seedlings samples were collected from each source. Seedling height and root collar diameter were measured. Then the seedlings were separated into shoot and root and oven-dried. Seedlings quality (root-to-shoot ratio (R/S), sturdiness quotient (SQ) and Dickson’s quality index (DQI) were calculated for each source (Table 1). R/S is root dry weight divided by shoot dry weight, SQ is obtained as seedling height divided by root collar diameter, while DQI is total seedling dry weight divided by the sum of sturdiness quotient and R/S ratio. These parameters later correlated with seedlings field performance.

**Setting up the experiment in the field**

In late 2012, the seedlings were transplanted in the field. After six months of field establishment, the experiment was set-up and data collection monitored for two years during the period 2013/14–2014/15. The split plot design with three replicates was used with watering treatment representing the main plots, and the sources as subplots. The watering treatments used were water frequencies at the rate of once every week, once every two weeks and once every three weeks. All the treatments were irrigated with the same amount of water up to the field capacity using drip irrigation system.

**Tree growth**

Trend in tree height and diameter (at root collar) was measured eight consecutive times for two years. The first measurement commenced after six months of growth in the field and the remaining seven measurements were done on a three-month basis in order to evaluate growth trend with time. Relative monthly height increment (RMHI) was calculated using the equation

\[
RMHI = \frac{(H_{\text{final}} - H_{\text{first}})}{(\text{month}_{\text{final}} - \text{month}_{\text{first}})},
\]

where \(H_{\text{final}}\) and \(H_{\text{first}}\) are tree height at final month and first month respectively. Relative monthly diameter increment (RMDI) was calculated as

\[
RMDI = \frac{(D_{\text{final}} - D_{\text{first}})}{(\text{month}_{\text{final}} - \text{month}_{\text{first}})},
\]

where \(D_{\text{final}}\) and \(D_{\text{first}}\) are the diameter at final and first months respectively.

**Stomatal resistance**

Six consecutive Rs measurements were carried out. In each measurement two trees per source per watering treatment were selected and from each tree five leaves

<table>
<thead>
<tr>
<th>Source</th>
<th>R/S ratio</th>
<th>SQ</th>
<th>DQI</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSA</td>
<td>1.63b</td>
<td>11.88b</td>
<td>1.54a</td>
</tr>
<tr>
<td>SD5.1</td>
<td>2.17a</td>
<td>13.94a</td>
<td>1.47a</td>
</tr>
<tr>
<td>SD6.2</td>
<td>1.73b</td>
<td>12.67b</td>
<td>1.51a</td>
</tr>
<tr>
<td>Source effect</td>
<td>**</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

* a, b represents mean separation tests. Means with different letters are significantly different at \(P < 0.05\) using new Duncan’s multiple range test.
were measured at the apical site of the leaf using steady state leaf porometer\textsuperscript{33}. The measurements done on November, January, March, May, July and September to cover the weather in all seasons.

Specific leaf weight

For determination of SLW we collected five fully grown leaf samples from each tree during the same day of Rs measurement. Leaves were collected from 27 trees (one tree per source per watering treatment per block). The freshly collected leaves were immediately photographed using a high-resolution camera (Olympus). The photographs were processed using a digital image analysis software package\textsuperscript{33} to obtain leaf area. Then the leaves were oven-dried at 65°C for 72 h to get the dry weight. SLW (g cm\textsuperscript{-2}) was calculated as the ratio of leaf mass to leaf area.

Vapour pressure deficit and air-to-leaf temperature difference

Relative humidity (RH), ambient air temperature and leaf temperature were measured during the same time of stomatal resistance measurements (HD5001 Extech) Psychrometer and 30:1 Infrared thermometer plant stress. The leaf temperature was measured on the same five leaves per tree on which Rs was measured with five corresponding air temperature and relative humidity values. $\Delta T$ was calculated as the difference between air temperature and leaf temperature, where air vapour pressure and leaf vapour pressure were derived using vapour pressure calculator\textsuperscript{34}. VPD was calculated as

$$\text{VPD} = e_{\text{leaf}} - e_{\text{air}}$$

where $e_{\text{leaf}}$ is the saturated water vapour pressure at the leaf temperature and $e_{\text{air}}$ is the actual vapour pressure outside\textsuperscript{35}.

Data analysis

The main effects of watering, sources and their interactions on the studied parameters were tested by analysis of variance (ANOVA) and the means separated with new Duncan multiple range test. SAS version 9.2 software was used for data analysis\textsuperscript{36}.

Results and discussion

Tree growth

The results revealed that Balanites trees show wide variability in growth among the different intraspecific sources. This in accordance with earlier studies that showed wide range of variability in this species on seed and fruit morphometric characteristics\textsuperscript{3}, response of seedlings to imposed drought stress\textsuperscript{37}, and adaptive mechanisms to withstand salinity and water stresses\textsuperscript{4}. The results revealed that watering stress had significant effects on tree height only in the last two measurements (Table 2). However, the interaction effects of watering treatment with source was not significant in all measurements, resulting in no change in source ranking at this stage in tree height under different watering regimes (Table 2). In all measurements and across all watering treatments, SD5.1 and SD6.2 showed highest growth compared to KSA. However, under irrigation every three weeks SD5.1 showed exceptionally high growth than the other two sources. Unlike tree height, watering treatment has a significant effect on tree diameter in almost all measurements. In addition, the interaction effect of irrigation with source in diameter growth was significant in all eight measurements (Table 3). This was resulted in source ranking change under different watering treatment. During normal irrigation (once a week), SD6.2 had significantly higher diameter compared to the other sources. The situation changed when the trees were irrigated once every three weeks in which case SD5.1 ranked better across all measurements. This indicates that diameter is an important trait for water stress tolerance in the species. The watering treatments and geographical source of the seeds have a major impact on relative monthly height and diameter increments. The high watering frequency lowered both RMHI and RMDI compared to watering the trees once every week. In plots irrigated once a week SD6.2 source obtained higher RMDI, while plots irrigated once every three weeks induced more RMDI on seeds from SD5.1 compared to the other sources.

The nursery seedlings R/S ratio as seedlings quality trait was well reflected on growth performance later in the field. SD5.1 with higher seedling R/S ratio in the nursery obtained better field growth across all watering regimes. This is in agreement with Mahmoud\textsuperscript{3} who reported that R/S ratio is a good indicator for Balanites survival under drought stress conditions. Extensive deep root system is a characteristic of this species to thrive under arid conditions in its natural range\textsuperscript{36}. DQI may not be an important seedlings quality factor to affect growth among sources under water stress conditions. In an earlier study on these sources\textsuperscript{3}, it was found that DQI is more associated with salinity stress rather than water stress. The high variability observed between the three sources in growth traits calls for selection of better seed sources. This may also suggest a range wide provenance or provenance/progeny trial of this valuable tree species in different locations to identify best provenances and genotypes for every ecological zone.

Stomatal resistance

The watering treatment applied in the experiment had a significant effect on Rs among the three Balanites...
among three different
provenances in all six measurements (Table 4). Whereas
the source factor revealed significant differences in five
measurements. The interaction effect of the watering
treatment and source was significant only in the last
measurement (Table 4). Although, Rs varied among the
different sources studied, it was more sensitive to water
stress and seasonal atmospheric weather changes. The
response to seasonal variation may be due to rise in air
temperature accompanied with increase in RH. This at-
mospheric condition, in addition to low soil moisture un-
der higher watering frequency, increased leaf temperature
leading to lower $\Delta T$ and higher VPD that induced higher
Rs. Under water stress conditions some plants tend to in-
crease stomatal resistance as part of their survival stra-
tegy$^{24}$. The reduction aims to minimize water loss under
low water availability in the soil$^{25}$. This strategy is clearly
represented in the present study. The source SD5.1,
which had higher Rs under higher watering frequency,
accounted for better growth rate under water stress. Thus,
Rs may serves as physiological response trait for this
species to reduce water loss under low water availability.
Earlier findings explained the association of Rs with VDP
and $\Delta T$ as an adaptive mechanism to regulate stomatal
opening and closure$^{26}$, especially under water stress con-
ditions$^{28}$.

Specific leaf weight

The effect of watering frequency on SLW was significant
in all six measurements. While variability among the dif-
ferent geographical sources of *Balanites* in SLW showed
no significant result in all measurements (Figure 1). Fig-
ure 1 shows the effect of irrigation frequency on SLW. Simi-
lar to Rs, SLW is highly responsive to watering
treatment for this species. Watering the tree at a
Frequency of once every three weeks produced higher SLW, compared to watering once a week or once every two weeks. This may be explained by the fact that during the high watering frequency, plants tend to reduce leaf area and increase leaf weight resulting in higher SLW. Reduction of leaf area and increase in weight may be a strategy to withstand harsh environments. The insignificant variability among the three sources in SLW in all measurements and the large difference among the water treatments may be attributed to the fact that SLW is a water stress adaptive trait to withstand water shortage. SLW was also affected by seasonal variation during the year. In the hottest months of May and July (corresponding to the fourth and fifth measurements), SLW values were higher, while the lowest value was obtained in the cooler month of January (second measurement).

Air-to-leaf temperature difference and vapour pressure deficit

Tables 5 and 6 reveal that both ΔT and VPD are significantly affected by the watering treatment applied. However, VPD is more responsive to watering stress compared to ΔT. The impact of seasonal atmospheric weather changes and the watering frequency on ΔT and VPD is high. The hot weather and irrigating the trees once every three weeks decrease the value of ΔT and increase VPD. The difference between leaf and outside air temperatures is highly responsive to the seasonal atmospheric weather variation. It tends to induce stomatal closure under water stress conditions. The aim is to reduce the evaporative water loss from the leaves during shortage of water.

The Rs values increased positively with increasing VPD and decreasing ΔT. It may also have some relationship with RH. It is observed that hot weather with high RH increases VPD and decreases ΔT. The increase in temperature and RH increases leaf temperature, which in turn, leads to a decline in ΔT and an increase in VPD. The decrease in ΔT and increase VPD leads to an increase in Rs. Some plants regulate their stomata as part of their strategy to withstand water stress. This regulation is influenced by ΔT and VPD. In turn, VPD depends on seasonal atmospheric conditions during the year and plant stress status. This is associated with an increase in stomatal resistance.

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Table 4. Effect of watering frequency on stomatal resistance (Rs, m$^2$/s/mol) among three different intraspecific sources of B. aegyptiaca grown under field conditions

<table>
<thead>
<tr>
<th>Observation</th>
<th>Source</th>
<th>Rs 1</th>
<th>Rs 2</th>
<th>Rs 3</th>
<th>Rs 4</th>
<th>Rs 5</th>
<th>Rs 6</th>
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<td>Once a week</td>
<td>KSA</td>
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<td>1.97</td>
<td>2.28</td>
<td>5.27</td>
<td>5.91</td>
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</tr>
<tr>
<td></td>
<td>SD5.1</td>
<td>3.38</td>
<td>2.22</td>
<td>4.33</td>
<td>5.60</td>
<td>9.78</td>
<td>4.86</td>
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<tr>
<td></td>
<td>SD6.2</td>
<td>3.97</td>
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<td>3.41</td>
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<td>10.34</td>
<td>5.46</td>
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<td>KSA</td>
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<td>2.15</td>
<td>3.38</td>
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<td>8.03</td>
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<td>3.38</td>
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<td>4.90</td>
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<td>5.22</td>
<td>9.65</td>
<td>13.26</td>
<td>6.74</td>
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CV

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<tr>
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<th>Rs 3</th>
<th>Rs 4</th>
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<td>ns</td>
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<td>**</td>
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<td>CV</td>
<td>Source effect (P)</td>
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</tbody>
</table>

*P = ≤0.05; **P = ≤0.01; ns, not significant.

Figure 1. Effect of irrigation frequency on specific leaf weight (SLW) in Balanites aegyptiaca. Higher values were obtained in hot months of May and July (measurements 4 and 5), and lower value in cooler month of January (measurement 2). Means with different letters in the same column are significantly different using new Duncan’s multiple range test at $P = 0.05$. 

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CURRENT SCIENCE, VOL. 112, NO. 6, 25 MARCH 2017
FRUITS AND SEEDS WITHIN AND AMONG PARKLAND AGROFORESTS

SEEDLINGS FROM THREE DIFFERENT SOURCES, NS, NOT SIGNIFICANT.

EFFECT OF DROUGHT AND SALT STRESS ON NIGRA.

ADAPTIVE MECHANISMS TO MINIMIZE WATER LOSS DURING HOT WEATHER AND LOW WATER AVAILABILITY IN THE SOIL.


4. Elfeel, A. A. and Abouhassan, R. A., Response of Balanites aegyptiaca (L.) Del. var. aegyptiaca seedlings from three different sources of B. aegyptiaca grown under field conditions.

**P = ≤0.05; **P = ≤0.01; ns, not significant.

Table 5. Effect of watering frequency on air-to-leaf temperature difference (ΔT) among three different intraspecific sources of B. aegyptiaca grown under field conditions.

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Source</th>
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<th>ΔT2</th>
<th>ΔT3</th>
<th>ΔT4</th>
<th>ΔT5</th>
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Table 6. Effect of watering frequency on vapour pressure deficit (VPD) among three different intraspecific sources of B. aegyptiaca grown under field conditions.

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Source</th>
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<th>VPD2</th>
<th>VPD3</th>
<th>VPD4</th>
<th>VPD5</th>
<th>VPD6</th>
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<td>2.90</td>
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<td>0.76</td>
<td>1.31</td>
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<td>2.97</td>
<td>1.43</td>
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**P = ≤0.05; **P = ≤0.01; ns, not significant.

Conclusion

The present study reveals high intraspecific variability among three geographical sources of B. aegyptiaca in field growth performance under arid saline soil. This indicates that selection of proper source is necessary for domestication of this tree under arid climate of Saudi Arabia. Root collar diameter serves as a good indicator of tree growth function variability under water stress conditions. The environmental conditions associated with the season of the year have a strong influence on ΔT and VPD. The impact is increase in Rs and SLW with increasing watering frequency due to increase in VPD and decrease in ΔT. The responses may be related to adaptive mechanisms to minimize water loss during hot weather and low water availability in the soil.

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