Initial growth of sunflower plants under different water regimes

Crescimento inicial de plantas de girassol sob diferentes regimes hídricos

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ABSTRACT
The present study aimed to identify through a range of morphophysiological aspects the effect of water availability in the establishment and growth of Helianthus annuus plants. The experiment was carried out in a greenhouse in the State University of Goiás, Ipameri.
It was set up in completely randomized design with six treatments (0%, 25%, 50%, 100%, 200% and 400% of evapotranspiration) and six replications of BRS 323 cultivar in 15 liters pots with 12 kg of substrate containing soil, sand and manure in 3:1:1 proportion respectively. The treatments were established 18 days after the seedling emergence and the analysis were performed on 25th day. Thus, the *H. annuus* plants tolerate drought due to the high stomatal sensitivity which limits the loss of water to the atmosphere and by the increase of root system depth. Also, it stands out that *H. annuus* plants did not show stress symptoms in the excess of water, because the high transpiration rate removed to atmosphere the amount of excess of water from irrigation.

**Keywords**: *Helianthus annuus*, drought, flood.

**1 INTRODUCTION**

Sunflower (*Helianthus annuus* L.) it’s one of the most consumed oilseed crop in the world, which, Ukraine, Russia and Argentina are the major producers, around 65% of the world production (CONAB, 2017). Sunflower contains a high quality vegetable oil, the oil consist 40% of the seed. In Brazil, the planted area has increased by 41% in the 2023 off-season compared to the previous year. The Midwest Region is the main producer of sunflower in Brazil, with emphasis on the states of Mato Grosso and Goiás (CONAB, 2023). In Mato Grosso, the planted area has been reduced over the years due to competition with corn planting. In Goiás, the state with the highest sunflower production, the planted area in 2023 was 35,000 hectares with a very favorable scenario, where sunflower is being replaced by other crops such as sorghum and corn in the off-season (CONAB, 2023).
Brazil has a elevated potential for sunflower cultivation by a great availability of area, high technology and propitious climate. Central west is seen as a powerful region for sunflower cultivation due to edaphoclimatic conditions and technological availability, however, it’s important to promote research that secures the sunflower growth under abiotic stress conditions to prevent that sunflower cultivation areas not be replaced for other tolerant crops. According to Burio et al. (2016), the sunflower is sensitive to varitations in water availability, because results in growth and yield reduction, nonetheless, the specie is considered drought resistant due to a great ability to drain soil water.

The abiotic stresses: drought, salinity, heat and low oxygen are relevant for delay the growth and provoke yield loss. The cultivars development and verification of management practices capables to provide better adaptation to the species to these type of unfavorable conditions consist in a challenge for science, and according to Faria et al. (2020) the extreme events has been increased throughout the years with climate changes and generates significant economic damages in agriculture.

The water is the most abundant factor in nature and the most limited in farmlands due to rainfall seasonality. The water deficiency it’s the most frequent stress in nature, 35% cropland areas in the world are considered arid and semi-arid as a result of the lack of water (MATOS et al., 2019). The shortage of water availability in soil generates restriction in cellular expansion, leaf area limitation, reduced stomatal opening, leaf drop, low biomass accumulation besides the decay of photosynthesis and yield (TAIZ et al., 2017).

The excess of water in the soil causes reduction in growth and development in sunflower plants, in these conditions the plant show similar effects to drought by the inhibition of aquaporins activity, important proteins in water transport in the membranes, the saturation occasionated by the waterlogging doesn’t allow the root system respiration and making more difficult the solute transport (HUANG et al., 2019; RAHMAN et al., 2019).

Despite of sunflower be a crop cultivated in the summer season, rainy season, and winter season, shortage of water, the specie it’s not preferred in summer season (soybean) and has been losing territory in the winter season for sorghum and maize in Goiás state. According to Costa et al. (2021) and De Brito et al. (2022), despite having important drought and salinity tolerance mechanisms, sunflower shows reduced growth when
subjected to these stress conditions. The present study exposes two major problems as reasons to the lost of territory of the sunflower cultivation to be clarify: i) The cultivation in the summer crop season not allows financial gains comparing with soybean, ii) In the winter crop season the sunflower is less tolerant to drought than other species. Thus, the present study aimed to identify through a range of morphophysiological aspects the effect of water availability in the establishment and growth of Helianthus annuus.

2 MATERIAL AND METHODS

The study was carried out in a greenhouse covered with transparent plastic and shade cloth on the sides with 50% interception of the light, in State University of Goiás, Ipameri campus (Lat. 17° 42’ 59,12 S, Long. 48°08’40,49” West, Alt. 773 m), Ipameri, GO. The region has tropical climate with dry winter and wet summer (Aw) according to Köppen classification and average temperature of 20°C (ALVARES et al., 2013). Three seeds of Helianthus annuus BRS 323 cultivar were sown per pots with 15 liters capacity full with 12 Kg of substrate containing oxisol, sand and manure at 3:1:1, respectively. The chemical analysis of the soil showed the following aspects: pH (CaCl$_2$) 5.4; 16 g dm$^{-3}$ of organic matter; 68 mg dm$^{-3}$ of P; 6.81 mmolc dm$^{-3}$ of K (Mehlich-1); 22 mmolc dm$^{-3}$ (SMP Method) of H + Al; 31 mmolc dm$^{-3}$ of Ca; 15 mmolc dm$^{-3}$ of Mg; 53 mmolc dm$^{-3}$ Base Saturation; 75 mmolc dm$^{-3}$ Cation Exchange Capacity and 71% Base Saturation.

Eighteen days after emerging (DAE) the plants were thin out, just one plant remained in each pot and the treatments were set up during seven days. The experiment was set up in a completely randomized design with six treatments and six replications. Eighteen DAE the plants were irrigated with water volumes according to 0%, 25%, 50%, 100%, 200% and 400% of substrate holding capacity.

The seedlings were irrigated daily, until eighteen DAE, with 100% of substrate holding capacity. The crop coefficient (kc) for sunflower has not been determined for Ipameri region, GO, it was used kc=1 following the estimates from FAO 56 (ALLEN et a., 1998) for a group of crops at early stages of development.

The water volumes were estimated setting the reference evapotranspiration and the crop coefficient. To determine the crop evapotranspiration, was used the equation:

$$ETc = ET0 \times kc$$
Where:

Etc = crop evapotranspiration  
Kc = crop coefficient  
ETo = Reference evapotranspiration  

The ETo daily measurement was realized by Penman-Monteith method, recommended by FAO (Smith et al., 1991) using the daily maximum and minimum temperature, relative humidity, solar radiation and wind speed obtained from Ipameri INMET weather station.

At 25 DAE the following analysis was executed: leaf number, plant height, stem diameter, biomass, root mass ration (RMR), stem mass ration (SMR), leaf mass ratio (LMR), photosynthetic pigments, water relative content (RWC), transpiration ratio and chlorophyll $a$ fluorescence.

**Growth Variables:** Plant height was measured from the root-stem transition region at soil level (crown) to the tip of the stem using graded rule. The stem diameter was measure at the crown with a digital pachymeter. The number of leaves was obtained by counting. The roots, stems and leaves were separated and dried in an oven at 72 °C until constant dry weight and the weighed. The dry matter data were used to calculate the RMR, LMR and SMR and biomass.

**Photosynthetic Pigments:** To determine the total chlorophylls and carotenoids concentrations, 0.6 mm diameter leaf discs were removed from completely opened leaves and placed in test tubes containing dimethyl sulfoxide (DMSO). Then extraction was carried out in a water bath at 65 °C for one hour. Aliquots were removed for spectrophotometric reading at 480, 649 and 665 nm. Then contents of chlorophyll $a$ (Cl $a$), chlorophyll $b$ (Cl $b$) and total carotenoids (Car) were determinate according to the equation proposed by Wellburn (1994).

**Relative Water Content:** To obtain the relative water content, ten 12 mm leaf discs were removed, weighed and placed for four hours to saturate in petri dishes with distilled water. The discs were again weighed and placed to dry at 70 °C for 72h to obtain the dry matter weight.

**Transpiration:** The total daily transpiration of the plant was determined from the difference in the mass of the pots. The set of pots with plants were placed in individual plastic bags fixed with a rubber band at the stem of the plant, leaving the canopy (leaves and stem) exposed. The pots were weighed at 12 o’clock (mass 1) and again 24 hours
The total transpiration was estimated based on the difference between mass 1 and mass 2.

**Fluorescence:** The analysis of chlorophyll $a$ fluorescence were performed using a portable fluorometer JUNIOR-PAM (Walz, Germany) at 4 am with light saturation pulse emission of 0.3 seconds, under 0.6 KHz frequency, at 30 days after implement the water regimes. The data of fluorescence were computed using software WinControl-3.

**Statistical Procedures:** An analysis of variance and a linear and quadratic regression were performed and in the cases of significance of regression by the F test, the coefficient of determination ($R^2$) was calculated by the ratio of the sum of the squares of the regression over the total sum of squares. Multivariate analysis was carried out by multiple regression using the forward stepwise model (SOKAL and ROLF, 1995) and principal component analysis was performed using a permutational multivariate analysis of variance (PERMANOVA - ANDERSON, 2001). The Statsoft (STATISTICA, 2019) and SigmaPlot 10.0 (Systat Software, 2006) software was used to carry out these analyses.

### 3 RESULTS

The fluorescence and photosynthetic pigments variables did not fit significant to any regression model, so that, the variations in water availability in the present study did not promote explainable variations to biologically understandable models. The results of the regression analysis for plant height, leaf number, leaf area, biomass, stem diameter, and transpiration are shown in figure 1.

Based on the mean values of the variables, the highest plant height occurred in the greatest water regime corresponding to 400% of evapotranspiration (ET) and 23% superior than the plants with 100% of ET and 31% higher comparing to severe drought, the plants didn’t receive water (0% of ET). The highest number of leaves was obtained in the 400% of ET irrigation, superior in 15% and 20.75% when compared to the plants with 100% and 0% of ET respectively. The leaf area in 400% of ET was 58% and 43% superior to the plants under severe drought (0% of ET) and the proper water supply (100% of ET) respectively. The biomass showed the greatest value when the plants were irrigated with 400% of ET and 54% and 71% superior to 100 and 0 of ET regimes respectively.

Stem diameter was superior when the plants were irrigated with 400% of ET and superior in 15% and 21% when compared with the plants irrigated with 100% and 0% of ET respectively. The highest transpiration rate was obtained when the plants were
irrigated with 400% of ET and superior in 71% and 95% than the plants at 100% and 0% of ET respectively.

Figure 1. Graphs with regression equations for height, leaf number, leaf area, biomass, stem diameter and transpiration of sunflower plants under different water levels. * Significant at 5% probability; ** significant at 1% probability.

The results from the regression analysis for the ratios of stem (SMR), root (RMR) and leaf (LMR) are shown in figure 2. All mentioned variables fitted in the linear regression model. The stem and leaf ratios decreased with the increments in water availability, so that, in SMR the greatest values were obtained in plants at severe drought,
0% of ET, with values 10% and 36% superior than plants at 100% and 400% of ET respectively.

The LMR was superior in plants under severe drought (0% of ET) in 5% and 22% compared to plants irrigated with 100% and 400% of ET respectively. The principal component analysis explain 70.8% of the data variations and reveals that the greatest root length in the plants under severe drought with decrease as the water availability increases, whilst biomass is greater in plants irrigated with 400% of ET and decreases as the water availability decline. The RMR was superior when plants was irrigated with 400% of ET in 49% and 52% compared to plants at 100% and 0% of ET respectively.

Figure 2. Graphs with regression equations for stem mass ratio (SMR), root mass ration (RMR) and leaf mass ration (LMR) of sunflower plants under different water levels. * Significant at 5% probability; ** significant at 1% probability.

The multiple regression analysis shown in table 1 represents 89% of biomass variation and shows that the root and leaf dry mass and stem diameter were the major variables in biomass growth.
Table 1. Multiple regression analysis to identify the variables with major importance in biomass accumulation in sunflower plants under different water regimes.

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Beta</th>
<th>Std.Err. of Beta</th>
<th>B</th>
<th>Std.Err. of B</th>
<th>t (28)</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-21.325</td>
<td>7.591</td>
<td>-2.809</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transpiration</td>
<td>0.237</td>
<td>0.15</td>
<td>0.007</td>
<td>0.004</td>
<td>1.587</td>
<td>0.124</td>
</tr>
<tr>
<td>Dry root mass</td>
<td>0.485</td>
<td>0.138</td>
<td>0.289</td>
<td>0.082</td>
<td>3.509</td>
<td>0.002**</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>0.384</td>
<td>0.106</td>
<td>1.934</td>
<td>0.534</td>
<td>3.623</td>
<td>0.001**</td>
</tr>
<tr>
<td>Dry leaf mass</td>
<td>0.164</td>
<td>0.074</td>
<td>0.348</td>
<td>0.158</td>
<td>2.209</td>
<td>0.036*</td>
</tr>
<tr>
<td>Biomass leaf</td>
<td>0.111</td>
<td>0.098</td>
<td>0.083</td>
<td>0.073</td>
<td>1.136</td>
<td>0.266</td>
</tr>
<tr>
<td>Relative water content</td>
<td>-0.07</td>
<td>0.068</td>
<td>-0.034</td>
<td>0.033</td>
<td>-1.026</td>
<td>0.313</td>
</tr>
</tbody>
</table>

* Significant at 5% probability; ** significant at 1% probability.


The results referring to the canonical variables are shown in Figure 3. This analysis explains 94.8% of the data variation. Growth variables related to plant vigor were grouped to the left of axis 1 along with treatments with higher volumes of water, while variables related to plant protection against water stress such as carotenoids were grouped to the right of axis 1.

4 DISCUSSION

The initial growth stage, still in establishment, represents a huge vulnerability stage to the abiotic stress, mainly the drought, due to low root system development still unable to drain water from greater depth. During the establishment, the lack or excess of water for a long term results in plant death, not just the low yield, when the stresses occurs in moments after the development. According to Matos et. (2019), a vigorous initial development is fundamental to tolerate stresses in mature vegetative stage, however, the severe stress in initial stage has as consequence the plant death.

The results demonstrated that the *H. annuus* plants under different water regimes showed significant growth changes, under severe water deficit, without water supply, the plants showed reduced height, leaf number, leaf area, stem diameter, SMR and biomass, however, in the interval between the severe water deficit (without irrigation) and the water availability of 100% of ET the plants has a discreetly growth owing to the rise of the cited variables. According to Costa et al. (2021) despite having drought tolerance mechanisms, sunflower shows reduced growth under water deficit.

In the other hand, the gap between the water volumes of 100% and 400% of ET, the increments in the cited variables were emphasized. According to Taiz et al. (2017), the turgor pressure is a *sine qua non* condition to cell expansion by the demand of tension of the protoplasm in the relaxed cell wall. The greatest growth in this interval is due to the water availability in the cell and didn’t show excess, because the *H. annuus* plants performed a higher transpiration rate and eliminated the amount of excess water.

The narrow connection between transpiration and biomass is common in many plant species and crucial for growth studies (MATOS et al., 2019). The results demonstrated that the water availability modified the stomatal conductance, because the low water availability the *H. annuus* plants showed high stomatal sensitivity to avoid water loss, however, as a consequence occurred lower CO2 influx to leaf mesophyll resulting in lower photosynthetic rate and biomass.

The *H. annuus* plants in establishment stages showed higher percentage of biomass allocation in the root system instead of stem and shoot, and the stage justify the need of root growth for drain more water and nutrients from the soil, however, under severe drought is noticed low investments in root and higher biomass partitioning to the shoot. Is possible that in the absence of irrigation, the root system, a water status indicator in soil, has suffered more restriction than the shoot, because the shoot can maintain...
hydrated for a certain period of time with the atmosphere humidity, mainly in the night and early morning period. Conform De Oliveira et al. (2019), hydration of plant tissues in the early morning is more suitable for growth. In addition, because it is a pot experiment, there is a limitation of the development of the root system.

It should be noted that the greater root development is in line with the greater hydraulic conductivity proven by the greater diameter of the stem in plants irrigated with high volumes of water as described in the analysis of canonical variables, however, under water deficit and limited space in the container, the root system of the sunflower plants had limited development. The results corroborate those found by Dos Anjos et al. (2017) who identified variations in the partition of assimilates in the root and oleaginous shoot Jatropha curcas under variation in water availability.

The multiple regression analysis demonstrated that the leaf and root dry masses and stem diameter were the major variables to increase *H. annuus* biomass, so that, the plants under water deficit committed the resources in shoot and root length and under high availability of water results in increases in root mass, shoot and biomass, thus, the *H. annuus* plants tolerates drought due to the high stomatal sensitivity wich limits water loss to atmosphere and the enhanced root depth. Also, it stands out that the *H. annuus* plants did not show symptoms of stresses caused by the excess of water, because the high transpiration rate removed to atmosphere the amount of extra water.

5 CONCLUSION

The *H. annuus* plants are tolerant to water deficit and shows as a strategy the high stomatal sensitivity and increase of root length.

The *H. annuus* plants are tolerant to excess of water relative to 400% of evapotranspiration due to the high transpiration rate wich removes to atmosphere the excess of water from irrigation.

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