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Stomatal conductance and transpiration in *Allium sativum* L. under water deficiency

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Abstract
The study aimed to verify the water stress indicators of garlic (*Allium sativum* L.) submitted to water deficit levels, simulating the effects of possible climatic changes. Considering that *A. sativum* is a crop known worldwide for its medicinal, culinary properties and applications in the ecological management of agricultural pests and, knowing that the planet is undergoing constant climatic changes, this study becomes essential to understand the influence of these changes on this species cultivation. The treatments were established with water conditions of 100, 75, 50, and 25% of the pot capacity (PC). According to the pot capacity, three bulbils were planted per pot (5 L) containing Plantmax® substrate, irrigated every two days. During the first and last week of the greenhouse treatments, the chlorophyll index was evaluated using a porometer with a seven-day interval between the analysis. The first analysis was performed in the first week of water stress and the last one in the last days of stress. Garlic plants showed sensitivity to water deficit, resulting in lower conductance and transpiration compared to treatments 50% and 75% water deficit, apparently without causing changes in production. In view of the results of this study, it is concluded that *Allium sativum* L. will not be totally harmed by the climate changes expected in the future according to atmospheric changes in the region of Laranjeiras do Sul.
Keywords: climate change; water deficit; medicinal plants.

Condutância estomática e transpiração em *Allium sativum* L. sob deficiência hídrica

Resumo
O objetivo do estudo foi verificar os indicadores de estresse hídrico do alho (*Allium sativum* L.) submetido a níveis de déficit hídrico, simulando os efeitos de possíveis mudanças climáticas. Considerando que *A. sativum* é uma cultura conhecida mundialmente pelas suas propriedades medicinais, culinárias e aplicações no manejo ecológico de pragas agrícolas e, sabendo que o planeta está passando por constantes alterações climáticas, este estudo torna-se fundamental para entender a influência destas mudanças sobre o cultivo destas plantas. Os tratamentos foram constituídos com condição hídrica de 100; 75; 50 e 25% da capacidade de pote (CP). Foram plantados três bulbilhos por vaso (5 L) contendo substrato Plantmax®, irrigados a cada dois dias, de acordo com a capacidade de pote. Avaliou-se o índice de clorofila durante a primeira e última semana da realização dos tratamentos em casa de vegetação, e análise com porômetro com sete dias de intervalo entre uma análise e outra. A primeira análise foi realizada na primeira semana de estresse hídrico e, a última análise, nos últimos dias de estresse. As plantas de alho demonstraram sensibilidade ao déficit hídrico, resultando da condutância e transpiração nos tratamentos 50% e 75% do déficit hídrico, porém sem causar alterações na produção. Diante desse estudo, conclui-se que a o *A. sativum* não será totalmente prejudicado pelas mudanças climáticas previstas futuramente.
Palavras-chave: mudanças climáticas; déficit hídrico; plantas medicinais.

Introduction

Climate change comprises phenomena resulting from global warming and has invaded daily life, becoming a complex reality. Climate is a fundamental characteristic of the terrestrial environment that exhibits dynamic force influencing Earth's regions configurations, soil structure, and agricultural production. Consequently, it induces the forms of life that inhabit a certain planet's area (CORTESE, 2014).

The term climate change is defined by the Intergovernmental Panel on Climate Change (IPCC) as a statistically significant variation in an average climate parameter or its variability, persisting over an extended period (typically decades or more) (CORTESE, 2014). This phenomenon is associated with extremes, such as drought periods, floods, heat, and cold waves, affecting biodiversity and causing agricultural impacts across the planet. Thus, climate change is a worrying factor that alters biological processes, producing enormous economic and life losses (CERA; FERRAZ, 2015; MARENGO, 2006).

At this point, agriculture will be severely affected by compromising food production. Based on this information, it is important to study the water deficit hypothesis. Consequently, perennial species may have greater difficulty supporting stress due to lack of water during the year's driest period, being more affected than annual crops.

According to Graciano (2009), water deficit can cause physiological disorders such as changes in plant development and metabolism imbalance, compromising agricultural production. Therefore, in this situation, the plant must develop adaptive physiological or biochemical mechanisms to help resist these variations. A plant can complete its life cycle during the wettest period, or else the plants can maintain tissue hydration, delaying dehydration in drier periods (SALAMONI, 2008). Other physiological mechanisms include turgor reduction, leaf abscission, leaf area reduction, roots deepening, stomatal closure, and photosynthesis limitation, besides the abscisic acid (ABA) synthesis.

Stomata represent the main resistance to the diffusive flow of water vapor through the leaves. Measurements of stomatal resistance or its inverse, stomatal conductance, are important for establishing the influence of stomata on transpiration and/or exchanging other gases between leaf tissues and the surrounding atmosphere (OLIVEIRA *et al.*, 2004).

When the water deficit in the soil causes stomata to close, the radiation intercepted by the leaf tends to promote leaf heating, reaching levels that are harmful to the plant's metabolism (COSTA, 2001; OLIVEIRA *et al.*, 2004). Chlorophyll rates can also vary in the face of stress, compromising survival and reducing photosynthesis, compromising the plant's vital processes.

Knowing the defense mechanisms of vegetables in response to water deficit and considering it an eventual process in the near future, we must understand how plants' responses will be, especially for medicinal plants, as they are essential for life. According to Lozano, Bagne and Hora (2015), thanks to scientific studies with medicinal plants, their consumption and the substitution of synthetic drugs for natural ones have significantly increased over the years. Costa *et al.* (2007) Scientific research and its evidence on medicinal plants' potential, influenced by the search for healthy habits, made the use of herbal medicines frequently questioned by researchers, with garlic being the target of these analyses according to their origin and indications.

Allium sativum L., popularly known as garlic, is related to the onion family (Liliaceae), one of the most sought-after plants for phytotherapeutic purposes. Its medicinal properties, already tested and proven, include antioxidants, anticancer, antimicrobials, hepatoprotective, immunomodulator, and cardioprotector, presenting more than 2000 active compounds like phenolic compounds (phenylpropanoids) (TUAN *et al.* 2010).

Therefore, the current study aimed to investigate the effect of water deficit on garlic crop, an important medicinal plant, evaluating its stomatal conductance and transpiration rates as a physiological response to different water-deficit levels.

Material and Methods

The experiments were carried out in the greenhouse of the Federal University of Fronteira Sul – UFFS, Laranjeiras do Sul Campus, PR. The garlic bulbils of the species *Allium sativum* L. were all purchased in the same location (same access) with the farmers of Nova Laranjeiras in the form of heads and the bulbil (garlic cloves) separated (EMBRAPA, 1993), which were propagated in a greenhouse. Three bulbils were planted per pot, filled with Plantmax® substrate, rich in organic matter, with ten pots for each treatment, at a depth from 2 to 3 centimeters (EMBRAPA, 1993). After one month of acclimatization, the plants were submitted to different water-deficit levels. The plants were kept under treatment for 45 days, according to development.

Exposure to water deficit

The pot capacity (PC) was adopted as the water content drained by the soil after suffering saturation through the action of gravity until the cessation of this drainage (SOUZA *et al.*, 2001). The level of stress adopted was 25%, 50%, 75%, and 100% of PC. The pots' irrigation was controlled by the weighing procedure. The pots were weighed every two days on an electronic scale to replace later the evapotranspiration water in the period. This procedure keeps the pots close to the field capacity and omits the irrigation in 25%, 50%, 75%, and 100% of the pot capacity in those under stress conditions treatments, starting 30 days after sowing (SOUSA, 2013). Irrigation was performed using a bucket with measurement in mL at 2-day intervals. During the period of water stress, the plants were subjected to stomatal conductance analysis and chlorophyll tests.

Stomatal conductance assessments

The crop stomatal conductance measurements were made using a porometer of the Delta T brand, AP4 model in a dynamic equilibrium state with direct measurements during the water stress. Five random pots were separated for each treatment, and one plant per pot. Three leaves exposed to solar radiation were selected from each plant to perform such measures (PAIVA *et al.* 2005). First, the measurements were made on the youngest leaf, the second leaf was marked with a bow, and the third one was the oldest. Such analyses were carried out in the adaxial region in three regions of each leaf of the garlic crop, always occurring from the bottom upwards, the lower region first, followed by the middle one, and the upper one. The measurements were carried out between 8 am and 2 pm at high temperature, low humidity and intense light.

As it corresponds to the interval in which the peak of solar radiation is located in the Laranjeiras do Sul region during the sampling period (TONELLO; TEIXEIRA FILHO, 2013; PAIVA *et al.*, 2005). The measurements were performed once a week starting 35 days after planting, totaling five measurements during the water stress period (TONELLO; TEIXEIRA FILHO, 2013). The temperature variations inside the greenhouse varied between 22 to 28°, and the relative humidity of the air 65%, in a light intensity that varied in
approximately 90 mmol.m2 .S-1, verified at each analysis.

Chlorophyll index assessments

The relative chlorophyll content (SPAD) data were determined using two readings with a chlorophyll meter (Falker ClorofiLog CFL 1030 model), according to the methodology described by Leonardo *et al.* (2013). Five pots were chosen at random for the analyses. One plant was selected from each pot, with three leaves being analyzed in three regions of each leaf, always occurring from the bottom upwards, with the lower region first, followed by the middle one, and lastly, the upper one. The evaluations took place in the first and last week of the beginning of the treatments.

Statistical analysis

The experimental design used was completely randomized, consisting of ten pots per treatment and three seedlings per pot, totaling 30 seedlings per treatment. From these treatments, five replications were obtained equivalent to the treatments 25%, 50%, 75%, and 100%, respectively. The data were submitted to analysis of variance by the SISVAR software, and the means were compared by the Tukey test at 0.05% significance.

Results And Discussion

The results regarding the chlorophyll contents of the apex, median and basal regions of the *Allium sativum* plant leaf subjected to different water deficit concentrations are shown in Table 1. According to the table, only the treatment with a 50% water deficit induced a 46% reduction in chlorophyll levels in the basal leaf region of *A. sativum* compared to the control (100% pot capacity). Also, in the treatment with 50% of the water deficit, some of the evaluated leaves' yellowing was observed.

Dutra *et al.* (2012) obtained results similar to garlic when studying sunflower during water stress, where the chlorophyll content of plant leaves was limited due to the low availability of water. This finding can be understood since chlorophylls are physiological state indicators of plants, essential to their growth and adaptation to the most varied environments (ARAÚJO, 2011). Argenta *et al.* (2004) evaluated the chlorophyll concentration index (CCI) in four corn plants' development stages. They verified the variation in their levels, which were higher in the median leaf region and lower in the basal region. The concentrations with the lowest volume of water (25%) and the highest volume of water (100%) obtained the lowest chlorophyll concentration index values, very similar to the chlorophyll levels in the median and basal regions observed in the current study (Table 1).

Table 1. Chlorophyll concentration index in the apex, median, and basal regions of the leaves of *Allium sativum* growing under different water-deficit levels.

Level (%)	Chlorophyll concentration index (CCI)			
	Apex	Median	Basal	Mean
25	45.64 abB	42.02 abB	27.80 abA	38.48 a
50	42.56 aB	45.56 abB	18.66 Ab	35.59 a
75	52.65 bB	48.63 aA	32.13 bA	44.47 b
100	46.73 abB	36.05 aA	34.44 bA	39.07 ab
Mean	46.90 B	43.06 B	28.26 A	
C.V. (%)		15.15		

Means followed by the same lowercase letter in the column and uppercase in the line do not differ by the Tukey test at 5% probability.

Uddling *et al.* (2007) and Jesus and Marenco (2008) justify the lack of linearity between the chlorophyll levels observed in their studies. The chlorophyll distribution on the leaf surface in the referred studies presents unevenness, especially in well-greenish leaves, leading to underestimating the index values in leaves with high substance levels. The same authors also described that some plants have a thicker leaf blade probably contributed to the chlorophyll meter's lower efficiency, which may have happened in what refers to the basal part of the garlic leaf. The chlorophyll indexes of garlic verified by the chlorophyll meter may have been influenced by the greenhouse's shading. The chlorophyll and carotenoids levels in the leaves are used to estimate the plants' photosynthetic potential due to their direct connection with luminous energy absorption, transfer, growth, and adaptation to different environments, as reported by Baldo *et al.* (2009).

Chlorophylls are pigments responsible for capturing light used in photosynthesis, being essential in the light radiation conversion into chemical energy in the form of ATP and NADPH. Thus, chlorophylls are related to plants' photosynthetic efficiency and, consequently, their growth and adaptability to different environments (JESUS and MARENCO, 2008). The chlorophyll levels obtained by Carvalho *et al.* (2003) when studying Artemisia plants grown in pots with substrate maintained at field capacity, 90%, 70%, and 50% were reduced over time in any water level, indicating a tendency to senescence. Despite the continuous reduction in chlorophyll levels, Artemisia plants maintained at 50% of field capacity had, throughout the time, a relative chlorophyll content about 30% higher compared to plants maintained at 90% of field capacity. The higher relative chlorophyll content in the expanded leaves of plants under water deficiency can be used as a stress indicator (CARVALHO *et al.*, 2003).

The inner greenhouse temperatures ranged from 22 to 28 °C, with a relative air humidity of 65%, in a light intensity varying approximately 90 mmol m^2 s^{-1}. These data were verified in each analysis with the porometer's aid. According to Calbo (2005), the porometer is an instrument developed to measure water vapor conductance in plants. The instrument makes it possible to determine the transpiration rate divided by the deficit in vapor pressure and the product mass.

Costa and Marenco (2007) describe that the opening and closing stomata process is mainly related to the light intensity and the leaf's hydration status. According to the authors, the stomata and the leaf area influence the plant yield. The first factor controls the absorption of CO_2, and the second determines light interception. The leaf water potential indicates its energy status. If the plant loses water at a higher rate than its absorption and transport capacity, the leaf water potential decreases, leading to stomatal closure and reduced photosynthesis (COSTA and MARENCO, 2007).

When submitting stomatal conductance analyzes with the porometer, it was possible to verify that the activities were higher in the treatment with 50%, mainly in the leaf's middle region. The 75% and 50% treatments were the most significant, with the leaf basal apex not having relevant differences in any treatments (Table 2). Plants inevitably lose water through the leaves by absorbing CO_2. This water loss occurs mainly through stomata, which have mechanisms to control their opening degree. This control is attributed to stomatal leaf conductance, often used as a water deficiency indicator (PAIVA, 2005).

According to Tonello and Teixeira Filho (2013), for plants growing under moderate water stress or dry season conditions, the combined effect of several factors influencing transpiration and stomatal conductance generally results in a daily occurrence pattern of variation as observed in garlic. This daily pattern is characterized by the occurrence of maximum values of stomatal conductance in the early morning and late afternoon, with a characteristic of decrease around noon. In severe water deficit conditions, the maximum stomatal opening occurs only in the early morning, closing at noon and reopening slightly in the afternoon.

As Costa and Marenco (2004) reported, during the hottest hours of the day, stomatal conductance decreases to the point that the leaf water potential does not fall below levels considered adequate for the water transport system stability. In the garlic case, the analyses were made in the morning, beginning around 8:00 am and ending between 2:00 pm and 3:00 pm. Besides, luminosity ranged within the greenhouse, explaining the variations observed in the porometer results due to the garlic's exposure conditions.

Transpiration is influenced by climate, soil, age of plants, and water availability in the soil. Transpiration intensifies with a decrease in the relative humidity of the air and an increase in temperature. Due to the strong radiation, the leaf's heating also increases the transpiratory rates because it increases the difference in vapor pressure between the air and the leaf, causing transpiration even with saturated air (SOUZA *et al.*, 2011).

Table 2. Stomatal conductance of the apex, middle and basal regions of the *Allium sativum* leaf growing under different water-deficit levels.

Level (%)	Region of *Allium sativum leaf* (mmol.m^2.S^{-1})			
	Apex	Median	Basal	Mean
25	3925.36 aA	3828.76 aA	3503.86 aA	3752.66 a
50	5741.67 aA	8112.26 bAB	8956.62 cB	7603.52 b
75	5970.50 aA	7257.84 bA	7949.68 bcA	7059.34 b
100	6197.46 aA	6197.58 abA	5309.98 abA	5901.67 b
Mean	5458.75 A	6349.11 A	6430.03 A	
C.V. (%)		33.43		

Means followed by the same lowercase letter in the column and uppercase in the line do not differ by the Tukey test at 5% probability.

It was found that, at 25% concentration, the stomatal conductance values were reduced in *A. sativum*, although an increase was observed in the concentration of 75% (Table 2). Literature data agree with the data obtained in the present study for stomatal conductance, for instance, de Oliveira *et al.* (2004) studying the papaya tree production, Santos *et al.* (2016) who found little variation in the stomatal conductance of pineapples, and Paiva *et al.* (2005) in his study with beans. This finding indicates that when the soil water deficit is not very pronounced, the stomatal conductance variations follow the same water unrestricted plants' trend. Therefore, for garlic, such results suggest that a condition of good water availability in the soil, with greater stomatal conductance, results in greater gas exchange, greater CO_2 incorporation from the atmosphere, and greater plant photoassimilates production.

Five analyses were performed with the porometer, with seven days between them. The first analysis happened in the first week of water stress, and the last one in the last days of stress.

Table 3 displays that on the first analysis day, the 75% and 100% concentrations showed significant stomatal conductance rates compared to the other days of analysis. As the days of stress passed, the treatment with 75% obtained higher stomatal conductance rates than the other treatments. However, on the last day of analysis, the 25% and 50% concentrations obtained higher rates than the rest of the treatments.

According to Martins *et al.* (2006), irrigation of *A. sativum* is essential to reach the maximum yield potential and the final product's highest quality. Despite the irrigation relevance for most vegetable quality, no studies reported have provided the irrigation effect on garlic's chemical composition and quality so far.

Martins *et al.* (2006) report that cultivation conditions can significantly affect the chemical composition of garlic and could be employed to ensure adequate handling of bioactive compounds' content and, consequently, the quality of the final products. Thus, Ankri and Mirelman (1999) state that the chemical compounds found in garlic, such as alliin, allicin, and ajoene, with herbicidal, fungicidal, and antibacterial action, despite not being related to factors such as water absorption and photosynthesis.

Thus, garlic's chemical composition did not interfere with the results obtained for stomatal conductance since the equipment (porometer) can estimate the leaf conductance for gas diffusion. This parameter is useful to determine the plants' water status by determining the resistance or the conductance existing in the leaf to water vapor passage from inside the leaf to the atmosphere (VEIGA JUNIOR *et al.*, 2005).

Veiga Junior *et al.* (2005) observed when studying tomatoes that there is a relationship between the balance of radiation, transpiration, and stomatal conductance of plants without water restriction. According to the authors, this reflects the stomata's response to energy availability and evaporative demand on field-grown plants.

Table 3. Comparison of the stomatal conductance index of *Allium sativum* leaves growing under different water-deficit levels.

Level (%)	Comparison with Porometer analyzes					
	Day 03	Day 10	Day 14	Day 24	Day 31	Mean
25	3900.37 Aab	2303.11 aA	3509.11 Aab	878.64 aA	5755.62 bB	3269.37 a
50	3843.57 aAB	3016.62 aA	3083.93 aA	3241.51 aAB	6212.26 bB	3879.58 a
75	4019.04 aA	5015.33 aA	3498.31 aA	2218.71 aA	4464.37 abA	3843.15 a
100	4138.77 aA	3150.42 aA	192769 aA	3316.46 aA	2069.40 aA	2920.55 a
Mean	3975.44 BC	3371.37ABC	3004.76 AB	2413.83 A	4625.41 C	
C.V(%)	50.82					

Means followed by the same lowercase letter in the column and uppercase in the line do not differ by the Tukey test at 5% probability.

Paiva *et al.* (2005), when studying the stomatal conductance of beans, proved that water availability affects the plants' growth by controlling the opening of stomata and, consequently, phytomass production. According to the same authors, water decrease in the soil reduces the water potential in the leaf and its stomatal conductance, promoting stomata's closure. This closure blocks the flow of CO_2 to the leaves, affecting photoassimilates' accumulation, reducing productivity.

Veiga Junior *et al.* (2005) state that plants grown in environments with high temperatures and vapor pressure deficits did not change their stomatal conductance, measured with the porometer, justifying the little variation observed in the present study. The same authors considered that this response was due to the culture's acclimatization, which developed since the beginning of the cycle in a high evaporative demand environment. Sousa (2013) explains that light intensity, humidity, and temperature can influence the plant's stomatal conductance rates.

Scalon *et al.* (2011) cite that the available water to the plant in the soil is defined for a particular soil-crop combination. The chemical, physical, and biological characteristics of the soil directly influence the amount of water available to plants. Thus, if there was any physical, chemical, or biological restriction in any soil profile layer, changing the root system development, the availability will be affected because the plants could not extract the stored water.

However, Nayyar and Gupta (2006) describe that water stress profoundly impacts agricultural systems because the ecosystem's function can be altered due to the plant functional groups' relative abundance changes. Plant reactions to water stress differ significantly at various organizational levels, depending on the intensity and duration of stress, as well as plant species and their of developmental stage.

Conclusion

It is concluded that water deficit induces changes in the chlorophyll index and stomatal conductance in *Allium sativum*. Still, such changes did not cause drastic effects on the crop and, therefore, such plants will be less affected given the expected changes with climate change in the future.

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