Irrigation levels in Chrysanthemum [*Chrysanthemum morifolium* (Ramat. & Kitam.)] mother plants under protected conditions

Niveles de riego en plantas madre de Crisantemo [*Chrysanthemum morifolium* (Ramat. & Kitam.)] bajo condiciones protegidas

Níveis de irrigação em plantas matrizes de Crisântemo [*Chrysanthemum morifolium* (Ramat. & Kitam.)] sob condições protegidas

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Abstract

In Mexico, chrysanthemum [*Chrysanthemum morifolium* (Ramat. & Kitam.)] is one of the species of greatest floricultural importance. However, information regarding water management to produce vegetative material is limited, especially in Yucatán, where there has been significant interest in the production of propagation material. The objective of this study was to evaluate five irrigation levels for obtaining chrysanthemum vegetative material under protected conditions. From September to January 2023-2024, five levels of available water (20, 30, 40, 50, and 60 %) were evaluated in chrysanthemum under a protected structure at the Instituto Tecnológico de Conkal, Mexico. A randomized complete block design with four replications was used, and parameters of plant water relations and growth were evaluated. The results indicated that increasing available water levels up to 60 % available water (AW) in the soil improved plant water potential, relative water content up to 89.34 %, and survival percentage (97 %). With the application of 60 % AW, plants exhibited greater height and stem diameter with 30.13 cm and 4.59 mm, respectively; however, for total dry biomass, the 40 % and 50 % treatments stood out, obtaining 25.01 and 21.82 g plant^{-1}. Based on the results, it is feasible to reduce available water to 30 %, as plant development and survival are not affected.

Keywords: Available water, Water stress, Water relations, Relative water content, Growth.

Resumen

En México, el crisantemo [*Chrysanthemum morifolium* (Ramat. & Kitam.)] es una de las especies de mayor importancia florícola. Sin embargo, la información sobre el manejo hídrico para la producción de material vegetativo es limitada, especialmente en Yucatán, donde existe un gran interés en la producción de material de propagación. El objetivo de este estudio fue evaluar cinco niveles de riego para la obtención de material vegetativo de crisantemo bajo condiciones protegidas. De septiembre a enero de 2023-2024, se evaluaron cinco niveles de agua disponible (20, 30, 40, 50 y 60 %) en crisantemos bajo una estructura protegida en el Instituto Tecnológico de Conkal, México. Se utilizó un diseño de bloques completos al azar con cuatro repeticiones, y se evaluaron parámetros de relaciones hídricas y de crecimiento de las plantas. Los resultados indicaron que incrementar los niveles de agua hasta un 60 % de agua disponible (AD) en el suelo mejoró el potencial hídrico de la planta, el contenido relativo de agua hasta un 89.34 %, y el porcentaje de supervivencia (97 %). Con la aplicación del 60 % de AD, las plantas mostraron mayor altura y diámetro del tallo con 30.13 cm y 4.59 mm, respectivamente; sin embargo, para la biomasa seca total, destacaron los tratamientos del 40 % y 50 %, obteniendo 25.01 y 21.82 g planta^{-1}. Con base en los resultados, es factible reducir el agua disponible al 30 %, ya que el desarrollo y supervivencia de la planta no se ven afectados.

Palabras clave: Agua disponible, Estrés hídrico, Relaciones hídricas, Contenido relativo, Crecimiento.

Resumo

No México, o crisântemo [Chrysanthemum morifolium (Ramat. & Kitam.)] é uma das espécies de maior importância na floricultura. No entanto, informações sobre o manejo hídrico para a produção de material vegetativo são limitadas, especialmente em Yucatán, onde tem havido um interesse significativo na produção de material de propagação. O objetivo deste estudo foi avaliar cinco níveis de irrigação para a obtenção de material vegetativo de crisântemo sob condições protegidas. De setembro a janeiro de 2023-2024, cinco níveis de água disponível (20, 30, 40, 50 e 60 %) foram avaliados em crisântemos sob uma estrutura protegida no Instituto Tecnológico de Conkal, México. Utilizou-se delineamento de blocos casualizados com quatro repetições, e parâmetros de relações hídricas e crescimento das plantas foram avaliados. Os resultados indicaram que aumentar os níveis de água disponível para 60 % de água disponível (AD) no solo melhorou o potencial hídrico da planta, o conteúdo relativo de água para 89,34 % e a porcentagem de sobrevivência (97 %). Com a aplicação de 60 % de AD, as plantas apresentaram maior altura e diâmetro do caule com 30,13 cm e 4,59 mm, respectivamente; entretanto, para a biomassa seca total, destacaram-se os tratamentos de 40 % e 50 %, obtendo 25,01 e 21,82 g planta^{-1}. Com base nos resultados, é viável reduzir a água disponível para 30 %, pois o desenvolvimento e a sobrevivência das plantas não são afetados.

Palavras-chave: Água disponível, Estresse hídrico, Relações hídricas, Conteúdo relativo de água, Crescimento.

Introduction

Globally, chrysanthemum [*Chrysanthemum morifolium* (Ramat. & Kitam.)] is important within the context of the nursery sector due to the economic interest it has achieved, which has been increasing as a result of greater demand in the ornamental industry (Fanourakis et al., 2022; Wang et al., 2022), ranking second worldwide as a cut flower (Hao et al., 2022; Mekapogu et al., 2022). Furthermore, this species has traditionally been used to treat diseases, improve health, and as food (Hao et al., 2022; Mekapogu et al., 2022; Liu et al., 2024), which enhances the value of this important plant.

In Mexico, the production value of chrysanthemum, at the close of the 2023 agricultural cycle, reached 1,724.8 thousand pesos, cultivated across 2,802.84 ha. The State of Mexico is the main producer with 2592.17 hectares (92.5 %), followed by Puebla with 118.00 hectares (4.2 %), Morelos with 59.0 hectares (2.1 %), Chiapas with 10.27 hectares (0.4 %), and San Luis Potosí with 9 hectares (0.3 %) (SEIA, 2023).

In the state of Yucatán, there are no records of chrysanthemum production because very little land is allocated to this activity. In this region, its main use is as a cut flower; however, with the aim of supplying the local and national industry, this form of production is becoming an attractive business (Fanourakis et al., 2022). To produce vegetative material, it is necessary to provide mother plants with special care, highlighting aspects related to water requirements, since water is essential for plant life, and without the required attention, the risk of economic losses can affect production.

Considering the great importance of water in plants, it is necessary to consider that a limited or excessive amount of water for chrysanthemum can constitute a limiting factor for growth and influence plant survival. Therefore, proper irrigation management ensures improved production and propagule quality, especially in a protected environment where water is obtained solely through irrigation (Pereira et al., 2021).

However, irrigation management in floriculture has been characterized by empiricism and often involves excessive or deficient water applications (Pereira et al., 2021); in this sense, chrysanthemum has been no exception. All of this leads to the need to establish ideal irrigation conditions for obtaining chrysanthemum vegetative material under protected conditions. Therefore, the objective of this study was to evaluate five irrigation levels for obtaining vegetative material in chrysanthemum [*Chrysanthemum morifolium* (Ramat. & Kitam.)] under protected conditions.).

Materials and Methods

Experimental Site

The research was conducted in a greenhouse at the Tecnológico Nacional de México, Conkal Campus, in Yucatán, Mexico (21° 05 N y 89° 32 W). According to the classification proposed by Köppen and modified by García (1981), the climate of this region is AwO (X') (I') g, which corresponds to warm sub-humid with summer rains. The site presents a mean annual temperature of 26.6 °C and an average annual precipitation of 900 mm, with an altitude of 8 a.m.s.l.

Experiment Establishment

For the establishment of the experiment, the protected structure and the substrate were disinfected with Furadan (a.i. carbofuran) at a dose of 1.5 mL L^{-1}. The beds were filled with a substrate based on 30 % local soil known as "Kankab" (Rhodic Luvisol) mixed with 70 % henequen bagasse. Planting was carried out using a spacing of 14 x 14 cm between rows and plants, respectively. Chemical fertilization was performed by applying a 50-25-100 treatment of N-P-K, respectively, using urea (46-00-00), phosphoric acid H_3PO_4 (85 %), potassium nitrate KNO_3 (12-0-45), and monoammonium phosphate MAP (12-61-00) as fertilizer sources.

Irrigation level Estimation

The treatments received the same amount of water at the beginning of the study. To achieve this, the field capacity (Θcc) and permanent wilting point (Θpmp) of the substrate were estimated (Eq. 1). These values were obtained in the Water-Soil-Plant Laboratory of the Instituto Tecnológico de Conkal.

$$\mathrm{Am} = (\Theta\mathrm{cc} - \Theta\mathrm{pmp}) * \mathrm{Cd} * \mathrm{Bd} \qquad \text{Eq. 1}$$

Donde:

Am = Available moisture (cm)

Θ_{cc} = field capacity (%)

Θ_{pmp} = permanent wilting point (%)

Cd = Container depth (m)

Bd = Bulk density (g cm^{-3})

Following the calculation of the irrigation level, the water volume required to wet the plots was estimated, considering the plot area (Eq. 2).

$$\mathrm{Iiv} = \mathrm{Am} * A \qquad \text{Eq. 2}$$

Where:

Iiv = Initial irrigation volume (cm^3)

Am = Available moisture (cm)

A = Plot area (cm^2)

For subsequent irrigations, experimental treatments were applied every other day, corresponding to different percentages of available water according to the treatments: 20% (T1), 30% (T2), 40% (T3), 50% (T4), and 60% (T5). The treatments were initiated when the plants had developed three vegetative shoots.

Evaluated Variables

Moisture Content: This was estimated using the gravimetric method, which consisted of weighing a wet soil sample and subsequently drying it in a forced-air oven at 65 °C until constant weight was reached. For this purpose, a soil sample was obtained per experimental unit at a depth of 5 cm using a Veihmeyer-type auger.

Water Potential: This variable was measured monthly between 11:00 and 13:00 h using a Scholander pressure chamber. For this, a fully developed leaf was selected and cut from one plant per experimental unit. The leaf petiole was placed inside a cork ring and inserted into the pressure chamber so that the cut end of the petiole remained outside and completely visible. Pressure was then applied using compressed air until a film of water was observed on the petiole surface. At that moment, the leaf water potential was considered equal to the chamber pressure; it is considered that as the chamber pressure equals the tension of the liquid in the leaf, sap is forced out of the petiole cut. The reading was recorded in Megapascals (MPa), representing the leaf water potential.

Relative Water Content (RWC): This was determined by taking a randomly selected leaf from the middle canopy of the plant. A 1 cm diameter disk was cut and weighed (fresh weight). Subsequently, disks were placed in Petri dishes with distilled water and left to sit for four hours before being weighed again (turgid weight). Samples were then placed in a forced-air oven at 65 °C to obtain dry weight. Relative water content was calculated applying Eq. 3

$$RWC = \frac{FW-DW}{TW-DW} * 100 \qquad \text{Eq. 3}$$

Where:

RWC = Relative water content (%)

FW = Fresh weight (mg)

TW = Turgid weight (mg)

DW = Dry weight (mg)

Survival: To determine the survival percentage, transplanted plants were counted per treatment at the beginning of the experiment and recounted weekly to record the number of surviving plants.

Plant Growth: The parameters considered were diameter, final height of the main stem, leaf area, and plant biomass. In this case, 20 established plants in the experiment were measured. Diameter was measured with a 15 cm digital caliper, evaluated at 1 cm from the soil surface. Height was measured with a tape measure, defined as the length from the surface to the highest apex of the plant. For leaf area, 4 plants per treatment were sampled, totaling 20 plants. This variable was determined at the end of the experiment using a LI-COR 3100 leaf area meter. Biomass was evaluated on 20 plants at the end of the experiment; for this, plant organs (stem and leaf) were separated and placed in separate paper bags. Subsequently, they were dried in a forced-air oven at 65 °C until constant weight. Total biomass was obtained from the sum of the plant organs.

Experimental Design and Statistical Analysis

A randomized complete block design was used with five treatments and four replications. The experimental unit consisted of 42 plants. Data were analyzed through a two-way analysis of variance using the SAS ver. 8.1 statistical package for Windows, and means were compared using Tukey's test ($p \leq 0.05$) (SAS Institute, 2000).

Results and Discussion

Soil Moisture

Figure 1 presents the results of soil moisture content (%) in the chrysanthemum crop under the effect of five levels of available water (AW). Moisture content increased in direct proportion to the increase in applied irrigation levels, as a greater amount of water increased the soil moisture content. A statistically significant difference was detected between the applied irrigation levels of 20 % and 60 % AW. The highest moisture content (38.59 %) was found with 60 % AW; however, this was statistically equal to the treatments of 30, 40, and 50 %

AW.

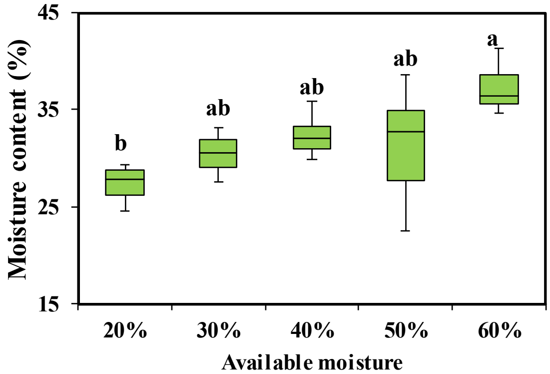

Figure 1. Soil moisture contents in chrysanthemum cultivation under the effect of five levels of available water. Means with the same letter are statistically equal (Tukey, $p \leq 0.05$).

Soil moisture content is an important factor for optimizing crop value (yield, biomass accumulation, flower production, fruit size, and quality, among others), as the concept of the production function is based on the theory that plant growth and development are affected by variations in the soil moisture regime (Blanco and Kalcsits, 2021). The effect of water content on soil water potential is well known. The use of different irrigation levels is a valuable criterion for understanding the effects of irrigation on plant growth (Gonzalez-Nieto et al., 2023). With these results, it is possible to determine the effect of AW contents on the growth and development of chrysanthemum plants and establish adequate irrigation management for the crop in the state of Yucatán, as different moisture contents and, consequently, different levels of water retention in the substrate were achieved. Water is a factor of great importance for plant growth, as deficient water levels can affect cell turgor, which is a determinant factor for cell expansion and the growth of plants and fruits (Gonzalez-Nieto et al., 2023; Cosgrove, 2024). Soil moisture is a factor determined by soil type and water retention capacity, hydraulic conductivity, texture, and soil structure, among other factors; therefore, it is important to establish these parameters in each region to achieve optimal irrigation for each species to be established.

Effect of Available Water Levels on Water Relations in *Chrysanthemum* Plants (Water Potential and Relative Water Content)

Regarding the water potential of chrysanthemum plants, results indicated a positive correlation ($R^2 = 0.94$) between soil moisture content and water potential (Figure 2B); that is, as substrate moisture increased, water potential was higher. The application of 20 % AW resulted in the lowest soil moisture at 28.81 % (Figures 1 and 2B) and presented the lowest water potential in chrysanthemum plants (-0.59 MPa) (Figure 2A), showing statistically significant differences (Tukey, $p \leq 0.05$) compared to the AW levels of 40, 50, and 60 %.

It was observed that the application of different irrigation levels affected the Relative Water Content (RWC) in chrysanthemum plants (Figure 2C). Statistically significant differences (Tukey, $p \leq 0.05$) were detected among the evaluated treatments. The highest RWC (89.34 %) was obtained with 60 % AW; however, it was statistically equal to the treatments of 50 % and 40 % AW, like the results for water potential (Figure 2A). Nevertheless, differences were detected between the 60 % AW level and the 30 % and 20 % AW levels, which obtained RWC values of 83.05 % and 79.51 %, respectively.

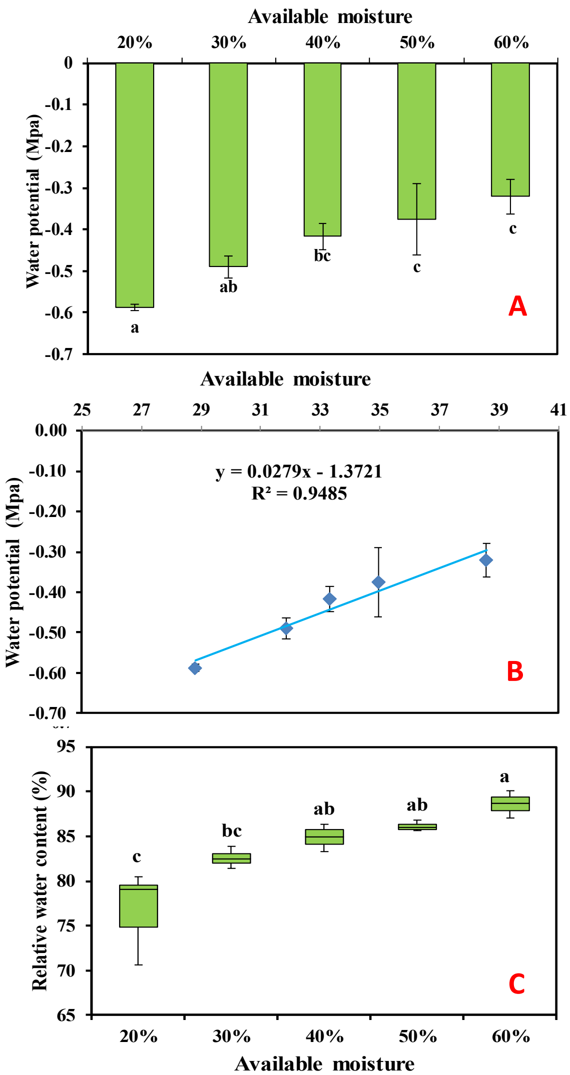

Figure 2. Effect of irrigation level (A) and soil available water (B) on water potential and relative water content (C) of the chrysanthemum plant. Means with the same letter are statistically equal (Tukey, $p \leq 0.05$).

It is widely documented that Ψ is a sensitive indicator and thus indicative of water stress in plants (Pagay, 2022); therefore, continuous monitoring of this indicator offers highly valuable information for irrigation scheduling in crops (Blanco and Kalcsits, 2021). The minimum values, which became progressively more negative as the irrigation level and AW decreased, may be due to the combination of high evaporative demand and lower water availability in the soil with the decrease in substrate moisture (Blanco and Kalcsits, 2021). This may indicate that chrysanthemum plants subjected to AW levels below 31.86 % (Figure 1), which in this study corresponded to an irrigation level below 30 %, might have been stressed by water deficiency, as they could not reach their maximum water status during the development of this experiment. RWC is an indicator of water absorption and plant water status (Bacarrillo-López et al., 2021). The decrease in this variable with the reduction of AW levels is indicative of difficulty on the part of chrysanthemum plants to absorb water at lower AW levels. This indicates that irrigation levels of 20 % and 30 % AW, which provided a soil moisture content of 31.86 % and 28.85 %, respectively, affect water absorption and the maintenance of water relations in chrysanthemum plants.

Survival

Regarding the survival percentage in chrysanthemum plants under the effect of different AW levels, statistical differences ($p \leq 0.05$) were found among the evaluated treatments (Figure 3). A higher survival percentage (97 %) was obtained with 60 % AW irrigation, with no statistical differences compared to the 30, 40, and 50 % AW treatments; however, this result was statistically different from the 20 % AW level, which resulted in the lowest survival percentage at 81 % (Figure 4).

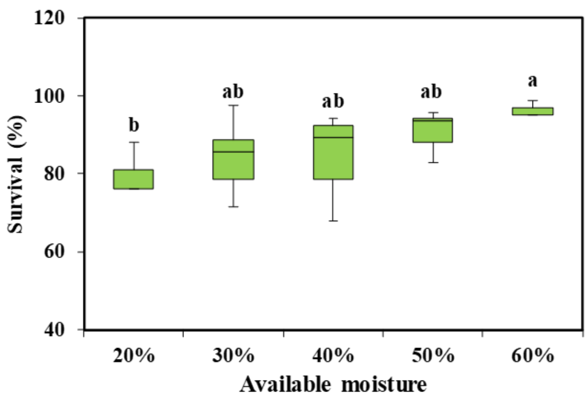

Figure 3. Effect of available water levels on the survival percentage in chrysanthemum plants. Means with the same letter are statistically equal (Tukey, $p \leq 0.05$).

Survival results correspond to those detected for soil moisture content, where differences were detected only when a 20 % AW level was applied (Figure 1). However, survival does not correspond to the results for water potential and relative water content, given that these indicators were more sensitive to water deficit caused by the decrease in soil water content, with their behavior being affected from the 30 % AW level (Figures 2A, B, and C). Water loss can be a limiting factor for plant growth and development through damage caused to metabolism in general, provoking physiological disorders and causing death (Wu *et al.*, 2022). If water is insufficient to cover the plant's water requirements, the final consequence can be death; therefore, lower plant numbers were evidenced in survival at non-optimal irrigation levels, such as 20 % AW. The effects of water stress can vary with plant variety, degree and duration of stress, and crop growth stage, but in susceptible plants, a reduction in soil water content affects many physiological processes such as growth, and under severe deficit, senescence of mature leaves is accelerated, which can result in plant death (Meng *et al.*, 2021).

Water also fulfills important functions in plant physiology (Yang *et al.*, 2020). Most plants, as a stress response mechanism in the first minutes of not covering their water requirement, send hormonal signals (ABA production) that can improve stomatal conductance and photosynthetic activity. However, as the magnitude of water deficiency increases, stomatal closure can occur, causing a decrease in stomatal conductance and CO2 fixation, which decreases photosystem activity (Sharma *et al.*, 2020). The medium and long-term effects of decreased photosynthesis include the degradation of energy reserves required by the plant to sustain its metabolism, leading to death (Yang *et al.*, 2020; Sharma *et al.*, 2020). The results of this work, as reducing the amount of water in the soil produced a decrease in the survival percentage because of an imbalance in the physiological processes of the plant.

Effect of Available Water Levels on Growth Variables (Plant Height, Stem Diameter, Leaf Area, and Biomass Accumulation)

For the variable of chrysanthemum plant height under the effect of different irrigation levels (% AW), the results showed statistical differences ($p \leq 0.05$) among the evaluated treatments (Figure 4A). Plant height was affected by the application of the 20 % AW level; in turn, this treatment differed statistically from the application of the 60 % AW level, while the latter did not differ from the 50 % and 40 % irrigation levels, results like those of water potential and RWC. Height ranged between 30.13 and 14.75 cm for the 60 % and 20 % AW treatments, respectively. Therefore, the effect of reducing soil water content decreased plant height by 52 % (Figure 4A), evidenced by the adverse effect of decreasing substrate water content and that chrysanthemum plants were subjected to water deficit stress conditions.

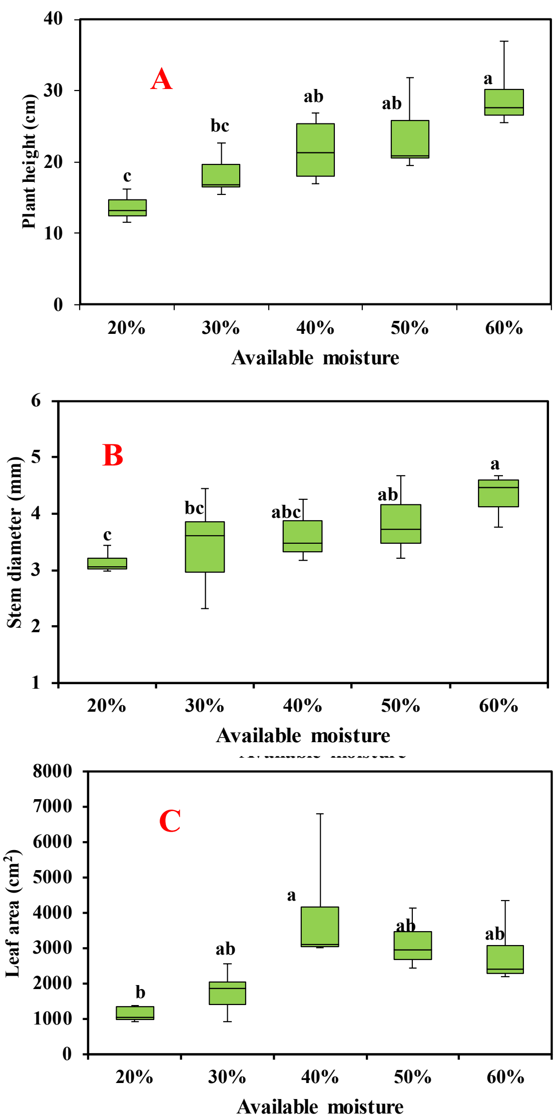

Figure 4. Plant height (A), Stem diameter (B), and Leaf area (C) of chrysanthemum plants with the application of five levels of available water. Means with the same letter are statistically equal (Tukey, $p \leq 0.05$).

For stem diameter, the behavior was like that of plant height, with significant statistical differences ($p \leq 0.05$) found among the evaluated available water levels. The highest value for stem diameter, as with height, was obtained by applying 60 % AW with 4.59 mm. However, it was statistically equal to the 40 % and 50 % AW treatments but showed significant differences compared to the 20 % and 30 % AW levels, which obtained the lowest values with 3.62 and 3.22 mm, respectively (Figure 4B). Figure 4C presents the results for leaf area in chrysanthemum under the effect of AW levels. Statistically significant differences ($p \leq 0.05$) were found in chrysanthemum plants subjected to different percentages of AW via the application of different irrigation levels. With the 40 % AW level, chrysanthemum plants presented the greatest leaf area with 4147.96 cm²; however, this was statistically equal to the rest of the treatments except for the 20 % AW level, which obtained the lowest leaf area with 1327.31 cm². Regarding the levels of 50 %, 60 %, and 30 % AW, leaf area values of 3472.45, 3057.05, and 2046.73 cm², respectively, were obtained. As a result of water deficiency, a decrease in growth was confirmed, expressed as a decrease in stem, leaf, and total biomass per plant, where statistically significant differences ($p \leq 0.05$) were detected among treatments for all these variables (Table 1).

Table 1. Biomass production in chrysanthemum plants subjected to different levels of available soil moisture.

Treatments (% Available moisture)	Biomass (g plant^{-1})		
	Leaves	Stem	Total
20	3.49 ± 1.48 b	3.23 ± 1.74 b	6.73 ± 2.09 b

Treatments (% Available moisture)	Biomass (g plant^{-1})		
	Leaves	Stem	Total
30	6.90 ± 2.71 ab	11.49 ± 4.55 a	18.40 ± 6.17 ab
40	14.69 ± 7.87 a	10.32 ± 3.77 a	25.01 ± 10.87 a
50	11.81 ± 2.93 ab	10.01 ± 2.71 a	21.82 ± 5.28 a
60	5.88 ± 1.52 b	12.56 ± 1.63 a	18.44 ± 3.05 ab

Mean ± Standard error. Means with the same letter between columns are statistically equal (Tukey, $p \leq 0.05$).

Stem biomass was only statistically affected when AW was reduced to 20 %; meanwhile, leaf biomass was affected to a lesser extent by the decrease in water content, as greater biomass was accumulated with 40 % AW. Total biomass showed a trend like leaf biomass, where the greatest accumulation was evidenced with 40 % AW, which differed statistically from 20 % AW (Table 1). The results show that for aerial biomass in chrysanthemum plants, a decrease in soil moisture content had a negative effect on production. This effect occurs because soil with scarce moisture causes the plant to exert greater effort to absorb the necessary water due to the adjustments required to generate a more negative water potential. This effort can be costly from an energetic point of view, causing a loss of metabolic integrity and affecting growth.

Furthermore, in an experiment conducted on chrysanthemum, results like those of this work were obtained, finding that the dry weight of the aerial part in treatments receiving water deficit was 40 % lower compared to the control treatment (without water deficit). This decrease is attributed to lower dry matter production in stressed plants since one of the first factors affected by water stress is stomatal opening, which directly impacts photosynthesis and, therefore, growth (Wu et al., 2022).

In this research, it was perceived that water stress is quickly reflected in the water relations of the chrysanthemum plant, starting from the application of moderate levels of water content reduction, affecting water potential and RWC. On the other hand, at the whole-plant level, adverse effects were observed for some parameters, such as plant height and stem diameter, starting from 30 % AW. However, in other growth indicators, such as leaf, stem, and total biomass accumulation, effects were only observed when reduced to 20 % AW, an aspect that aligns with plant survival. This reveals an important result, as during the management of chrysanthemum plants, despite the perception of stress due to water deficit from moderate levels of irrigation reduction under the conditions of this study, it may be possible to apply the 30 % AW level. This would save considerable water without causing plant death or significantly affecting growth.

Despite these considerations, based on the results up to this stage of the research, it is important to reason that the study should be extended to the flower production stage, as this is an indicator that is usually affected in chrysanthemums when plants are subjected to water deficit stress (Pereira et al., 2021; Zhai et al., 2021), this being the ultimate goal for plants produced under these irrigation conditions. With this, the effect at the level of the productive variable will be known, and the research will be completed, reflecting the magnitude of irrigation management on crop productivity considering economic variables. The results revealed, for the first time for irrigation management conditions in chrysanthemum cultivation in Yucatán, important considerations for water management in this species. These can be a critical starting point regarding the extent to which water is misused in agriculture, given that it is an exhaustible natural resource.

Conclusions

Increasing soil moisture improves water potential and leads to higher relative water content in chrysanthemum leaves. No changes were observed in the survival percentage of chrysanthemum plants with respect to the irrigation level; however, irrigating with 60% available water produced taller plants. In general, to produce chrysanthemum cuttings, it is not advisable to reduce soil water content to less than 30% AW, as plant development is impaired, leading to a decrease in biomass production and reduced survival.

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