

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

- Yohanna Vassura^(1), Evaristo Mauro de Castro⁽¹⁾, Orivaldo Benedito da Silva⁽¹⁾, Marcio Paulo Pereira⁽¹⁾, Orlando Gonçalves Brito⁽²⁾, Valter Carvalho de Andrade Junior⁽²⁾, and Fabricio José Pereira⁽³⁾
- ⁽¹⁾ Universidade Federal de Lavras, Departamento de Biologia, Setor de Botânica Estrutural, Trevo Rotatório Professor Edmir Sá Santos, s/nº, Caixa Postal 3037, CEP 37203-202 Lavras, MG, Brazil. E-mail: yohanna.vassura@outlook.com, emcastro@ufla.br, orivaldo.bio@gmail.com, marciopaulop@hotmail.com
- ⁽²⁾ Universidade Federal de Lavras, Departamento de Agricultura, Setor de Olericultura, Trevo Rotatório Professor Edmir Sá Santos, s/n^e, Caixa Postal 3037, CEP 37203-202 Lavras, MG, Brazil. E-mail: orlandocefet@yahoo.com.br, valter.andrade@ufla.br
- ⁽³⁾ Universidade Federal de Alfenas, Instituto de Ciências da Natureza, Rua Gabriel Monteiro da Silva, nº 700, Centro, CEP 37130-001 Alfenas, MG, Brazil. E-mail: fabricio.pereira@unifal-mg.edu.br

☑ Corresponding author

Received May 22, 2022

Accepted December 06, 2023

How to cite

VASSURA, Y.; CASTRO, E.M. de; SILVA, O.B. da; PEREIRA, M.P.; BRITO, O.G.; ANDRADE JUNIOR, V.C. de; PEREIRA, F.J. Leaf anatomy and physiology of garlic cultivars related to tolerance to environmental factors. **Pesquisa Agropecuária Brasileira**, v.59, e03368, 2024. DOI: https://doi.org/10.1590/ S1678-3921.pab2024.v59.03368. Horticultural Science/ Original Article

Leaf anatomy and physiology of garlic cultivars related to tolerance to environmental factors

Abstract - The objective of this work was to evaluate the physiological and anatomical characteristics of garlic cultivar leaves that indicate tolerance or susceptibility to environmental factors. The experiment was conducted in a greenhouse in a completely randomized design, with eight treatments (cultivars). The evaluated cultivars were Amarantes, BRS Hozan, Cacador, Crespo, Chinês Folha Fina, Chonan, Gigante Roxo Escuro, and Ito. The following physiological variables were evaluated: net photosynthetic rate, stomatal conductance to water vapor, transpiration, internal and external carbon, and water use efficiency. The anatomical characteristics were analyzed with a microscope coupled to a camera. The Chinês Folha Fina, Chonan, Gigante Roxo Escuro, and Crespo cultivars showed higher mean photosynthetic rates and thicker photosynthetic tissues than the others. The Chonan and Crespo cultivars stood out for their higher photosynthetic rates, higher stomatal indices, thicker cuticle and epidermis, and larger mesophyll intercellular spaces, which are characteristics common to plants tolerant to water deficit. The characteristics of the Chonan and Crespo garlic cultivars are related to drought tolerance, and those of BRS Hozan, Ito, and Caçador to susceptibility.

Index terms: *Allium sativum*, chlorophyll parenchyma, photosynthesis, transpiration.

Anatomia e fisiologia foliar de cultivares de alho relacionadas à tolerância a fatores ambientais

Resumo – O objetivo deste trabalho foi avaliar as características fisiológicas e anatômicas de folhas de cultivares de alho que indiquem tolerância ou susceptibilidade a fatores ambientais. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, com oito tratamentos (cultivares) e quatro repetições. As cultivares avaliadas foram Amarantes, BRS Hozan, Caçador, Crespo, Chinês Folha Fina, Chonan, Gigante Roxo Escuro e Ito. Avaliaram-se as seguintes variáveis fisiológicas: taxa fotossintética líquida, condutância estomática ao vapor d'água, transpiração, carbono interno e externo, e eficiência no uso da água. As características anatômicas foram analisadas com microscópio acoplado a uma câmera. As cultivares Chinês Folha Fina, Chonan, Gigante Roxo Escuro e Crespo apresentaram taxas fotossintéticas médias maiores e tecidos fotossintéticos mais espessos que as demais. As cultivares Chonan e Crespo destacaram-se por maiores taxas fotossintéticas, índices estomáticos, espessuras da cutícula e da epiderme, e espaços intercelulares do mesofilo, características comuns em plantas tolerantes ao deficit hídrico. As características das cultivares de alho Chonan e Crespo são relacionadas à tolerância à seca, e as de BRS Hozan, Ito e Caçador à suscetibilidade.

Termos para indexação: *Allium sativum*, parênquima clorofiliano, fotossíntese, transpiração.



Introduction

Garlic (*Allium sativum* L.), from the Amaryllidaceae family and native of Central Asia, has a great economic and social importance, being the second most consumed plant of the genus *Allium* worldwide due to its characteristic flavor and medicinal properties (Sasi et al., 2021). In Brazil, garlic is grown under different technological levels, with the largest production in the Southeastern and Midwestern regions of the country (Maciel et al., 2021), which still depends on garlic imports to ensure the supply for domestic consumption (Buta & Silva Junior, 2021).

Although Brazil's edaphoclimatic conditions are favorable for garlic cultivation, for it to achieve self-sufficiency, specific facilities are needed for the vernalization of garlic seeds and postharvest processing, which requires more investments than competing crops, such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], to reach a good profitability, hindering the increase in cultivation areas (Buta & Silva Junior, 2021).

Among the abiotic factors that affect agricultural production, water deficit stands out (Fatima et al., 2018) as it is directly related to photosynthesis, which is extremely important for a plant's primary metabolism and, therefore, yield (Ouyang et al., 2017). In addition, plant growth and development are also affected by light, a source of energy for photosynthesis that can cause changes in the anatomy of leaves (Arrigoni-Blank et al., 2022), which play a key role in controlling water loss during the process of photosynthesis (Oliveira et al., 2022). Moreover, the anatomical characteristics of the leaves influence gas exchange, meaning that a greater development of photosynthetic tissues contributes to an increase in plant photosynthesis and, consequently, in yield (Silva et al., 2005). Therefore, to increase crop yield, it is fundamental to investigate the relationships between leaf anatomy and plant physiology (Ouyang et al., 2017).

In the literature, studies have associated garlic agronomic yield with morphology (Oliveira et al., 2018; Andrade Júnior et al., 2019), phenotypic characteristics (Santos et al., 2022), and bulb quality (Bessa et al., 2020; Marodin et al., 2020), highlighting the species' high genetic diversity (Barboza et al., 2020; Bacelar et al., 2023). However, researches focused on relating garlic anatomical leaf characteristics to the crop's cultivation are still scarce, although associations between physiological characteristics, such as gas exchange, and biomass yield under water deficit conditions have been made (Sánchez-Virosta & Sánchez-Gómez, 2019; Sánchez-Virosta et al., 2021).

In this context, variations in the anatomical and physiological characteristics of garlic leaves can be used to characterize and identify different genotypes, indicating the best conditions for growing a specific cultivar and providing a basis for further studies with this crop.

The objective of this work was to evaluate the physiological and anatomical characteristics of garlic cultivar leaves that indicate tolerance or susceptibility to environmental factors.

Materials and Methods

The experiment was conducted at the Center for Technology Transfer and Development of Universidade Federal de Lavras, located in the municipality of Ijaci, in the south of the state of Minas Gerais, Brazil. According to Köppen-Geiger's classification, the climate is Cwb, subtropical, with rainy summers and cold and dry winters. In the region, the average annual rainfall is 1,486 mm and the average annual temperature is 19.9°C.

Eight garlic cultivars from Embrapa Hortaliças were evaluated: BRS Hozan, Amarantes, and Gigante Roxo Escuro, from the semi-noble group; Ito, Chonan, and Caçador from the noble group; and Chinês Folha Fina and Crespo.

Sowing was performed from May 22 to October 21, 2019, in a soil classified as a Latossolo Vermelho distroférrico típico, according to Brazilian Soil Classification System (Santos et al., 2013). The soil analysis showed the following chemical characteristics: pH H₂O 6.2, 278.06 mg dm⁻³ K, 60.96 mg dm⁻³ P, 0.00 cmol_c dm⁻³ Al, 4.12 cmol_c dm⁻³ Ca, 1.02 cmol_c dm⁻³ Mg, 3.20 cmol_c dm⁻³ H+Al, 17.40 mg L⁻¹ remaining P, 14.80 mg dm⁻³ Zn, 34.10 mg dm⁻³ Fe, 17.90 mg dm⁻³ S, sum of bases of 5.85 cmol_c dm⁻³ B, 1.20 mg dm⁻³ S, sum of 5.85 cmol_c dm⁻³, effective cation exchange capacity of 5.85 cmol_c dm⁻³, aluminum saturation of 0.00%, base saturation of 64.67%, 2.34 dag kg⁻¹ organic matter, 550 g kg⁻¹ clay, 110 g kg⁻¹ silt, and 340 g kg⁻¹ sand.

Virus-free bulbils were sown in beds directly on the ground under a protected environment (greenhouse),

Leaf anatomy and physiology of garlic cultivars

consisting of a prefabricated modular structure, with the top covered with polyethylene and one of the sides with an anti-aphid fabric, at ambient temperature and relative humidity.

The cultivars were arranged in a completely randomized design, with eight treatments (cultivars) and four replicates, using 400 bulbils per plot (10 m^2), spaced at 0.10 m from each other in four crop rows 0.20 m apart. Irrigation was carried out using a drip system, keeping soil moisture close to field capacity. On the same day the bulbils were planted, a fertilizer, consisting of 150 g simple superphosphate, 10 g potassium chloride, and 15 g ammonium sulfate, was applied per linear meter. Topdressing fertilization (10 g per linear meter of a 2:1 ammonium sulfate: potassium chloride mixture) was applied at 30 and 60 days after the bulbils were planted. Throughout the cycle, deltamethrin was applied once and copper oxychloride thrice to all plots to control Thrips tabaci and Alternaria porri, respectively.

Gas exchange was analyzed at 100 days after planting during the maximum vegetative growth and intense bulbification of the crop. For this, the fourth fully-expanded leaf of four garlic plants per plot were evaluated between 8 and 11 a.m., using the LI-6400XT infrared gas analyzer equipped with a 6.0 cm² camera and the 6400-02B artificial red/blue LED light source (Li-COR Biosciences, Lincoln, NE, USA). The photosynthetic photon flux density was standardized at 1,000 µmol m⁻² s⁻¹. In addition, vapor pressure deficit in the leaf was 1.63 kPa, pump flow was 500 µmol s⁻¹, and block temperature was 28.26°C, while the mixer function of the equipment was used to provide 380 ppm CO₂ during the analyses. Net photosynthetic rate, leaf stomatal conductance to water vapor, transpiration, internal carbon, internal to external carbon (Ci/Ca) ratio, and water use efficiency (WUE, net photosynthetic rate/transpiration) were also evaluated.

For the anatomical analysis, the fourth fullyexpanded leaf of two plants per plot was collected at 100 days after the bulbils were planted, following the gas exchange analysis. The collected leaves were fixed in FAA 70 (formaldehyde, acetic acid, and ethanol 70%) for 48 hours and subsequently stored in 70% ethanol (Johansen, 1940). The paradermal cuts were made manually in the median region of the leaves using a steel blade, cleaned with 50% sodium hypochlorite for 20 min and, subsequently, washed in distilled water. The sections were stained with 1% safranin (m v^{-1}) and then mounted on semipermanent slides with 50% glycerol (Kraus & Arduin, 1997), totaling four slides per treatment.

To obtain the cross sections, samples of the mid region of the leaf were processed and embedded in HistoResin (Leica Embedding Kit, Leica Biosystems, Germany). following the Nussloch. standard procedures. The samples were dehydrated with increasing concentrations of ethanol (70, 80, 90, and 100%) at 2 hour intervals, at room temperature, according to Johansen (1940), with modifications. Afterwards, the samples were immersed in a pre-infiltration solution containing 100% ethanol and a base resin at a 1:1 ratio for 24 hours, at 4°C, following the manufacturer's instructions. After this period, the material was subjected to infiltration in the base resin for another 24 hours at 4°C. Polymerization was performed in an oven, at 50°C, for 12 hours, also according to the manufacturer's instructions. The YD-335 semiautomatic rotary microtome (Jinhua Yidi Medical Equipment CO., LTD, Zheiang, China) was used to slice 8.0 µm thick cross sections, which were then stained in a 1% toluidine blue solution at pH 6.7 for 3 min (Feder & O'Brien, 1968), washed in distilled water, and then mounted on permanent slides with Entellan (Merck KGaA, Darmstadt, Germany), totaling four slides per treatment.

The slides were photographed with a camera coupled to the LED Eclipse E100 microscope (Nikon, Tokyo, Japan) and analyzed using the ImageJ image analysis software (Schneider et al., 2012). In the transversal section, the following leaf traits were evaluated due to their influence on photosynthetic efficiency and water loss control: thickness of the mesophyll, palisade parenchyma, aerenchyma chambers of the mesophyll, and central vein; average diameter of the metaxylem vessels; area of the vascular bundle of the central vein; epidermal thickness of the adaxial and abaxial surfaces; and cuticle thickness of the adaxial vein and abaxial surface. For the abaxial and adaxial paradermal sections, three measurements were taken per replicate of the following traits: stomatal density, using the equation (number of stomata \times 10⁶)/section area, in which the section area is equal to 408771.075 μm²; stomatal length and width; stomatal index, through the equation ((number of stomata)/ (number

of stomata + number of epidermal cell)) \times 100; and stomatal functionality, obtained by dividing the polar diameter by the equatorial diameter of the leaves. One plot was evaluated per replicate.

The gas exchange and anatomical data (cross sections and paradermal sections) were tested for normality and then subjected to the analysis of variance, and means were compared by the Scott-Knott test, at 5% probability. The statistical analyses were carried out using the Sisvar 5.0 software (Ferreira, 2019). The results were analyzed, and the cultivars were compared and grouped by the cluster analysis using the results of the anatomical evaluations. For the cluster analysis, the Ward method was performed with the PAST software (Hammer et al., 2001).

Results and Discussion

The eight evaluated garlic cultivars differed significantly regarding leaf gas exchange (Figure 1) and anatomical structures (Figures 2, 3, 4, 5, and 6). The Chonan cultivar stood out due to its high photosynthetic rate, low transpiration rate, low stomatal conductance, and high WUE, as well as to its high stomatal functionality, larger intercellular spaces in the mesophyll, and thicker palisade parenchyma, which are structures directly involved in the photosynthesis process, showing a leaf anatomy related to the plant's physiological response.

Through the cluster analysis, the cultivars were divided into two groups: drought tolerant, with higher gas exchange values and anatomical characteristics favorable to water loss control; and susceptible to drought, with lower photosynthesis values and a reduced drought tolerance potential (Figure 7). The group of drought-tolerant cultivars included Chonan, Crespo, Chinês Folha Fina, and Gigante Roxo Escuro, with the last two showing more similar characteristics and the first the highest photosynthetic potential. The group of cultivars susceptible to drought included Amarantes, BRS Hozan, Ito, and Caçador. The last three presented lower gas exchange values and anatomical characteristics unfavorable to the water deficit environment, such as lower stomatal functionality values, lower stomatal density, and thicker palisade parenchyma and mesophyll, important structures for gas exchange; however, the first stood out for presenting more favorable anatomical characteristics,

as a greater stomatal density and thicker cuticle, epidermis, palisade parenchyma, and mesophyll.

The Chinês Folha Fina, Chonan, Gigante Roxo Escuro, and Crespo cultivars showed the highest photosynthetic rates and, with the exception of Chonan, the highest stomatal conductance (Figure 1). Since a lower stomatal conductance rate is interesting when associated with a high photosynthetic rate, a low transpiration rate, and a high WUE, the Chonan cultivar is more efficient in the photosynthetic process. In addition, the high stomatal functionality and larger intercellular spaces of the mesophyll observed for Chonan are an indicative that this cultivar has great potential for cultivation under a low water availability (Figures 2, 3, and 4).

Another important behavior of the Chonan cultivar was its low Ci/Ca ratio, which resulted in a high photosynthetic rate and a low stomatal conductance. According to Leakey et al. (2019), this reduction in the Ci/Ca ratio may be related to the increase in the efficiency or carboxylation capacity by Rubisco or to a reduction in the losses by photorespiration since stomatal conductance was maintained, allowing of CO_2 to flow through the mesophyll. These results were confirmed by the cluster analysis, highlighting the high efficiency of the Chonan cultivar (Figure 7).

A lower transpiration rate and a higher photosynthetic rate, as those of the Chonan cultivar (Figure 1), i.e., the relationship between transpiration and CO₂ assimilation, is an indication of WUE (Ribeiro et al., 2018). Plants with a low WUE are more susceptible to drought, and this susceptibility is related to high transpiration rates (Cruz et al., 2019). The Chinês Folha Fina cultivar showed a low WUE, a high transpiration rate, and a high photosynthetic rate, meaning that a higher transpiration may cause a greater water loss during stomatal opening since transpiration is directly related to this process (Oliveira et al., 2022). Therefore, although this cultivar has a high potential, the conditions for its cultivation must include a high water availability to avoid negative effects on the plants. Consequently, the Chinês Folha Fina cultivar requires more irrigation than Chonan, which is the most adaptable to water stress.

The size of the stomata varied between the studied cultivars. The Chonan and Ito cultivars presented the smallest equatorial diameter on the abaxial and adaxial surfaces of the epidermis (Figure 2), which was



Figure 1. Evaluated physiological characteristics of eight garlic (*Allium sativum*) cultivars: A, photosynthesis (A); B, stomatal conductance to water vapor (g_{sw}); C, transpiration (E); D, concentration of intercellular CO₂ (Ci); E, intercellular to atmospheric carbon (Ci/Ca) ratio; and F, water use efficiency (WUE). Different letters differ significantly by the Scott-Knott test, at 5% probability. N = 32.

possibly related to an environmental factor, causing the stomata to become more elliptical and functional during their opening and closing. The highest polar diameter was observed for the Caçador and BRS Hozan cultivars on the abaxial surface and for BRS Hozan and Amarantes, followed by Chonan and Caçador, on



Figure 2. Evaluated anatomical characteristics of the leaves of eight garlic (*Allium sativum*) cultivars: abaxial equatorial diameter (A), adaxial equatorial diameter (B), abaxial polar diameter (C), adaxial polar diameter (D), abaxial stomatal functionality (E), adaxial stomatal functionality (F), stomatal density on the abaxial surface (G), stomatal density on the adaxial surface (H), stomatal index of the abaxial surface (I), and stomatal index of the adaxial surface (J). Different letters differ significantly by the Scott-Knott test, at 5% probability. N = 32.

the adaxial surface. The highest averages of stomatal functionality were found for cultivar Chonan on the abaxial surface and for Chonan, Amarantes, Caçador, and BRS Hozan on the adaxial surface. The Chonan cultivar stood out because it presented the highest ratio between the polar and equatorial diameters of the stomata on both surfaces of the epidermis and the lowest transpiration rate. In this line, Chaves et al. (2022) found that, as stomata become more elliptical due to the polar:equatorial diameter ratio, stomatal functionality increases.

The Crespo cultivar showed the highest stomatal density on the abaxial surface of its epidermis (Figure 2) and, together with Amarantes, the highest averages on the adaxial surface, whereas BRS Hozan had the lowest stomatal density on both surfaces of the epidermis and the largest polar diameter, which may indicate a better adaptation of this cultivar to conditions of high-water availability. Contrastingly,



Figure 3. Cross section of the leaf internerval region of eight garlic (*Allium sativum*) cultivars, showing: thickness of the cuticle of the abaxial surface (A), thickness of the cuticle of the adaxial surface (B), thickness of the epidermis of the abaxial surface (C), thickness of the epidermis of the adaxial surface (D), mesophyll thickness (E), palisade parenchyma thickness (F), and area of the mesophyll aerenchyma chambers (G). Different letters differ significantly by the Scott-Knott test, at 5% probability. N = 32.



Figure 4. Cross sections from the median region of the leaf blade of the Chinês Folha Fina (A), Chonan (B), Gigante Roxo Escuro (C), Crespo (D), Amarantes (E), Ito (F), Caçador (G), and BRS Hozan (H) garlic (*Allium sativum*) cultivars, showing: stomata (ST), adaxial cuticle (ADC), abaxial cuticle (ABC), adaxial epidermis (ADE), abaxial epidermis (ABE), palisade parenchyma (PP), intercellular space (IS), and vascular bundle (VB). Images were obtained with a camera coupled to the LED Eclipse E100 microscope (Nikon, Tokyo, Japan).



Figure 5. Evaluated anatomical characteristics of the leaf midrib of eight garlic (*Allium sativum*) cultivars: area of the vascular bundle (A); mean diameter of xylem vessels (B); and area of the intercellular space of the midrib (C). Different letters differ significantly by the Scott-Knott test, at 5% probability. N = 32.

a greater stomatal density associated with a smaller polar diameter may indicate a better adaptation of the plant to water deficit (Ribeiro et al., 2012).

The stomatal index of the abaxial surface did not differ significantly between the cultivars (Figure 2). However, that of the adaxial surface was higher for cultivars Crespo, Gigante Roxo Escuro, and Chonan. The Chonan cultivar showed a high stomatal index, in addition to a low stomatal density and a high density of epidermal cells, as also observed by Ribeiro et al. (2012). Oliveira et al. (2022) related stomatal density to the control of water loss by the plant and of leaf gas exchange. In the present study, Chonan showed a low transpiration rate, a high WUE, and a high rate of stomatal functionality, which may be related to the low stomatal density of the cultivar, causing the stomata to be more functional.

Cuticle thickness was similar among most cultivars, except for Chonan and Ito, which showed the lowest values on both sides of the epidermis (Figures 3 and 4). Likewise, the epidermal thickness of the abaxial and adaxial surfaces did not differ among most cultivars, with lower means for Chinês Folha Fina and Crespo on the abaxial surface and for Chonan, Ito, and Caçador on the adaxial surface. In addition to a low transpiration rate (Figure 1), cultivar BRS Hozan presented a thick cuticle and epidermis, as well as a low stomatal density, on both leaf surfaces (Figures 2, 3, and 4). According to Khelil et al. (2016), the dense deposition of wax layers under the epidermal cells prevents tissue dehydration and reflects solar radiation, consequently reducing water transpiration by the cuticle, which will occur through the stomata, often reduced in number.

In comparison with the other cultivars, Crespo had a thicker mesophyll, followed by Amarantes and Gigante Roxo Escuro (Figures 3 and 4); however, Crespo and Gigante Roxo Escuro had higher photosynthetic rates than Amarantes (Figure 1). Zheng & Van Labeke (2017) highlighted that leaf thickness directly influences light absorption and gas exchange due to larger intercellular spaces and a thicker palisade parenchyma, which increases the area of CO_2 reception and dissolution in the leaf. Therefore, the higher photosynthetic efficiency of the Crespo, Gigante Roxo Escuro, and even Amarantes cultivars may also be related to the thickness of their mesophyll and parenchyma.

The Amarantes cultivar had the thickest palisade parenchyma and a moderate photosynthetic rate



Figure 6. Cross sections from the median region of the central vein of the Chinês Folha Fina (A), Chonan (B), Gigante Roxo Escuro (C), Crespo (D), Amarantes (E), Ito (F), Caçador (G), and BRS Hozan (H) garlic (*Allium sativum*) cultivars, showing: stomata (ST), adaxial cuticle (ADC), abaxial cuticle (ABC), adaxial epidermis (ADE), abaxial epidermis (ABE), palisade parenchyma (PP), intercellular space (IS), vascular bundle (VB), xylem (XI), and phloem (FL). Images were obtained with a camera coupled to the LED Eclipse E100 microscope (Nikon, Tokyo, Japan).

(Figures 3 and 4), whereas Crespo, Ito, Caçador, and BRS Hozan had the thinnest palisade parenchyma. Batista et al. (2010) concluded that elongated cells in the palisade parenchyma are associated with a reduced mesophyll resistance to CO_2 and with an increase in the factors that potentially limit the photosynthetic process, such as enzymatic activity, electron transport, and stomatal conductance. By the cluster analysis, among the group of cultivars susceptible to drought, Amarantes stood out due to its interesting anatomical characteristics for future research under water-deficit conditions (Figure 7).

Another structure related to gas exchange in the most efficient cultivars (Chonan, Crespo, Chinês, and Gigante Roxo Escuro) is the aerenchyma chamber in the mesophyll, which facilitates gas exchange between the internal environment of the leaf and the external environment (Oliveira et al., 2022). The larger the intercellular spaces are, the greater the diffusion area of CO_2 and mesophyll conductance in the leaf, increasing plant photosynthesis (Terashima et al., 2011; Oliveira et al., 2022). Of the evaluated cultivars, Chonan and



Figure 7. Analysis of the clusters of eight garlic (*Allium sativum*) cultivars formed according to efficiency and susceptibility parameters related to leaf gas exchange and anatomy using the Ward method.

Crespo showed the largest aerenchyma area (Figures 3 and 4) and the highest photosynthetic rate (Figure 1), a result that may have been related to the aerenchyma of their leaves.

The Ito, Caçador, and BRS Hozan cultivars presented the lowest intercellular CO_2 concentrations and the lowest photosynthetic rates (Figure 1). According to Ribeiro et al. (2018), stomatal closure decreases the intercellular concentration of CO_2 in the leaf mesophyll due to the production of a diffusion barrier to CO_2 and, consequently, reduces the photosynthetic rate. These three cultivars also had the smallest aerenchyma chambers (Figures 3 and 4), which may have reduced CO_2 conductivity and diffusion in the mesophyll since intercellular spaces are important for these parameters (Terashima et al., 2011; Oliveira et al., 2022). The results of leaf gas exchange and anatomy confirmed the grouping of these cultivars as less efficient by the cluster analysis (Figure 7).

The evaluated cultivars presented similar values for the area of the central vein vascular bundle, with the exception of Ito and BRS Hozan, which presented lower averages (Figures 5 and 6). There was also no significant difference between the cultivars for xylem vessel diameter. However, for the area of the intercellular spaces of the midrib, cultivars Chonan, Crespo, and Caçador showed the highest averages. The results obtained in the present study for the evaluated garlic cultivars can be used as indicators for future research with the species.

Conclusions

1. The Chonan and Crespo garlic (*Allium sativum*) cultivars present characteristics related to drought tolerance, and BRS Hozan, Ito, and Caçador to drought susceptibility.

2. The Chonan cultivar shows the best traits for cultivation under water-deficit conditions.

3. The Chonan, Crespo, Chinês Folha Fina, and Gigante Roxo Escuro cultivars stand out because they present anatomical characteristics favorable to the control of leaf gas exchanges.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financing, in part, this study (Finance Code 001); to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for scholarship granted; to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), for financial support (00065-22 and 01732-23); and to researcher Francisco Vilela Resende of Embrapa Hortaliças, for providing the cultivars for the research.

References

ANDRADE JÚNIOR, V.C. de; GUIMARÃES, A.G.; FIRME, T.D.; COSTA, A.A.A.; COSTA, M.R. da; LOPES, T.K.; SOUZA, R.J. de; RESENDE, F.V. Associations between morphological and agronomic characteristics in garlic crop. **Horticultura Brasileira**, v.37, p.204-209, 2019. DOI: https://doi.org/10.1590/ S0102-053620190211.

ARRIGONI-BLANK, M. de F.; SILVA, J.H.S.; GOIS, I.B.; CASTRO, E.M. de; BLANK, A.F.; NIZIO, D.A. de C.; NOGUEIRA, P.C. de L.; MENEZES-SÁ, T.S.A. Change in leaf anatomy, physiology, and essential oil of *Varronia curassavica* Jacq. accessions under two light conditions. **Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas (BLACPMA)**, v.21, p.771-785, 2022. DOI: https://doi.org/10.37360/blacpma.22.21.6.47.

BACELAR, P.A.A.; FEITOZA, L.L.; VALENTE, S.E.S.; GOMES, R.L.F.; MARTINS, L.V.; ALMEIDA, P.M.; SILVA, V.B.; LOPES, A.C.A.; CARVALHO, R.; PERON, A.P. Variations in heterochromatin content reveal important polymorphisms for studies of genetic improvement in garlic (*Allium sativum* L.). **Brazilian Journal of Biology**, v.83, e243514, 2023. DOI: https://doi.org/10.1590/1519-6984.243514.

BARBOZA, K.; SALINAS, M.C.; ACUÑA, C.V.; BANNOUD, F.; BERETTA, V.; GARCÍA-LAMPASONA, S.; BURBA, J.L.; GALMARINI, C.R.; CAVAGNARO, P.F. Assessment of genetic diversity and population structure in a garlic (*Allium sativum* L.) germplasm collection varying in bulb content of pyruvate, phenolics, and solids. **Scientia Horticulturae**, v.261, art.108900, 2020. DOI: https://doi.org/10.1016/j.scienta.2019.108900.

BATISTA, L.A.; GUIMARÃES, R.J.; PEREIRA, F.J.; CARVALHO, G.R.; CASTRO, E.M. de. Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. **Revista Ciência Agronômica**, v.41, p.475-481, 2010. DOI: https://doi.org/10.1590/S1806-66902010000300022.

BESSA, A.T.M.; NEGREIROS, M.Z. de; LOPES, W. de A.R.; PAIVA, L.G. de; SILVA, O.M. dos P. da. Quality of virus-free garlic grown under high altitude conditions in the semiarid region of the northeast of Brazil. **Revista Caatinga**, v.33, p.945-953, 2020. DOI: https://doi.org/10.1590/1983-21252020v33n409rc.

BUTA, G. de O.; SILVA JUNIOR, J.J. da. Competitividade internacional nas importações de alho: análise do período 2008-2019. **Informações Econômicas**, v.51, eie162020, 2021. DOI: https://doi.org/10.56468/1678-832X.eie1620.2021.

CHAVES, M.P.B.; SILVA, B.A.B.; SILVÉRIO, H.F.; RAMOS, F.N.; DUARTE, V.P.; CASTRO, E.M.; PEREIRA, F.J. Anatomy and growth of the epiphytic cactus *Epiphyllum phyllanthus* under

different radiation conditions. **Plant Ecology & Diversity**, v.15, p.39-49, 2022. DOI: https://doi.org/10.1080/17550874.2022.20782 45.

CRUZ, Y. da C.; SCARPA, A.L.M.; PEREIRA, M.P.; CASTRO, E.M. de; PEREIRA, F.J. Growth of *Typha domingensis* as related to leaf physiological and anatomical modifications under drought conditions. **Acta Physiologiae Plantarum**, v.41, art.64, 2019. DOI: https://doi.org/10.1007/s11738-019-2858-1.

FATIMA, R.T. de; JESUS, E.G. de; GUERRERO, A.C.; ROCHA, J.L.A.; BRITO, M.E.B. Crescimento e trocas gasosas em alface cultivada sob regimes hídricos e adubação fosfatada. **Revista Brasileira de Agricultura Irrigada**, v.12, p.2683-2691, 2018. DOI: https://doi.org/10.7127/rbai.v12n300854.

FEDER, N.; O'BRIEN, T.P. Plant microtechnique: some principles and new methods. **American Journal of Botany**, v.55, p.123-142, 1968. DOI: https://doi.org/10.2307/2440500.

FERREIRA, D.F. Sisvar: a computer analysis system to fixed effects split plot type designs. **Revista Brasileira de Biometria**, v.37, p.529-535, 2019. DOI: https://doi.org/10.28951/rbb.v37i4.450.

HAMMER, Ø.; HARPER, D.A.T.; RYAN, P.D. PAST: paleontological statistics software package for education and data analysis. **Paleontologia Electronica**, v.4, art.4, 2001.

JOHANSEN, D.A. Plant microtechnique. New York: McGraw-Hill, 1940. 530p.

KHELIL, R.; JARDÉ, E.; CABELLO-HURTADO, F.; OULD-EL-HADJ KHELIL, A.; ESNAULT, M.-A. Structure and composition of the wax of the date palm, *Phoenix dactylifera* L., from the septentrional Sahara. **Scientia Horticulturae**, v.201, p.238-246, 2016. DOI: https://doi.org/10.1016/j.scienta.2016.02.012.

KRAUS, J.E.; ARDUIN, M. Manual básico de métodos em morfologia vegetal. Seropédica: EDUR, 1997. 198p.

LEAKEY, A.D.B.; FERGUSON, J.N.; PIGNON, C.P.; WU, A.; JIN, Z.; HAMMER, G.L.; LOBELL, D.B. Water use efficiency as a constraint and target for improving the resilience and productivity of C3 and C4 crops. **Annual Review of Plant Biology**, v.70, p.781-808, 2019. DOI: https://doi.org/10.1146/ annurev-arplant-042817-040305.

MACIEL, J.C.; SOUSA, L.F. de; COSTA, M.R.; SANTOS, J.B. dos; FERREIRA, E.A.; ZANUCIO, J.C. Direct planting of *Allium sativum* before and after desiccation of *Urochloa brizantha* straw with glyphosate. **Scientia Horticulturae**, v.289, art.110478, 2021. DOI: https://doi.org/10.1016/j.scienta.2021.110478.

MARODIN, J.C.; RESENDE, F.V.; RESENDE, J.T.V. de; GABRIEL, A.; ZEIST, A.R.; CONSTANTINO, L.V.; SANZOVO, A.W.S. Virus-free garlic: yield and commercial classification as a function of plant spacing and seed size. **Horticultura Brasileira**, v.38, p.295-300, 2020. DOI: https://doi.org/10.1590/S0102-053620200309.

OLIVEIRA, J.P.V. de; DUARTE, V.P.; CASTRO, E.M. de; MAGALHÃES, P.C.; PEREIRA, F.J.P. Stomatal cavity modulates the gas exchange of *Sorghum bicolor* (L.) Moench. grown under diferent water levels. **Protoplasma**, v.259, p.1081-1097, 2022. DOI: https://doi.org/10.1007/s00709-021-01722-1.

OLIVEIRA, N.L.C. de; PUIATTI, M.; FINGER, F.L.; FONTES, P.C.R.; CECON, P.R.; MOREIRA, R.A. Growth and yield of 'Amarante' garlic accessions. **Revista Ceres**, v.65, p.481-490, 2018. DOI: https://doi.org/10.1590/0034-737X201865060003.

OUYANG, W.; STRUIK, P.C.; YIN, X.; YANG, J. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. **Journal of Experimental Botany**, v.68, p.5191-5205, 2017. DOI: https://doi.org/10.1093/jxb/erx314.

RIBEIRO, J.E. da S.; BARBOSA, A.J.S.; LOPES, S. de F.; PEREIRA, W.E.; ALBUQUERQUE, M.B. de. Seasonal variation in gas exchange by plants of *Erythroxylum simonis* Plowman. **Acta Botanica Brasilica**, v.32, p.287-296, 2018. DOI: https://doi.org/10.1590/0102-33062017abb0240.

RIBEIRO, M. de N.O.; CARVALHO, S.P. de; PEREIRA, J.F.; CASTRO, E.M. de. Anatomia foliar de mandioca em função do potencial para tolerância à diferentes condições ambientais. **Revista Ciência Agronômica**, v.43, p.354-361, 2012. DOI: https://doi.org/10.1590/S1806-66902012000200019.

SÁNCHEZ-VIROSTA, A.; SADRAS, V.O.; SÁNCHEZ-GOMÉZ, D. Phenotypic plasticity in relation to intercultivar variation of garlic (*Allium sativum* L.) functional performance and yield-stability in response to water availability. **Scientia Horticulturae**, v.285, art.110128, 2021. DOI: https://doi.org/10.1016/j.scienta.2021.110128.

SÁNCHEZ-VIROSTA, A.; SÁNCHEZ-GOMEZ, D. Inter-cultivar variability in the functional and biomass response of garlic (*Allium sativum* L.) to water availability. **Scientia Horticulturae**, v.252, p.243-251, 2019. DOI: https://doi.org/10.1016/j.scienta.2019.03.043.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos.; OLIVEIRA, V.A. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; CUNHA, T.J.F.; OLIVEIRA, J.B. de. **Sistema brasileiro de classificação de solos**. 3.ed. rev. e ampl. Brasília: Embrapa, 2013. 353p.

SANTOS, M.A.V.; ANDRADE JÚNIOR, V.C. de; GUIMARÃES, A.G. ; BRITO, O.G.; TAULA, A.J.V.; COSTA, R.A.; ALVES, J.P.R.; SILVA, N.O.; RESENDE, F.V. Correlations between agronomic characters in garlic. **Pesquisa Agropecuária Brasileira**, v.57, e02603, 2022. DOI: https://doi.org/10.1590/ S1678-3921.pab2022.v57.02603.

SASI, M.; KUMAR, S.; KUMAR, M.; THAPA, S.; PRAJAPATI, U.; TAK, Y.; CHANGAN, S.; SAURABH, V.; KUMARI, S.; KUMAR, A.; HASAN, M.; CHANDRAN, D.; RADHA; BANGAR, S.P.; DHUMAL, S.; SENAPATHY, M.; THIYAGARAJAN, A.; ALHARIRI, A.; DEY, A.; SINGH, S.; PRAKASH, S.; PANDISELVAM, R.; MEKHEMAR, M. Garlic (*Allium sativum* L.) bioactives and its role in alleviating oral pathologies. **Antioxidants**, v.10, art.1847, 2021. DOI: https://doi.org/10.3390/antiox10111847.

SCHNEIDER, C.A.; RASBAND, W.S.; ELICEIRI, K.W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, v.9, p.671-675, 2012. DOI: https://doi.org/10.1038/nmeth.2089.

SILVA, L.M.; ALQUINI, Y.; CAVALLET, V.J. Inter-relações entre a anatomia vegetal e a produção vegetal. Acta Botânica Brasileira, v.19, p.183-194, 2005. DOI: https://doi.org/10.1590/S0102-33062005000100018.

TERASHIMA, I.; HANBA, Y.T.; THOLEN, D.; NIINEMETS, U. Leaf functional anatomy in relation to photosynthesis. **Plant Physiology**, v.155, p.108-116, 2011. DOI: https://doi.org/10.1104/ pp.110.165472.

ZHENG, L.; VAN LABEKE, M.-C. Long-term effects of red- and blue-light emitting diodes on leaf anatomy and photosynthetic efficiency of three ornamental pot plants. **Frontiers in Plant Science**, v.8, art.917, 2017. DOI: https://doi.org/10.3389/fpls.2017.00917.