Bioagro 34(3): 319-326. 2022 doi: http://www.doi.org/10.51372/bioagro343.11

TECHNICAL NOTE

CHLOROPHYLL INDEX, MAXIMUM FLUORESCENCE, AND WATER USE EFFICIENCY IN SUGAR BEET UNDER WATER DEFICIT AND ASCORBIC ACID APPLICATION

Anderson de Melo Gonçalves[1], José S. de Melo Filho[2], Valéria Fernandes de O. Sousa[2], Toshik Iarley da Silva[3], Thiago Jardelino Dias[4], Aline das Graças Souza[2] and Joana Gomes de Moura[2]

ABSTRACT

Sugar beet (*Beta vulgaris* L.) is one of the most cultivated vegetables in Brazil, where water limitation is the main cause of yield loss. This limitation can be attenuated with the application of organic solutes, such as ascorbic acid (AA). The objective was to evaluate the response of sugar beet plants under the application of AA via irrigation water as a strategy to attenuate water deficit. The experiment was carried out at the Centro de Ciências Agrárias of the Universidade Federal da Paraíba, Areia, Brazil, using a randomized complete block design with nine combinations of irrigation depths based on five evapotranspiration percentages (40.0, 51.6, 80.0, 108.4 and 120 % ET) and five doses of AA (0, 0.29, 1.0, 1.71 and 2.0 mM), with three replications, generated from a central composite matrix. Water use efficiency (WUE) and chlorophyll fluorescence index were evaluated. Data were submitted to analysis of variance and, when significant, to a regression analysis. Irrigation depths influenced chlorophyll *a, b* and total, maximum fluorescence, instantaneous WUE and intrinsic WUE, but no effect was observed for AA. The beet cultivar Maravilha Top Tall Early Wonder exhibits certain physiological mechanisms of tolerance to water stress. The ascorbic acid, in the concentrations and application method used in the study, had no effect on the beet plant response.
Additional keywords: *Beta vulgaris*, photosynthesis, stomatal conductance, transpiration

RESUMEN

Índice de clorofila, fluorescencia y uso del agua en remolacha azucarera bajo déficit hídrico y aplicación de ácido ascórbico
La remolacha azucarera (*Beta vulgaris* L.) es una hortaliza muy cultivada en Brasil, donde la limitación de agua es la principal causa del bajo rendimiento. Este efecto puede atenuarse con la aplicación de solutos orgánicos, como el ácido ascórbico (AA). El objetivo fue evaluar la respuesta de plantas de remolacha azucarera ante la aplicación de AA vía agua de riego como estrategia para atenuar el déficit de agua. El experimento se llevó a cabo en el Centro de Ciências Agrárias de la Universidade Federal da Paraíba, Areia, Brasil, bajo un diseño de bloques al azar con nueve combinaciones de cinco láminas de riego basadas en porcentajes de evapotranspiración (40,0; 51,6; 80,0; 108,4 y 120 % de ET) y cinco dosis de AA (0,0; 0,29; 1.0; 1,71 y 2,0 mM), con tres réplicas, a partir de una matriz experimental compuesta central. Se evaluó la eficiencia de uso del agua (EUA) y los índices de fluorescencia de las clorofilas. Los resultados se sometieron a un análisis de varianza y, en las variables significativas, a un análisis de regresión. Las láminas de riego afectaron los índices de clorofila *a, b* y total, máxima fluorescencia, y eficiencia instantánea e intrínseca de la EUA, pero ni hubo efecto de la aplicación de AA. El cultivar Maravilha Top Tall Early Wonder exhibe mecanismos fisiológicos de tolerancia al estrés hídrico. El ácido ascórbico, en las concentraciones y método de aplicación utilizados, no afectó la respuesta de las plantas.
Palabras clave adicionales: *Beta vulgaris*, conductancia estomática, fotosíntesis, transpiración

INTRODUCTION

Sugar beet (*Beta vulgaris* L.), a biennial herbaceous of the Chenopodiaceae family, with varieties of different bark and pulp colors (Yolcu et al., 2022), is one of the most cultivated

Received: August 2, 2021 Accepted: May 25, 2022
[1] Dept. of Plant Science, Federal University of Roraima, Boa Vista, Brasil. e-mail: anderson.agroufpb@yahoo.com
[2] Center for Agric. Sci., Federal University of Paraíba, Areia, Brazil. e-mail: sebastiaouepb@yahoo.com.br; valeriafernandesbds@gmail.com (corresponding author); alineufla@hotmail.com; joanagomes1963@hotmail.com
[3], Federal University of Viçosa, Minas Gerais, Brazil. e-mail: iarley.toshik@gmail.com
[4] Dept of Agriculture, Center for Hum., Soc. and Agrarian Sci. Federal University of Paraíba. Bananeiras, Brazil e-mail: thiagojardelinodias@gmail.com

vegetables in Brazil due to the increasing demand, both *in natura* and industrial forms (Viciedo et al., 2019).

However, arid regions concentrated in the Brazilian Northeast are characterized lately with recurrent droughts (Marengo et al., 2020). Therefore, strategies that enable the cultivation of vegetable crops in conditions of water deficit are essential; the water is one of the limiting factors affecting plant growth, physiology and productive behavior of sugar beet (Silva et al., 2015). Water deficit in the soil can lead to plant morphological and physiological changes, such as reduction of cell expansion and leaf area, increase of leaf abscission, decrease in the ratio between root and shoot biomass, closure of stomata and decreases in photosynthesis (El-Bially et al., 2018). Due to the importance of water in plant development and its implications on various soil properties, the response of the crop to the water supply is always important, both in the scientific and economic aspects (Soares et al., 2014).

Under water deficit conditions, plants accumulate reactive oxygen species (ROS), which in large amounts trigger oxidative stress, promoting membrane damage and even cell death (Gallie, 2012). However, there are antioxidants, such as ascorbic acid that are used for the detoxification and neutralization of several reactive oxygen species (ROS), and, consequently, maintaining the stable content of chlorophyll for plant growth (El-Bially et al., 2018).

As ascorbic acid participates in a variety of processes, including photosynthesis, cell wall growth, cell expansion and resistance to environmental stresses (Athar et al. 2008; El-Bially et al., 2018), including water deficit, this study aimed to evaluate application of ascorbic acid as a mitigation of water deficit in beet using physiological markers.

MATERIAL AND METHODS

Local of research and experimental design. The experiment was carried out under a greenhouse environment at the Centro de Ciências Agrárias of the Universidade Federal da Paraíba, in the city of Areia, Paraíba, Brazil. The experimental design used randomized blocks arranged in a 5×5 factorial set of treatments combined according to a Central Composite experiment matrix, as used by Melo et al. (2019), and where low variation coefficient was pursued to meet the recommendation of Mateus et al. (2001). Treatments included irrigation with five irrigation crop factors with five doses of ascorbic acid applied everyday via irrigation water. The minimum (−α) and maximum (α) values for irrigation ranged, respectively, from 40 to 120 % of the reference evapotranspiration and 0 to 2.0 mM for AA, totaling nine treatments, with three replications (Table 1). The application of the treatments started in a conjugated way at 15 days after emergence (DAE) and was extended until the harvesting day (82 DAE).

Conduction of the experiment. Sugar beet seeds of the cultivar Maravilha Top Tall Early Wonder were used. Each experimental plot consisted of three conical pots with 5 L capacity, with holes in the bottom. Pots were filled with a sandy soil collected from 0-20 cm depths, with pH = 6.26; cation exchange capacity = 7.03 $cmol_c \cdot dm^{-3}$; organic matter = 1.75 %; total porosity = 48 %; field capacity = 7.8 %; permanent wilting point = 4.3 %.

The sowing was done directly, with five seeds per pot. After the stabilization of the emergency (15 DAE) the thinning was performed, remaining only the plant with greater vigor. For the fertilization, N, P_2O_5 and K_2O were used at 40, 180 and 90 $kg \cdot ha^{-1}$, respectively, with all the P_2O_5 applied at the beginning and the N and K_2O divided into three applications. There was no incidence of diseases during the conduction of the experiment. The max and min temperatures were 31.6 and 20.4 °C, while relative humidity was 92.3 and 49.3 %, respectively.

To adjust the irrigation treatments, the mean weight of nine pots (weighing lysimeters) placed in the middle of the treatments, were weighed daily in a precision scale, throughout the experiment. The pots were maintained with adequate soil moisture by 100 % replacement of evapotranspiration until the treatments were established.

To compute the frequency and irrigation depth applied to the pots, a reference with 100 % replacement of evapotranspiration was used (Figure 1), based on the values of field capacity, permanent wilting point and critical moisture established as 50 % of available water (Topak et al., 2011), crop evapotranspiration and water balance throughout the crop cycle. The applied irrigation treatments were 40.0, 51.6, 80.0, 108.4 and 120 % ET. The former values imply a sugar beet water deficit according to Davidoff and Hanks (1989), who pointed out that production of this crop has shown a linear relationship with evapotranspiration in a trial in Fort Collins, CO, USA (R^2=0.74) where relative dry matter and root yield were reduced by about half when changing from 100 % ET to 60 % ET.

The total irrigation required, in volume, was calculated according to Bernardo et al. (2008), considering 100 % application efficiency.

Table 1. Combinations of treatments generated through a central composite matrix

	Levels		Doses	
Treatments	ET (%)	AA (mM)	ET (%)	AA (mM)
T1	-1	-1	51.60	0.29
T2	-1	1	51.60	1.71
T3	1	-1	108.40	0.29
T4	1	1	108.40	1.71
T5	−α	0	40.00	1.00
T6	α	0	120.00	1.00
T7	0	α	80.00	2.00
T8	0	−α	80.00	0.00
T9	0	0	80.00	1.00

ET= irrigation based on percentage of ET; AA= ascorbic acid

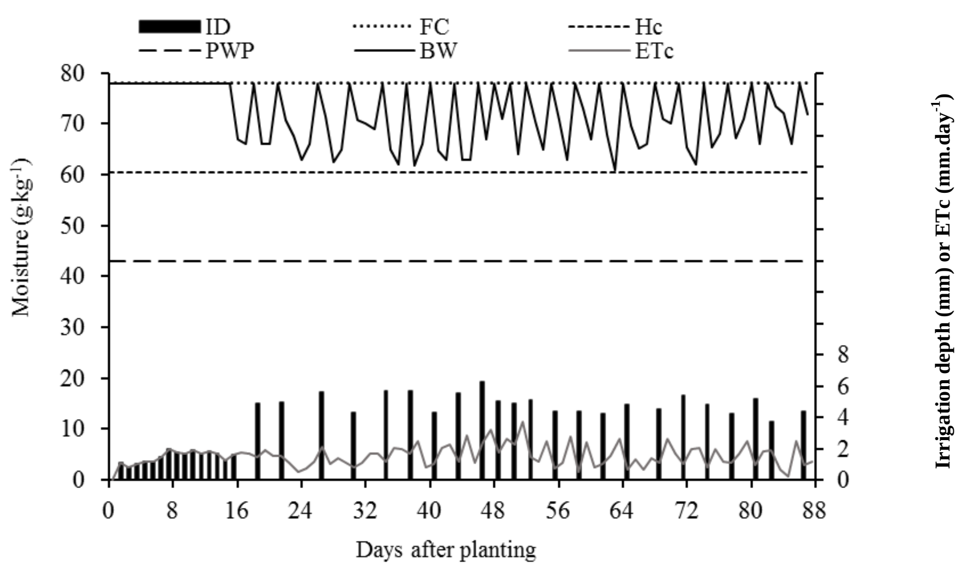

Figure 1. Water data in the weighing lysimeters with 100% replacement of evapotranspiration according to the days after planting the beet crop (*Beta vulgaris* L.). ID: frequency and irrigation depth; FC: field capacity; Hc: critical moisture; PWP: permanent wilting point; WB: water balance; ETc: crop evapotranspiration

Evaluations. Measurements were performed at 82 DAE between 09:00 and 11:00. The determination of the chlorophyll *a*, *b* index and total were taken from the middle part of all the leaves of the plants using a portable electronic chlorophyllometer (ClorofiLog Falker, model CFL 1030).

The chlorophyll fluorescence emission measurements were performed using a fluorescence modulator Plant Efficiency Analyzer–PEA II (Hansatech Instruments), determining the initial (F_0) and the maximum fluorescence (F_m).

Gas exchange determination was performed using an infrared gas analyzer (LI-COR–model LI-6400XT). The fourth leaf was analyzed from the upper part of the plant of each plot, always in the median region of fully expanded leaves, totally exposed to solar radiation. The following variables were analyzed: net CO_2 assimilation (A), stomatal conductance (Gs), and transpiration (E). After collecting the data, the instantaneous water use efficiency (WUE) was quantified by the relation A/E, and the intrinsic water use efficiency (iWUE) by A/Gs.

Statistical analysis. Data were submitted to analysis of normality using the Shapiro-Wilk test, and homogeneity of variances using the Bartlett test. After passing these tests, an analysis of variance was performed, and in the cases of significance, a regression analysis was conducted as a function of the irrigation treatment. For all analysis the SAS statistical program (Cary, NC, USA) was used.

RESULTS AND DISCUSSION

Irrigation treatments were independently significant ($P\leq0.05$) for indexes of chlorophyll *a* (Figure 2A), chlorophyll *b* (Figure 2B) and total (Figure 2C), maximum fluorescence (Figure 2D), instantaneous water use efficiency (Figure 2E), and intrinsic water use efficiency (Figure 2F). But no significant effect existed for the irrigation treatment over the rest of the measured variables. Likewise, there was no significant effect of the ascorbic acid treatment, and interaction with the irrigation treatment did not occur.

The levels of chlorophyll *a*, *b* and total reduced linearly as there was greater availability of water, corresponding to respective decreases of 5.51, 19.06 and 9.71 % when comparing the lowest water supply (40%) with the highest amount of water applied (120 %). Perhaps, with the increase in water content, there was less aeration in the substrate, which affect nutrient and water uptake (Sairam et al., 2008), which resulted mainly in plants with certain yellowish color or pale green at the highest irrigation percentage. Also, it can be thought that excessive irrigation may result in leaching of nitrogen out of the root zone (Sun et al. 2018). With nitrogen deficiency, there is a reduction in chlorophyll synthesis (El-Bially et al., 2018).

Similar results were obtained by Malmir et al. (2020) evaluating genotypes of *Beta vulgaris* L. in a protected environment in different irrigation depths, where they verified that the chlorophyll decreased with the increase of irrigation depths. The authors of the aforementioned research assumed that the DR1-HSF14-P.35 beet genotype has tolerance to water deficit. This could partially explain the different behavior of the cultivar Maravilha Top Tall Early Wonder in the present research. Beetroot cultivars that require lower water supply are fundamental in semi-arid conditions (Velarde, 2010), which suggests the importance of this cultivar.

There are several reports in the literature about the increase and reduction of chlorophyll content in the plant cell under deficit water conditions (Mendes et al. 2011, Cardoso et al. 2012). As in our results, the content of chlorophylls *a*, *b* and total were higher under water deficit conditions, suggesting an adaptive response of the plant to the water deficit, by activating protection mechanisms to the photosynthetic apparatus, these physiological indicators being primordial in the selection of water deficit-tolerant species (Mendes et al., 2011; Silva et al., 2014).

It is well known that chlorophyll is responsible for the capture of light energy, being the chlorophyll *a* the main pigment of the luminosity complex receptors for the photochemical reactions (Acosta et al., 2017). Likewise, in our study, the maximum value of fluorescence (Fm) was observed under the water deficit treatments, similar to the results of Celi et al. (2022). Values of Fm declined from 374.51 electrons·quantum^{-1} in the treatment of 40 % ET to 330.98 electron·quantum^{-1} with an irrigation of 120 % ET (Figure 2D). High Fm values indicate large flow of electrons, when the reaction centers reach their maximum capacity for photochemical reactions (Tatagiba et al., 2014; Sharma et al., 2015; Zhou et al., 2015). Thus, with higher water supply, there was a reduction in the capacity for photochemical reactions.

The instantaneous water use efficiency (WUE) and intrinsic water use efficiency (iWUE) were negatively affected as the irrigation depth increased (Figure 2E and 2F), this is justified by the increase in CO_2 concentration related to transpiration (E) and to the stomatal conductance (Gs) (Jadoski et al., 2015), ensuring the maintenance of the photosynthetic rate of the plant even with the partial closure of the stomata, due to water stress (Hu et al., 2010). Fabeiro et al. (2003) obtained the highest WUE (17.05 kg·m^{-3}) for sugar beet by applying the lowest irrigation depth

(45 % ET). These results for WUE corroborate those found in our work.

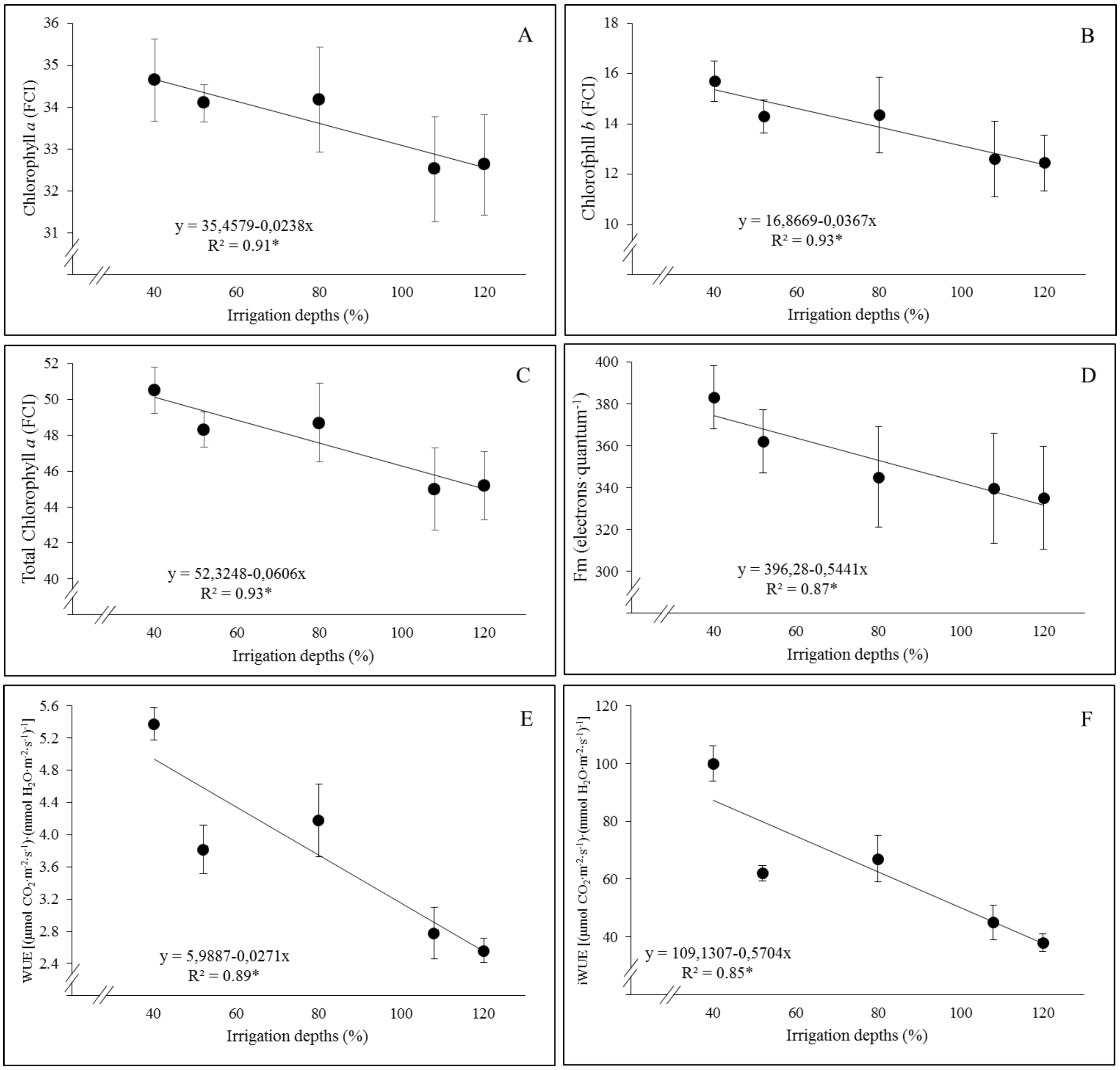

Figure 2. Indices of chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), maximum fluorescence (Fm) (D), instantaneous water use efficiency (WUE) (E) and intrinsic water (iWUE) (F) as a function of the irrigation based on different percentages of ET, in beet (*Beta vulgaris* L.). Vertical bars mean standard error.

CONCLUSIONS

The beet cultivar Maravilha Top Tall Early Wonder exhibits physiological mechanisms of tolerance to water deficits. The response of *Beta vulgaris* L. is not affected by the application of ascorbic acid via irrigation water at the concentrations used in this study.

ACKNOWLEDGMENTS

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the research sponsorship granted to the first author.

LITERATURE CITED

1. Acosta-Motos, J. R., M. F. Ortuño, A. Bernal-Vicente, P. Diaz-Vivancos, M. J. Sanchez-

Blanco and J. A. Hernández. 2017. Plant responses to salt stress: adaptive mechanisms. Agronomy 7: 1-37.

2. Athar, H., A. Khan and M. Ashraf. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environmental and Experimental Botany 63: 224-231.
3. Bernardo, S., A.A. Soares and E.C. Mantovani. 2008. Manual de irrigação. La Universidad Federal de Viçosa (UFV). Viçosa, Brasil.
4. Cardoso, N.D.S.N., L.M. Oliveira, L.G. Fernandez, C.R. Pelacani, C.L.M. de Souza and A.R. Oliveira. 2012. Osmo-condicionamento na germinação de sementes, crescimento inicial e conteúdo de pigmentos de *Myracrodruon urundeuva* fr. Allemão. Rev. Bras. de Biociências. 10: 457-461.
5. Celi-Soto, A., M.S. Mejía y L. Ríos-Rojas. 2022. Gas exchange and fluorescence in ‘sutil’lime’ (*Citrus aurantifolia* Swingle) under different soil moisture levels. Bioagro 34(2): 195-206.
6. El-Bially, M., H. Saudy, I. El-Metwally, M. Shahin. 2018. Efficacy of ascorbic acid as a cofactor for alleviating water deficit impacts and enhancing sunflower yield and irrigation water–use efficiency. Agricultural Water Management 208: 132-139.
7. Fabeiro, C., F. M. Santa Olalla, R. López and A. Domínguez. 2003. Production and quality of the sugar beet (*Beta vulgaris* L.) cultivated under controlled deficit irrigation conditions in a semi-arid climate. Agricult. Water Management 62: 215-227.
8. Gallie, D. R. 2012. The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. Journal of Experimental Botany 64: 433-443.
9. Hu, L., Z. Wang and B. Huang. 2010. Diffusion limitation sand metabolic factors associated within hibitionand recovery of photosynthesis from drought stress in a C3 perennial grass species. Physiology Plantarum 139: 93-106.
10. Jadoski, C.J., J.D. Rodrigues, R. P. Soratto, C. M. Santos and E. Ribeiro. 2015. Ação fisiológica da piraclostrobina na assimilação de CO_2 e enzimas antioxidantes em plantas de feijão condicionado em diferentes tensões de água no solo. Irriga 20: 319-333.
11. Malmir, M., R. Mohammadian, A. Sorooshzadeh, A. Mokhtassibidgoli and S. Ehsanfar. 2020. The response of the sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. Altissima Döll) genotypes to heat stress in initial growth stage. Acta Agricult. Slovenica 115: 39-52.
12. Marengo, J.A., A.P.M.A. Cunha, C.A. Nobre, G.G. Ribeiro Neto, A. R. Magalhães, R.R. Torres et al. 2020. Assessing drought in the drylands of northeast Brazil under regional warming exceeding 4 °C. Natural Hazards 103: 2589-2611.
13. Mateus NB., D. Barbin and A. Conagin. 2001. Viabilidade de uso do delineamento composto central. Acta Sci. Agron. 23: 1537-1546.
14. Melo-Filho, J.S.D., T.I.D. Silva, A.C.D. Melo-Gonçalves, L. Vieira de Sousa, M.L. Martins-Véras and T.J. Dias. 2019. Salt water and silicon application on growth, chloroplastid pigments, chlorophyll fluorescence and beet production. Revista Colombiana de Ciencias Hortícolas 13(3): 406-415.
15. Mendes, B.S.S., L. Willadino, P. C. Cunha, R. A. Oliveira Filho and T. R. Camara. 2011. Mecanismos fisiológicos e bioquímicos do abacaxi ornamental sob estresse salino. Rev. Caatinga 24: 71-77.
16. Sairam, R.K., D. Kumutha, K. Ezhilmathi, K., P.S. Deshmukh and G.S. Srivastava. 2008. Physiology and biochemistry of waterlogging tolerance in plants. Biol. Plant 52: 401
17. Sharma, D.K., S.B. Andersen, C.O. Ottosen and E. Rosenqvist. 2015. Wheat cultivars selected for high Fv/Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. Physiologia Plantarum 153: 284-298.
18. Silva, F.G., W.F. Dutra, A.F. Dutra, I.M. Oliveira, L. Filgueiras, A.S. Melo. 2015. Trocas gasosas e fluorescência da clorofila em plantas de berinjela sob lâminas de irrigação. Rev. Bras. de Eng. Agríc. e Ambiental. 19: 946-952.
19. Silva, M.D.A., C.M.D. Santos, H. D. S. Vitorino and A. F. D. L. Rhein. 2014.

Pigmentos fotossintéticos e índice SPAD como descritores de intensidade do estresse por deficiência hídrica em cana-de-açúcar. Bioscience J. 30: 173-181.

20. Soares, F.C., A.D. Robaina, M.X. Peiter, J.L. Russi and G.A. Vivan. 2014. Redes neurais artificiais na estimativa da retenção de água do solo. Ciênc. Rural. 44: 293-300.

21. Sun, M., Z. Huo, Y. Zheng, X. Dai, S. Feng and X. Mao. 2018. Quantifying long-term responses of crop yield and nitrate leaching in an intensive farmland using agro-eco-environmental model. Sci. Total Environ. 613: 1003-1012

22. Tatagiba, S.D., G.A.B.K. Moraes, K.J.T. Nascimento and A.F. Peloso. 2014. Limitações fotossintéticas em folhas de plantas de tomateiro submetidas a crescentes concentrações salinas. Rev. Eng. na Agricult. 22: 138-149.

23. Topak, R., S. Süheri and B. Acar. 2011. Effect of different drip irrigation regimes on sugar beet (*Beta vulgaris* L.) yield, quality and water use efficiency in Middle Anatolian, Turkey. Irrigation Science 29: 79-89.

24. Velarde, M.R. 2010. Water management in sugar beet. Sugar Tech. 12: 299-304.

25. Viciedo, D.O., R. de M. Prado, R.L. Toledo, L.C.N. Santos, A.C. Hurtado, L.L. Nedd and L.C. Gonzalez. 2019. Silicon Supplementation alleviates ammonium toxicity in sugar beet (*Beta vulgaris* L.). Journal of Soil Science Plant Nutrition 19: 413-419.

26. Yolcu, S., H. Alavilli, P. Ganesh, M. Asif, M. Kumar and K. Song. 2022. An insight into the abiotic stress responses of cultivated beets (*Beta vulgaris* L.). Plants 11: 12.

Zhou, R., X. Yu, K. H. Kjaer, E. Rosenqvist, C. O. Ottosen and Z. Wu. 2015. Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. Environmental and Experimental Botany 118: 1-11.