Irrigation management of *Punica granatum* in the sub-middle São Francisco River Valley: Morphophysiological aspects.

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Abstract

Water availability in arid and semi-arid areas has been a limiting factor to the expansion of agricultural production in several regions of the world. In this context, this research aimed to study the morphophysiological responses of pomegranate as a function of different irrigation depths in the Sub-Middle São Francisco River Valley (SSFV). The research was carried out in a commercial area located in Petrolina-PE, Brazil, with eight-year-old plants of lineage No. 12 of Embrapa Semi-Arid, spaced 4.0 x 2.0 m. Drip irrigation with a flow of 2.4 L h\(^{-1}\) emitter\(^{-1}\) was used every two days. The treatments consisted of D50 – continuous irrigation deficit with 50% ET\(_0\) replacement, D75 – continuous irrigation deficit with 75% ET\(_0\) replacement, D100 – continuous irrigation deficit with 100% ET\(_0\) replacement, and DF – farm irrigation. The experiment was conducted in randomized blocks with five replications. Plant height, cup diameter, number of flower buds, flowers, and fruits, relative chlorophyll content, free proline content in the leaves, gas exchange, water potential in the branch, and soil moisture were evaluated. The data were submitted for analysis of variance and regression at the 5% probability level, using the Sisvar program. We observed that the analyzed pomegranate can avoid excessive water loss to the atmosphere when subjected to water stress using different mechanisms. Moreover, the deficit of irrigation depths led to an anticipation and/or uniformity of flowering.

Keywords: *Punica granatum*; water relationships; pomegranate, Petrolina

Introduction

The sub-middle São Francisco River Valley is known as a major fruit producer in the Northeast of Brazil, especially grapes (*Vitis vinifera* L.) and mangoes (*Mangifera indica* L.), which are highly dependent on the price of the international market, subject to the risk of international crises, indicating the need for crop diversification in this region.

The pomegranate (*Punica granatum* L.) is one of the fruit trees known since antiquity, mentioned in the bible together with the vine and olive. It is native to Central Asia but ended up expanding to different soil and climate conditions. In addition, it is undemanding to water (500–700 mm year\(^{-1}\)), tolerant to drought and salinity, and can be irrigated with water whose electrical conductivity is up to 5 dS m\(^{-1}\) (AJAP, 2017).

The quantity and quality of water available for irrigation are limiting factors for the expansion of agriculture in arid and semi-arid regions (Nasrabadi et al., 2020).

Bearing in mind that water resource management strategies in Brazil, especially in the São Francisco River Basin, to maintain adequate reservoir levels for energy can intensify conflicts between water-demanding sectors (ANA, 2012) and the climate conditions of the semi-arid region, characterized by deficits in water balance and periodically prolonged drought events, studying water saving tactics is essential.

Galindo et al. (2018) proposed that one way to deal with water scarcity in the semi-arid Mediterranean region and predicted climate changes on a global scale is to use deficit irrigation strategies associated with emerging crops such as jujube (*Ziziphus jujuba* Mill. J.), loquat (*Eriobotrya japonica* Lindl.), pistachio (*Pistacia vera* L.), and pomegranate (*Punica granatum* L.), which
can withstand water restriction with minimal impact on production and fruit quality.

Pomegranate, for example, is recognized as a fruit rich in bioactive compounds, which have antioxidant and anti-inflammatory activity. The confirmation of these medicinal properties and high nutraceutical values have aroused interest in the pomegranate among farmers, the pharmaceutical industry, and researchers, thus encouraging new research and promoting an increase in the production and development of new products (Hussein & Gouda, 2018; Souza et al., 2018).

Therefore, studies have been carried out in several countries aiming to identify pomegranate cultivars tolerant to water stress, as well as their performance in terms of irrigation deficit strategies (Collado-González et al., 2015; Intrigliolo et al., 2013; Galindo et al., 2017; Noitsakis et al., 2016; Selahvarzi et al., 2017; Tatari et al., 2020).

Despite the tolerance to water scarcity being an intrinsic mechanism to the species, the ability to tolerate the water supply restriction is related to some factors, such as the region of the world, the cultivar, the phenological stage in which the restriction occurs, and the restriction level. In this perspective, the proline content, antioxidant enzymes, and ion displacement can be used as criteria in the selection of cultivars tolerant to water stress (Tatari et al., 2020).

However, few studies have evaluated the behavior of pomegranate plants under different irrigation strategies and the possible morphological and physiological responses under the conditions of the Brazilian semi-arid region, especially in the region of the São Francisco River Valley.

Thus, this research aimed to study the physiological, morphological, and flowering responses of the pomegranate as a function of different irrigation strategies in the Sub-Middle São Francisco River Valley.

Material and Methods

Experimental and orchard conditions

The study was carried out from 09/16/2020 to 03/16/2021 on a farm (9°23′ S; 40°30′ W; 380 m altitude), in the plot No. 1358 in the Senador Nilo Coelho Irrigated Perimeter, N10, Petrolina-PE, Brazil, whose climate classification is BSwh’ according to Köppen (Texeira, 2010).

Pomegranates from lineage No. 12 (Embrapa Semi-Arid), whose plants are approximately 2.0 m tall, have fruits with an intense red peel and pulp, soft seeds, and high soluble solids content in the pulp, were used.

The eight-year-old orchard, grown in ridges, is spaced 4.0 x 2.0 m, with plant material remaining from pruning kept in the planting rows to form a mulch and help to retain moisture and conserve the soil.

The fertilizers potassium sulfate, calcium nitrate, and MAP were used, totaling 23 kg ha⁻¹ of N, 14 kg ha⁻¹ of P, and 150 kg ha⁻¹ of K applied throughout the cycle by fertigation and split into biweekly frequencies.

Irrigation treatments and meteorological variables

Irrigation was applied on Mondays, Wednesdays, and Fridays by a drip system with a double line, eight emitters per plant, a mean emitter flow of 2.4 L h⁻¹, and an estimated application efficiency (Merrian and Keller, 1978) of 82%.

Four irrigation treatments were considered for the study to define the best irrigation strategies for pomegranate and water saving, as follows:

- D50 – continuous deficit with programmed irrigation to replace 50% of the reference crop evapotranspiration (ET₀).
- D75 – continuous deficit to replace 75% of the ET₀.
- D100 – continuous irrigation to replace 100% of the ET₀.
- DF – irrigation adopted on the farm, corresponding to the replacement of a fixed depth of 14.6 mm day⁻¹, with a mean value of 154% of the ET₀.

The irrigation deficit was used from 19 days after pruning (DAP). Soil matric potential was monitored from that date onwards using tensiometers installed at a depth of 35 cm, with three replications per treatment, with a digital tensiometer, before starting irrigation.

Data on temperature and air humidity were obtained using a thermo-hygrometer (model HMP45CL45-PT, Campbell), global solar radiation using a pyranometer (model LP02-L12-PT, Campbell), and wind speed at 2 m of the surface by an anemometer (03002-L45, Campbell), which were connected to a datalogger (CR800, Campbell) with readings taken in an interval of 1 second and mean values stored every 15 minutes.

Reference evapotranspiration (ET₀) was estimated using the Penman-Monteith equation (Allen et al., 1998) and precipitation was defined using a pluviometer. (Figure 1) shows the micrometeorological data throughout the study.

Evaluated variables

Physiological and morphological responses of pomegranate were studied under different irrigation strategies.

The physiological responses consisted of determining the free proline content in the leaves
Irrigation management of *Punica granatum* L. (BATES et al., 1973) at 92 DAP, CO₂ assimilation rate (A) ([µmol m⁻² s⁻¹]), stomatal conductance (gs) ([mol m⁻² s⁻¹]), transpiration (E) ([mmol H₂O m⁻² s⁻¹]), and the internal CO₂ concentration (Ci) ([µmol m⁻² s⁻¹]) at 105 DAP, using a Wallz GFS3000 infrared gas analyzer (IRGA). These data allowed quantifying the instantaneous carboxylation efficiency (A/Ci) ([µmol m⁻² s⁻¹] / [µmol m⁻² s⁻¹]).

The water potential in the branch was evaluated at 87 DAP, using a Scholander chamber (Scholander, 1965).

The relative chlorophyll a and b were acquired at 37, 51, 70, 84, 92, and 113 DAP with a Falkor ChlorofiLOG digital chlorophyll meter.

The morphological measurements were taken at 35, 51, 63, and 79 DAP, plant height (m), diameter (m), and crown volume (m³) at 100 and 126 DAP, and the leaf area of 20 leaves per plant (Nasrabadi et al., 2020) at 60 DAP. Additionally, flowering was evaluated by counting flower buds and flowers.

**Statistical design and data analysis**

The experimental design consisted of randomized blocks, with five replications per treatment. Each plot had four rows with five plants each, and the central plant was considered as the experimental unit.

DAP also became a source of variation for statistical analysis for the variables evaluated more than once, that is, over the days after pruning, and were used in the analysis for a better understanding of the impacts of treatments of irrigation on the variables over time.

Thus, the effect of different irrigation management on pomegranates was evaluated by analysis of variance, followed by regression analysis when it was significant, choosing the model that best fitted the data, considering the coefficients of determination r² and adjusted r² and the value of the F-test of the analysis of variance. The statistical program Sisvar was used (FERREIRA, 2011).

**Results and Discussion**

**Meteorological conditions, applied water, water status, and gas exchange of pomegranate**

Reference evapotranspiration ranged from 1.32 to 7.27 mm day⁻¹ (Figure 1B) and the lowest values (less than 2 mm day⁻¹) occurred due to precipitation (Figure 1B) and/or intense cloudiness. As expected for this period of the year (October and November) in the region, there is a higher atmospheric demand up to 60 DAP, a consequence of the higher values of vapor deficit (Figure 1A), coinciding with the flowering stage. In general, ET₀ remained between 5 and 6 mm between 65 and 180 DAP when the fruiting, growth, and ripening stages prevailed.

(Figure 2) shows the amount of water applied to each irrigation, accumulated and the reduction in the applied water depth compared to farm irrigation over the days after pruning pomegranate plants of lineage No. 12 from 19 to 180 DAP in Petrolina-PE, Brazil. 2020–2021.

Soil matric potential values ranged from −0.50 to −37.90 kPa (for irrigation based on 100% ET₀) and −0.33 to −14.25 kPa with irrigation management according to farm programming (DF). Soil matric potentials were mostly less negative than the potential at field capacity (−10 kPa), especially when using DF, throughout the entire pomegranate cycle (from pruning to final harvest). It indicates that water application was excessive and unnecessary (DF) or higher than the crop demand, which could have been lost due to deep percolation, although it was not evaluated. The highest water extraction by the plants was observed between 77 and 150 DAP, when the fruit growth stage prevailed (Figure 3), due to the higher water consumption of pomegranate at this stage and the high atmospheric demand (Figure 1B).

Soil matric potential and the branch water potential, the proline content, transpiration (E), and stomatal conductance (gs) of the pomegranate crop were significantly influenced by the irrigation strategies (Table 1). (Figure 4) shows the models that best define the...
Irrigation management of Punica grana...

A linear and negative relationship was observed between the soil matric potential and irrigation depths (Figures 3 and 4A). Water restriction led to more negative values, confirming the reduction of soil water availability. Continuous irrigation deficits in D75 and D50 resulted in a decrease of 16 and 46% in the matric potential of the soil cultivated with pomegranate, respectively, at 145 DAP compared to 100% ET₀.

The decrease in water availability due to the adopted irrigation strategy promoted changes in the water potential of pomegranate branches (Figure 4B) at noon. There was an adjustment to the linear model with the highest value of ψ_branch observed in plants under DF irrigation, with a gradual decrease under water restriction, and the lowest mean was associated with a depth of 50% ET₀.

Similarly, Intrigliolo et al. (2013) observed that pomegranate plants had a minimum water potential in the branches of −2.40 MPa for the continuous deficit strategy with 50% ETc replacement and −1.50 MPa for the control treatment (100% ETc), whose soil water content ranged from 85 to 100% of field capacity moisture.

Water stress caused by excess water in the soil also affects the water status of pomegranate. According to Olmo-Vega et al. (2017), stomatal closure is also used under these conditions, as there was a marked reduction (from −1.2 to −3.8 MPa) in the water potential of the branches of the cultivar Valenciana under flooded soil conditions.

These changes in ψ_branch as a function of irrigation differentiation, especially under soil water deficit, are

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**Figure 3.** Matric potential of soil cultivated with pomegranate of lineage No. 12 at a depth of 35 cm as a function of irrigation strategies (D50, D75, D100, and DF) and days after pruning, Petrolina-PE, Brazil, 2020–2021. Accumulated depths per treatment: D50: 430.18 mm; D75: 576.50 mm; D100: 719.81 mm; DF: 1066.83 mm.

**Table 1.** Summary of analysis of variance for free proline content, soil matric potential (Ψm), branch water potential (Ψbranch), proline content, CO₂ assimilation rate (A), transpiration (E), stomatal conductance (gs), internal CO₂ concentration (Ci), and the ratio of CO₂ assimilation rate and internal CO₂ concentration (A/Ci) of pomegranate plants of lineage No. 12 as a function of irrigation strategies (D50, D75, D100, and DF% of ET₀), Petrolina-PE, Brazil, 2020–2021

<table>
<thead>
<tr>
<th>SV</th>
<th>Ψm soil</th>
<th>Ψbranch</th>
<th>Proline content</th>
<th>A</th>
<th>E</th>
<th>gs</th>
<th>Ci</th>
<th>A/Ci</th>
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<tbody>
<tr>
<td>Depth</td>
<td>21.24*</td>
<td>5.65*</td>
<td>20.85**</td>
<td>2.98*</td>
<td>4.27*</td>
<td>4.06*</td>
<td>0.380*</td>
<td>0.35*</td>
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<td>3.61*</td>
<td>0.59*</td>
<td>1.52*</td>
<td>2.73*</td>
<td>2.45*</td>
<td>0.66*</td>
<td>0.08*</td>
</tr>
<tr>
<td>CV(%)</td>
<td>21.32</td>
<td>9.26</td>
<td>9.58</td>
<td>37.05</td>
<td>36.71</td>
<td>37.08</td>
<td>94.21</td>
<td>62.1</td>
</tr>
</tbody>
</table>

*Significant at 1%; *Significant at 5%; ns not significant by the F-test.
plant responses, signals (hydraulic and non-hydraulic) that are translocated to the transpiration flow, starting to act in stomatal regulation and, consequently, conductance. Thus, minimizing water losses through transpiration is possible (STOLL et al., 2000; LIU et al., 2006; NOITSAKIS et al., 2016).

A quadratic trend can be observed for proline contents (Figure 4C), stomatal conductance (Figure 4D), and transpiration (Figure 4E) of pomegranate plants depending on the employed irrigation strategies. There was a higher proline accumulation in the leaves, a decrease in conductance, and, consequently, a reduction in transpiration in plants irrigated under water stress but with no effect on the assimilation and internal CO2 concentration (Table 1). The minimum proline point (34.93 µmol g⁻¹ FM) was obtained in pomegranate leaves subjected to irrigation to replace 131.94% ET0. Plants subjected to severe water deficit (D50) showed an increase of approximately 40% in the proline content when compared to the minimum depth. The proline content in plants irrigated with DF was 35.84 µmol g⁻¹ FM.

Tatari et al. (2020) also observed an increase in the proline content due to water stress. The cultivar M-Saveh showed an increase of 20.72% under stress conditions compared to the control treatment. Moreover, Olmo-Vega et al. (2017) verified an increase in proline concentration in the leaves due to water stress related to excess water in the soil when submitting the cultivars Valenciana, Mollar de Elche, and Wonderful to soil flooding.

Figure 4. Free proline content in the leaves at 92 DAP (A), soil matric potential at 145 DAP (B), water potential in the branch at 87 DAP (C), stomatal conductance (gs) (D), and transpiration (E) at 105 DAP of pomegranate plants of lineage No. 12 depending on the applied irrigation depths, cultivated in N10, Nilo Coelho Irrigated Perimeter, Petrolina-PE, Brazil, 2020–2021. Accumulated depths per treatment: D50: 430.18 mm; D75: 576.50 mm; D100: 719.81 mm; DF: 1066.83 mm.
The 98.1% ET\(_{c}\) irrigation depth was responsible for the highest stomatal conductance of pomegranate plants (52.9 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)). This value exceeded by 35% the stomatal conductance detected in pomegranate plants irrigated under continuous severe deficit (D50). Excessive irrigation (DF) led to a reduction of 25.77 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\) relative to the maximum conductance.

Parvizi et al. (2016) found a reduction of around 25% in the stomatal conductance of pomegranate plants between full irrigation and a 50% deficit at 170 days after flower induction, that is, during the fruit growth stage.

Olmo-Vega et al. (2017) observed an even higher reduction in conductance (around 70 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) as a response to pomegranate cultivars subjected to flooding. The authors also reported other effects of hypoxic and anoxic conditions in the soil, such as reduced vegetative growth and reduced net CO\(_2\) assimilation, which exert a direct influence on harvest yield. They concluded that the three tested cultivars were sensitive to flooding and did not identify morphological, physiological, or biochemical mechanisms of adaptation to these conditions.

The higher stomatal conductance associated with the 98% ET\(_{c}\) depth also resulted in a higher transpiration rate, with a maximum of 1.23 mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\). The moderate irrigation deficit (D75) caused a 9% reduction in transpiration, but the 50% water restriction, as well as the use of excessive depth (DF), led to a higher reduction in leaf transpiration (39 and 49%, respectively), which is one of the main pomegranate mechanisms to deal with water stress (ADIBA et al., 2020).

Therefore, the pomegranate crop showed the ability to minimize water loss to the atmosphere under water stress conditions, such as an increase in the proline content, as its accumulation in the cells, although influenced by the cultivar, adjusts the osmotic potential to decrease the water potential of the leaf cells and the consequent water loss through transpiration (ADIBA et al., 2020; TATARI et al., 2020). Importantly, the effect of water stress on gas exchange was a consequence of water availability in the soil (Figure 3) and meteorological conditions (Figures 1A and 1B) on the day of its evaluation (105 DAP). On that day, the atmospheric demand reached 5.97 mm, the mean air temperature was 27.32 °C, the mean RH was 66.11%, the solar radiation was 26.62 MJ m\(^{-2}\) day\(^{-1}\) and the soil matric potential was around −40 KPa in deficit treatments, −9.3 KPa for D100, and −5.97 KPa for DF.
continuous deficit (75% ETC) between years of deficit irrigation for the cultivars Shishecap and Malas-Yazdi.

(Figure 7) shows the relative chlorophyll indices of pomegranate plants as a function of irrigation strategies and evaluations (DAP). A higher change in CaRI and CbRI was observed with increasing days after pruning, a fact confirmed by the higher curvature of the DAP factor line on the response surface.

Regarding the factor depths, CaRI was higher for plants treated with D50 and D75 than those irrigated with D100 and DF. In general, an increase was observed in this index over the course of the evaluations up to 70 DAP, followed by a reduction for all depths. This increase over time followed by a decrease at 94 DAP was also observed for CbRI.

The maintenance of relative chlorophyll contents even with water restriction is advantageous for the plant, as these pigments are directly linked to photosynthesis so that higher chlorophyll contents allow higher light interception and higher photosynthetic rate, contributing to the expression of the crop’s productive potential (ADIBA et al., 2020).

The responses of the pomegranate lineage No. 12 indicate the ability of the plant not to reduce photosynthetic activity despite having undergone water stress, indicated by a reduction in the water potential of branches, an increase in proline, and a decrease in conductance and transpiration.

In contrast, Adiba et al. (2020) and Pourghayoumi et al. (2017) observed that chlorophyll a and b contents were negatively influenced by severe continuous irrigation deficits in pomegranate cultivars. The authors...
inferred that this reduction is advantageous in terms of tolerance to water stress, as there is a better distribution of light in the plant canopy, minimization of photochemical damage due to the absorption of light energy higher than the photosynthetic capacity, and reduction of the thermal load in the plant crown, thus providing less water required to cool the leaves.

Flowering components

The interaction between the applied irrigation depths and DAP significantly influenced the flowering and fruiting of the pomegranate. The depths promoted a significant effect on the number of fruits despite the depths alone did not influence the mean number of buds and flowers for the evaluated dates (Table 3).

(Figure 8) shows the mean values of flower buds (42 and 56 DAP) and the mean of fruits according to the irrigation depths, with a quadratic relationship, as, in general, the increase in the water depth resulted in lower means per plant.

Table 3. ANOVA of the number of flower buds, number of flowers, and number of fruits according to the different applied irrigation depths (D50, D75, D100, and DF) and days after plant pruning (42, 56, 72, 86, 98, and 116 DAP) in pomegranate plants of lineage No. 12 cultivated in N10, Nilo Coelho Irrigated Perimeter, Petrolina-PE, Brazil, 2020–2021

<table>
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<th>Effect</th>
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<td>36.5</td>
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</tr>
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<td>DAP</td>
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<td>22.4</td>
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<tr>
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</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>46.07</td>
<td>23.34</td>
</tr>
</tbody>
</table>

**Significant at 1%; *Significant at 5%; ns not significant by the F-test.**

A difference was observed for the number of buds in the first evaluation at 42 DAP (Figure 8A) when the minimum depth was 154.1% ET₀, whose mean number of buds was 55.96, while the restriction of 50% ET₀ resulted in a mean of 119.9 buds. D50 also significantly outperformed D154 at 56 DAP (Figure 8B), with 23% of flower buds and the lowest number of buds (61.26) under an irrigation depth of 108% ET₀.

The number of buds was similar between depths during the other evaluations (72, 86, and 116 DAP) (Figure 8). However, pomegranate flowering was favored by irrigation deficit (D50 and D75), as the mean number of fruits (Figure 8C) exceeded the treatments D100 and DF.

According to Silva et al. (2009), the regulated water deficit with the suspension of irrigation for 60 days at the growth stage was effective in standardizing coffee flowering, as stress induced the break of dormancy of reproductive buds with consequent synchronization of flowering, which was associated with high production.
collected 94.95 fruits for the continuous deficit treatment by applying 50% of ETc throughout the entire cycle, and 110 fruits for the control treatment (100% of ETc) in the first cycle with water restriction.

Conclusions

Pomegranate of lineage No. 12 grown in Petrolina-PE, Brazil, presents morphophysiological changes due to irrigation restriction, with no negative impacts on fruit set and with flowering uniformity under water deficit.

The pomegranate crop of lineage No. 12 can avoid the loss of too much water to the atmosphere under water stress conditions through accumulation of proline in the leaves, reduction of leaf water potential, stomatal conductance, and transpiration, without reducing CO₂ assimilation and photosynthetic efficiency.

The continuous water deficit (D50) allows for a water saving of 63% relative to the irrigation management of the crop carried out on the farm, resulting in a possibility of increasing the irrigated area by 2.64 ha.

The irrigation of 10 hectares of pomegranate plants under a continuous deficit of 50% of the crop evapotranspiration can generate savings of 63,644 m³ of water in a production cycle of six months, which is enough to meet the needs of 113.2 people per year, with consumption 154 L daily.

References


Leal et al. (2024) Irrigation management of Punica grana...


Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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