

JOÃO PAULO SOUSA BORGES

**AGRONOMIC, PHYSIOLOGICAL AND MULTIESPECTRATIVE ANALYSIS
OF SWEET CORN SUPPLEMENTED WITH SILICON AND MYCORRHIZAL
INOCULATION IN DROUGHT STRESS MITIGATION**

Dissertation presented to the Graduate Program
in Agriculture and Geospatial Information at
the Federal University of Uberlândia, *Campus*
Monte Carmelo, as a partial requirement for
obtaining the title of 'Master in Agriculture and
Geospatial Information '.

Advisor

Prof. Dr. Douglas José Marques.

MONTE CARMELO
MINAS GERAIS - BRAZIL

2023

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APPROVED on March 30, 2023

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Teachers can transfer knowledge in the classroom and are examples of professionals in the field.

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I leave here my thanks!

BIOGRAPHY

João Paulo Sousa Borges was born in Uberlândia, Minas Gerais, Brazil, 1997. He received a degree in agricultural engineering from the Federal University of Uberlândia (UFU), Minas Gerais, Brazil, in 2019. Master's student in the Graduate Program in Agriculture and Geospatial Information, focusing on agronomic, physiological, and multispectral imaging studies of drought stress responses in crop plants.

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ABSTRACT

BORGES, João Paulo Sousa. **Agronomic, physiological and multispectral analyzes of sweet corn supplemented with Si and mycorrhizal inoculation in mitigating drought stress.** 2023. 55p. Dissertation (Master in Agriculture and Geospatial Information) – Federal University of Uberlândia, Monte Carmelo. ¹

Water deficit in the soil-plant system causes changes in the development of sweet corn. The association of Silicon (Si) and arbuscular mycorrhizal fungus (AMF) can be an alternative for mitigating drought stress. We hypothesized that the presence of an association between Si and AMF would attenuate the effects of drought stress in sweet corn. This study aimed to investigate the association between Si and AMF in mitigating drought stress in sweet corn and their effects on biochemical, physiological, agronomic, and multispectral imaging activities. A randomized block design was used in a 2x2x2 factorial scheme, with four replications: first Si factor (with and without addition of Si as soil corrective) × second AMF factor (with and without AMF inoculation) × third irrigation factor (drought stress = 40 % and control = 100% water replacement in the soil at field capacity). Plant growth, productivity, gas exchange, Si and P content in the soil and plants, mycorrhizal colonization, and the use of multispectral images to determine photosynthetic pigments were evaluated. These results showed that applying Si and AMF mitigated the effects of drought stress on sweet corn plants. The combination of Si and AMF increased the levels of soluble solids. Drought stress affects dry mass, root volume, shoot mass, productivity, and nutrient uptake. The multispectral images obtained by the MAPIR camera allowed estimation of the SPAD, chlorophyll a, b and total values, using the GNDVI index and the green and near-infrared bands, and classification of the leaves by the simple linear regression model in the presence or absence of Si and AMF. We conclude that AMF inoculation and Si application, and the combination of these factors, mitigated the effects of drought stress on sweet corn plants, and multispectral images allowed the estimation of the levels of photosynthetic pigments.

Keywords: *Zea mays* L. Drought. Calcium silicate. Mycorrhiza. Vegetation index.

¹Advisor: Prof. Dr. Douglas Jose Marques

RESUMO

BORGES, João Paulo Sousa. **Análises agronômicas, fisiológicas e multiespectrais de milho-doce suplementado com Si e inoculação micorrizica na mitigação do estresse de seca.** 2023. 55p. Dissertação (Mestrado em Agricultura e Informação Geoespacial) – Universidade Federal de Uberlândia, Monte Carmelo. ¹

O déficit hídrico no sistema solo-planta provoca alterações no desenvolvimento do milho doce. A associação de Silício (Si) e fungo micorrízico arbuscular (FMA) pode ser uma alternativa para mitigar o estresse hídrico. Nossa hipótese é que a presença de uma associação entre Si e FMA atenuaria os efeitos do estresse hídrico no milho doce. Este estudo teve como objetivo investigar a associação entre Si e FMA na mitigação do estresse hídrico em milho doce e seus efeitos nas atividades bioquímicas, fisiológicas, agronômicas e de imagem multiespectral. Foi utilizado o delineamento em blocos casualizados em esquema fatorial 2x2x2, com quatro repetições: primeiro fator de Si (com e sem adição de Si como corretivo de solo) × segundo fator de FMA (com e sem inoculação de FMA) × terceiro fator de irrigação (estresse hídrico = 40 % e testemunha = 100% de reposição de água no solo na capacidade de campo). Foram avaliados crescimento vegetal, produtividade, trocas gasosas, teores de Si e P no solo e nas plantas, colonização micorrízica e uso de imagens multiespectrais para determinação de pigmentos fotossintéticos. Esses resultados mostraram que a aplicação de Si e AMF mitigou os efeitos do estresse hídrico nas plantas de milho doce. A combinação de Si e AMF aumentou os teores de sólidos solúveis. O estresse hídrico afeta a massa seca, o volume da raiz, a massa da parte aérea, a produtividade e a absorção de nutrientes. As imagens multiespectrais obtidas pela câmera MAPIR permitiram estimar os valores SPAD, clorofila a, b e total, usando o índice GNDVI e as bandas verde e infravermelho próximo, e classificar as folhas pelo modelo de regressão linear simples na presença ou ausência de Si e FMA. Concluímos que a inoculação de FMA e a aplicação de Si, e a combinação desses fatores, atenuaram os efeitos do estresse hídrico nas plantas de milho doce, e imagens multiespectrais permitiram estimar os níveis de pigmentos fotossintéticos.

Palavras-chave: *Zea mays* L. Seca. Silicato de cálcio. Micorriza. Índice de vegetação.

¹Orientador: Prof. Dr. Douglas José Marques

1. INTRODUCTION

Climate change has become a major challenge due to the intensification of global warming (KOCHAN, 2022). Consequently, drought stress significantly affects plant growth and yield owing to the agronomic and physiological changes caused by climate instability (ROU; SAM, 2021).

Drought stress in the soil-plant system causes changes in plant growth, shortening of the stem internode and leaf area, due to the low turgor of the cells, consequently affecting gas exchange, causing a reduction in productivity (FERRARI et al., 2015; PALHARINI, 2016; VIÇOSI et al., 2017).

AMF application can alleviate water deficiency in plants (SANTANA et al., 2023; SILVA et al., 2020; WU; ZOU, 2017). AMF colonize plant roots and their hyphae function as root extensions (WU; ZOU, 2017). This symbiosis promotes benefits such as greater root mass, plant growth, greater absorption of nutrients caused by increased root mass, better flowering conditions, and, consequently, greater tolerance to abiotic stresses (SILVA et al., 2020; WU; ZOU, 2017).

Si is one of the most abundant elements in soils (YAN et al., 2018), and although it is not classified as essential, it provides many benefits to plants (WANG et al., 2021a). Under drought stress conditions, the soil-plant system has some benefits, such as improved absorption of water and nutrients by roots, better control of water loss by leaves, alteration of signaling pathways, and stimulation of the antioxidant system (WANG et al., 2021a).

Si attenuates the symptoms of water deficit in plants (MENEGALE et al., 2015; NAWAZ et al., 2020) that when, in the cell walls of the leaves, there is an accumulation of Si, which results in a reinforced protective layer, making high water losses difficult, which causes a decrease in the rate of plant evapotranspiration (MENEGALE et al., 2015). In several studies in the literature, a favorable relationship was observed between the accumulation of Si in the leaves and the reduction in the transpiration rate for some crops when subjected to different levels of water deficit (YAN et al., 2018; MENEGALE et al., 2015; LANNA, 2021). Thus, its presence indicates a greater efficiency in soil water use (MENEGALE et al., 2015; YAN et al., 2018).

In plants, some proteins and genes are responsible for the transport of Si (YAMAJI et al., 2008, 2012, 2015; YAN et al., 2018). Silicon is transported via the symplast in all bundle sheath cells through the Lsi2 transporter (YAMAJI et al., 2012, 2015). The Lsi3 protein transports Si through the parenchymal cell layers. After overcoming the apoplastic barrier in bundle-sheath cells, Si can also be translocated via the apoplast, with the association of efflux currents through the parenchymal cell bridging Lsi2 and Lsi3 (YAN et al., 2018).

The Lsi6 protein is responsible for transporting silicic acid from the xylem to the xylem parenchyma cells, leading to the distribution of Si in the shoot (YAMAJI et al. 2008). Furthermore, similar to OsLis6 (rice), ZmLsi6 (maize) and HvLsi6 (barley) are expressed in both the roots and shoots and can show a polar position in the parenchyma cells of the xylem, which are adjacent to the vessels, both in the sheaths and laminae of the leaves (YAN et al., 2018).

Si, applied as calcium silicate (CaSiO_3), was used to correct soil acidity. Research indicates that it can be used instead of limestone, as most soils in Brazil have exchangeable acidity saturation and phosphorus (P) fixation in the soil (ALOVISI et al., 2018; TEIXEIRA et al., 2020). Additionally, silicates have greater mobility in soil because of the greater solubility of their products and their dissociation reactions (SANTOS et al., 2021).

The combination of Si and AMF has shown good results, and is an effective and economical way to mitigate the effects of drought stress on plants (ETESAMI et al., 2022). The benefits of Si-AMFs include improved nutrient absorption, decreased chlorophyll degradation, increased RUBISCO activity, attenuation of oxidative stress, and better plant growth and productive yield (VEGA et al., 2021). In addition, it improves the photosynthetic pigmentation of the plant, increases water potential in leaves and biomass production, and reduces the toxicity of mineral elements and proline production (VEGA et al., 2021).

Multispectral sensors measure the responses to the interaction of electromagnetic radiation with vegetation characteristics and can estimate information on the productivity, disease symptoms, pest attack, phenology, maturation, and physiological parameters of crops (ABREU JÚNIOR et al, 2022; KOAUDIO et al., 2021; NOGUEIRA et al., 2018;

PEREIRA et al., 2022; PICOLI et al., 2009). In recent research on microorganisms and remote sensing, subtle differences in images have been observed, creating difficulty in estimating parameters and suggesting the need for sensors with greater spatial, spectral, and radiometric resolutions (MARTINS et al., 2023).

Abiotic stress affects the metabolic reactions, development, and productivity of sweet corn crops (TAS; MUTLU, 2021). There are still no studies related to the association between Si and AMF applied to sweet corn crops; therefore, we hypothesized that the association between Si and AMF attenuates the effects of drought stress in sweet corn. This study aimed to investigate the association between Si and AMF in the alleviation of drought stress in sweet corn and its effects on biochemical, physiological, agronomic, and multispectral imaging.

2 MATERIAL AND METHODS

2.1 Plant growth conditions

The experiment was conducted in a greenhouse on the Campus of the Federal University of Uberlândia (UFU), located in the city of Monte Carmelo, Minas Gerais, Brazil. The experiment lasted from September 2, 2022, to December 20, 2022. The hybrid sweet corn crop GSS2577 was used, grown in pots with 12 dm³ using soil classified as Latosol Red Clay.

The temperatures and humidities were quantified in the greenhouse and collected daily using a digital thermo-hygrometer (Tomate, model PD-003), installed in the center of the greenhouse, with a resolution of 0.1°C temperature and 1% moisture.

Environmental conditions inside the greenhouse corresponded to maximum temperatures averaging 41°C, while the average minimum temperature was 18°C. The maximum humidity recorded was an average of 80%, whereas the minimum humidity averaged 12% during the experimental period (Figure. 1).

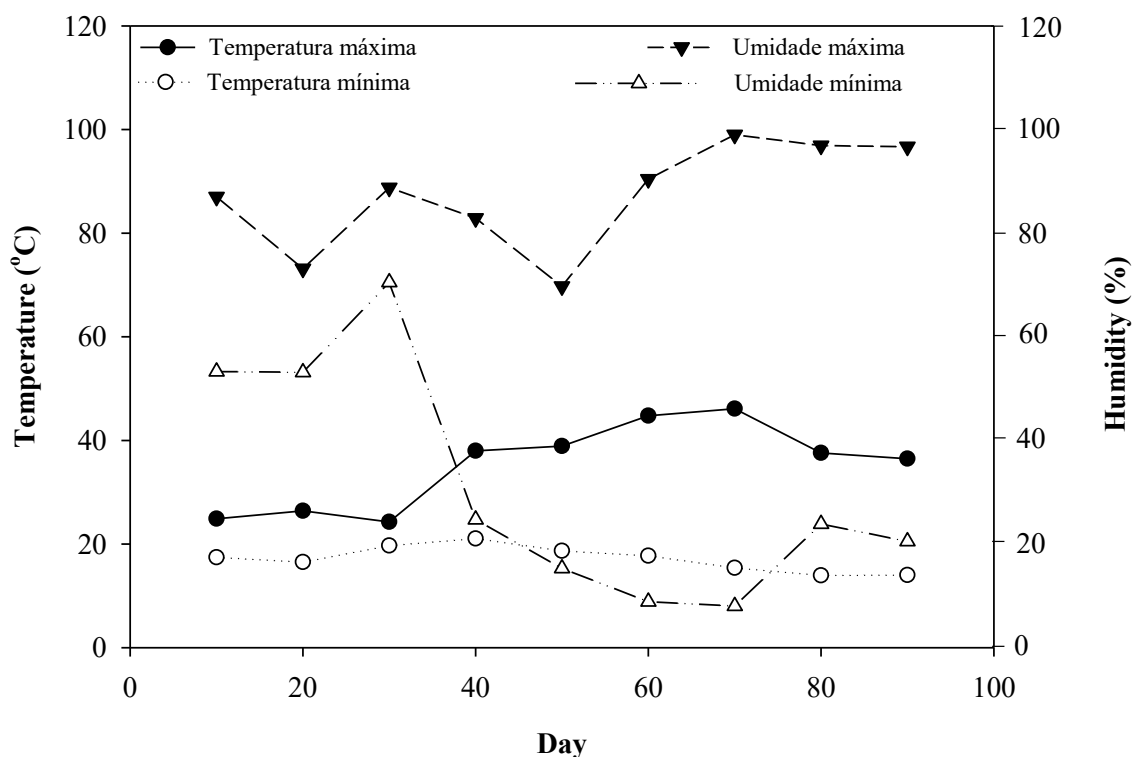


FIGURE. 1 Greenhouse environmental conditions, maximum and minimum temperature and humidity, from September to December 2022.

2.1 Experimental Design

The experiment was implemented in a randomized block design in a 2x2x2 factorial scheme, with four replications: first Si factor (with and without addition of Si as soil corrective) × second AMF factor (with and without AMF inoculation) × third irrigation factor (replacement of 40% and 100%, being drought stress, and control, respectively). For irrigation decision-making, 100% water replacement was defined as reference (control), and the critical value established for irrigation was -40KPa. The 100% replacement treatment corresponded to the amount of water required to reach field capacity, in which the irrigation time calculated for the control treatment was maintained for treatments under drought stress. To maintain time, drippers with different flow rates were used for each replacement level as follows: 40% ($2 \text{ L h}^{-1} \text{ vessel}^{-1}$) and 100% ($4 \text{ L h}^{-1} \text{ vessel}^{-1}$).

2.2 Soil characteristics and silicon supply

The soil used was collected in the municipality of Monte Carmelo, Minas Gerais, Brazil (18°32'33.76" S; 47°29'10.65" W; 799 msnm.), a sample was collected from the layer 0-20 cm of an oxisol, according to the classification of the United States Department of Agriculture (USDA) Soil Taxonomy, from a site with native vegetation and previously uncultivated soil. The soil was clayey, with a low silt content and low natural fertility, according to the soil chemical and physical parameters (Table 1).

TABLE 1. Chemical and textural traits of soil before correction and fertilization.

Chemical ¹	Unit	Value
pH in H ₂ O	-log [H ⁺]	5.50
pH in CaCl ₂	-log [H ⁺]	4.70
Phosphorus (Melich-1)	mg dm ⁻³	0.33
Exchangeable Potassium (K ⁺)	cmol _c dm ⁻³	12.00
Exchangeable Calcium (Ca ²⁺)	cmol _c dm ⁻³	0.20
Exchangeable Magnesium (Mg ²⁺)	cmol _c dm ⁻³	0.26
Sulphate (S-SO ₄ ²⁻)	mg dm ⁻³	3.75
Exchangeable Aluminium (Al ³⁺)	cmol _c dm ⁻³	0.00
Potential acidity (H ⁺ +Al ³⁺)	cmol _c dm ⁻³	1.93
Cation exchange capacity (T)	cmol _c dm ⁻³	2.42
Base saturation (V)	%	20.21
Organic matter (OM.)	%	0,63
Silicon (Si) ¹	mg dm ⁻³	7.20
Copper (Cu)	mg dm ⁻³	0.30
Iron (Fe)	mg dm ⁻³	29.47
Boron (B)	mg dm ⁻³	0.15
Manganese (Mn)	mg dm ⁻³	9.19
Zinc (Zn)	mg dm ⁻³	0.17
Physical	Unit	Value
Clay ²	%	17.00
Silt ²	%	9.00
Sand ²	%	52.80
Global density ³	g cm ⁻³	1.12

¹Determined with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0,01 mol L⁻¹; ²Determined with the pipette method ³Determined in an undisturbed soil sample.

The application of Si was performed with calcium silicate (CaO = 12%; SiO₂ = 78%; PRNT: 86%), while in the control vessels, doses of dolomitic limestone (CaO = 38.5%; MgO = 10.9%; PRNT: 95%). The amounts of both were based on their relative total neutralization power (PRNT) values, and their dosages were calculated according to Van Raij and Cantarella (1997) and adapted from Bianchini and Marques (2019) for the corn crop. Subsequently, water was added to the pots until the soil reached field capacity and incubated for 20 days for pH correction.

Base fertilization was carried out 30 d before sowing, and the coverings were applied 20 and 35 d after sowing using a nutrient solution according to Novais et al. (1991).

2.3 Inoculum of arbuscular mycorrhizal fungi

AMF was applied h before sowing. The AMF control treatment used only a mixture of sand and in the treatments with the presence of the fungus, a mixture of sand with the inoculum (1:1), and in the treatments with the presence of the inoculum and control, the amount applied per vase was 12 g, in 10 cm deep. The inoculum was obtained by growing Sorghum on a sterile sand substrate. Soil inoculum of AMF isolates were obtained from the International Culture Collection of Glomeromycota (www.furb.br/cicg) of the Blumenau Regional University (FURB) (Table 2).

TABLE 2. Number of spores of each AMF species in the whole inoculum used to inoculate sweet corn, cultivated in different levels of drought stress and presence and absence of Si.

AMF species and code	Number of spores (g ⁻¹ soil)
<i>Gigaspora albida</i> PRN200A	183
<i>Dentiscutata heterogama</i> MGR610A	227
<i>Rhizophagus clarus</i> SCT720A	22
<i>Rhizophagus intraradices</i> SCT736J	152
<i>Acaulospora mellea</i> SCT063B	14

<i>Acaulospora longula</i> PNB101A	144
Total	742 spores g ⁻¹

2.4 Irrigation management

The irrigation system was assembled with rigid PVC pipe coupled to hoses with self-compensating drippers (working pressure range: 0.7 - 4.0 bar) and coupled to the microtubes and fixed with stakes and the irrigation was activated using controller with a resolution of 1 min (RainBird Brand, Model ESP- RZXe), which controlled the opening and closing of the solenoid valve and the activation of the motor pump.

Previously, an undisturbed soil sample was collected to determine the water retention curve of the soil by obtaining physical-hydric data according to Van Genuchten's equation (1980). A form suitable for pots was created to obtain field capacity. The soil was saturated at the end of the afternoon, and the soil matrix potential was read the following morning, with an estimated value of -6kPa.

The onset of drought stress started 31 days after sowing and lasted up to 96 DAS. To monitor the matrix potential, daily readings were taken on the six randomly installed tensiometers: two tensiometers at 40% replacement and four tensiometers at 100% replacement. For irrigation decision making, 100% water replacement was defined as reference (control), and the critical value established for irrigation was -40KPa.

The 100% replacement treatment corresponded to the amount of water required to reach field capacity, in which the irrigation time calculated for the control treatment was maintained for treatments under drought stress. To maintain time, drippers with different flow rates were used for each replacement level as follows: 40% (2 L h⁻¹ vessel⁻¹) and 100% (4 L h⁻¹ vessel⁻¹). The drought and control treatments had average matrix potentials of -71 and -47 KPa, respectively (Figure. 2).

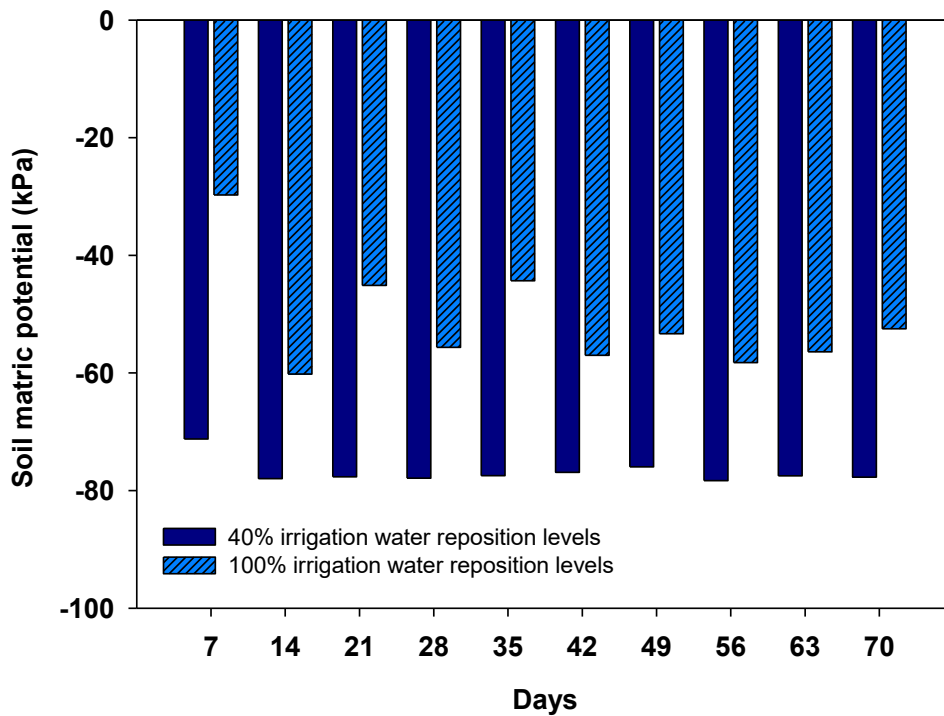


FIGURE 2 Soil matrix potential and water replacement at levels of 40% (drought stress) and 100% (control) from September to December 2022. For irrigation decision-making, a water replacement of 100 was defined as reference % (control) and the critical value established for irrigation was -40KPa.

A total irrigation time of 12.3 h was recorded during the drought-stress period. During this irrigation period, the total amount of water applied to the drought-stressed and control pots was 24.60 and 49.20 liters.

2.5 Agronomic evaluations (plants, leaves, ears, industrial yield and °Brix content)

The number of days male (DFM) and female (DFF) flowers were collected as reproductive structures appeared. Ears were collected and weighed when they reached the harvest point (when the stigmas were dry). After harvesting, the straw and heavy ears were removed. The number of rows and kernels per row of maize were counted. For industrial yield (IY), the grains were separated from the ears while still fresh, and the following equation was used for calculation: $IY = (\text{grain mass}/\text{ear mass}) \times 100$, expressed as a percentage. The soluble solids content (°Brix) of the harvested grains was evaluated using a refractometer (Instrutheme, model RTP-20 ATC).

Plant height (PH) was measured from the ground level to the flag leaf using a tape measure with a resolution of 1 cm. At the end of the cycle, the aerial parts of the plants were collected, dried in an oven at 60°C and weighed on a scale with a resolution of 0.001 g (Shimadzu, model UX6200H) to obtain the dry mass of the aerial parts (APDM). The roots were collected, washed, dried in an oven at 60°C, and weighed on a scale with a resolution of 0.001 g to determine root dry mass (MSR). Then, the roots were placed in a graduated cylinder of 1000 mL, with a known volume of 70% alcohol, and from the displaced volume, the root (RV) volume was obtained.

The soil temperature was evaluated before and after irrigation in the morning at 7:30 am, positioned 1 m away from the soil in the pots. For this measurement, a Cat S61 cell phone was used, which has a thermographic camera, with a scale from 0° to 400°C.

2.6 Physiological measurements (gas exchange, photosynthetic pigments and SPAD index)

Gas exchange was performed at the reproductive stage called R1 by standardizing the diagnostic leaf (physiologically active leaf) located below the ear. The plants were evaluated between 08:00 and 11:30 in the morning before irrigation. An IRGA infrared analyzer (model LC pro-SD) was used.

The SPAD index was used to quantify the green color in the leaves, which correlates with the N and chlorophyll content in the leaves (Model: SPAD-502, Konica Minolta brand). One reading per plant of each treatment was taken from leaves at the reproductive stage (R1) by standardizing the diagnostic leaf (physiologically active leaf) located below the ear. The mean of the obtained grade values was calculated. Both evaluations were carried out at the beginning of flowering, a period in which the plant's metabolic activities are high, considering the critical phenological stage for sensitivity to drought stress.

For the analysis of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids), leaves were collected at the reproductive stage (R1) by standardizing the diagnostic leaf (physiologically active leaf) located below the ear. The plants were evaluated between 08:00 and 11:30 in the morning before irrigation. The leaves were then washed with running water and dried with a paper towel, and the veins were removed. The leaves (0.5 g) were macerated using a mortar and pestle and placed

in glass test tubes. 1.5 mL of acetone PA and 1.5 mL of petroleum ether PA. The glass test tubes were wrapped in black cloth to prevent the passage of light and refrigerated for 24 h. Subsequently, a white solution was prepared with 1.5 mL of PA acetone and 1.5 mL of PA petroleum ether to adjust the standard curve. The readings were performed in the spectrometer at wavelengths of 470, 645, 652, and 663 nm (LICHTENTHALER; WELLBURN, 1983; WITHAM et al., 1971).

Leaves were collected at the reproductive stage (R1), standardizing the diagnostic leaf (physiologically active leaf) located below the ear to evaluate the leaf water potential. Evaluation was carried out between 05:00 am, with the plants before receiving irrigation. Still in the dark (before sunrise), they were wrapped in aluminum foil to avoid water loss and to prevent light from passing through and kept refrigerated while readings were taken, following collection, with a Scholander chamber, model 3115 (SCHOLANDER et al., 1965; SILVA et al., 2008).

2.7 Analysis of silicon and phosphorus

Si content in the soil

Soil samples were collected after ear harvesting at the end of the crop cycle. After harvesting, they were dried in an oven with forced air circulation at 45°C and crushed (Model: SL-102, brand SOLAB). A 10 mL aliquot of the soil extract was pipetted from each sample and placed in a 50 mL plastic beaker to prepare the samples. Next, 0, 2, 5, and 10 mL of a Si standard solution (20 mg L⁻¹) were pipetted and transferred to 100 mL flasks and the volume was completed with distilled water. Then, a 10 mL aliquot of each standard solution was collected and placed in a 50 mL plastic beaker (SILVA et al., 2023).

In plastic beakers, 1 mL of monosilicic acid (H₄SiO₄) was added. After 10 min, 2 mL of a tartaric acid solution (200 g L⁻¹) was added. After 5 min, 10 mL ascorbic acid solution was added. After 1 h, the absorbance was measured using a spectrophotometer (Model UV-M51, brand Bel) at 660 nm. The resulting values were expressed in ppm Si per sample (SILVA et al., 2023).

Si content in the leaf

The leaves were collected at the reproductive stage called R1 by standardizing the diagnostic leaf (physiologically active leaf) located below the ear, washed with distilled

water and dried in an oven with forced air circulation at 65°C. Then, they were ground and sieved through a 0.5 mm mesh, to homogenize the sample. 0.02 g were weighed on an analytical balance (Model: M214AIH, Bel brand) and digested with hydrogen peroxide (H₂O₂), 2 mL per sample and 3 mL sodium hydroxide (NaOH) per sample. Subsequently, the mixture was stirred for 5s and left in a water bath at a temperature between 70 and 80°C, autoclaved at 120°C for one hour. The mixtures were diluted once to 25 mL and a second time, a 1 mL aliquot of the first dilution, to a final volume of 10 mL. The dilutions were transferred to plastic containers and colorimetric reaction reagents were added to construct the standard curve. The silicon content of the sheets was calculated by measuring the absorption at 410 nm after a colorimetric reaction using molybdate (ELLIOTT; SNYDER, 1991).

P content in the leaf

Diagnostic leaves were collected at the reproductive stage (R1) by standardizing the diagnostic (physiologically active) leaves located below the ear. The leaves were washed with distilled water and dried in an oven with forced air circulation at 65°C. The leaves were crushed in a mill with a 0.5 mm mesh sieve to homogenize the sample. Solubilization (digestion) was carried out via wet, perchloric nitric acid solubilization, in which dry and ground samples (0.5 g) were weighed on an analytical balance, placed in a digester tube, 4 mL of nitric acid was added, and kept at rest for 12 h. Subsequently, the samples were heated to 120°C and kept at this temperature until the release of a brown vapor (NO₂) was completely exhausted. 2 mL of perchloric acid was added and the temperature was increased to 180°C, covering with a funnel to avoid loss of HClO₄ and to dry the extract (EMBRAPA, 2000).

Quantification was performed by vanadate yellow spectrometry, in which 5 mL of nitric-perchloric acid solution was used. The volume was made up to 20 mL with distilled water, and 4 mL of molybdate solution (50 g L⁻¹) and vanadate (2.5g L⁻¹). After 5 min, the absorbance was measured on the spectrometer (420 nm). The standard curve was adjusted and the P concentration in the solution was estimated (EMBRAPA, 2000). The following formula was used for determination: $P \text{ (g Kg}^{-1}\text{)} = \text{reading (mg L}^{-1}\text{)} \times 0.2$.

Accumulation of Si and P in the leaf

To determine the total concentration of Si in the leaves, the following equation was used: $NC = (NL/100) \times DM$. Where NC is the Si concentration, NL is the Si content expressed as a percentage, and DM is leaf dry mass.

To determine the total concentration of P in the leaves, the following equation was used: $NC = (NL/100) \times DM$. Where, NC is the P concentration, NL is the P level expressed as a percentage, and MS is the leaf dry mass.

2.8 Mycorrhizal colonization

After harvesting the ears and corn plants, the roots were collected at four points in the pot, 5 cm from the stem of the plant, and at a depth of 0-5 cm. The harvested roots were washed with water and the remaining soil was removed using a sieve. They were kept in Falcon tubes, with 70% alcohol and refrigerated. For root preparation, KOH (10%) was used for cleaning, and blue ink for coloring. The percentage of mycorrhizal colonization was then determined using the intersection of grid lines method, according to Giovanetti and Mosse (1980).

2.9 Multispectral imaging

The images were obtained using a multispectral camera, MAPIR Survey 3, which has three bands (red, green, and near-infrared) operating at wavelengths of 660, 550, and 850 nm. It has a focal length of 19 mm, a rectified sample, distance of 2.3 cm and resolution of 12 bits, positioned at a distance of about 1 m from the target (sweet corn leaves on a black surface) between 11 am and 1 pm, local time of higher incidence of electromagnetic radiation (MARTINS et al., 2021) (Figure 3).



FIGURE 3 Tripod mounted to attach the MAPIR camera (Survey 3) to collect multispectral images on corn leaves.

Multispectral images were used to create simple linear regression spectral models to estimate agronomic parameters that were directly measured in sweet corn leaves, and to provide guidance on the efficiency of the applied treatments. In addition, it allows the estimation of the potential of the images to discriminate between plants that present the presence of treatments (Si and AMF). The reflectance values of the images of each plant were displayed, allowing the construction of regression models capable of estimating the values of these parameters.

A simple linear regression model was used, which used the MAPIR bands and derived vegetation indices. This model uses 80% of the values to build the models (using formula) and 20% to validate the created models. This validation occurs through the correlation coefficient between the estimated and predicted variables (%) and the Root Mean Square Error (RMSE).

2.10 Vegetation indices

Vegetation indices are mathematical formulas used in image processing to better characterize treatments based on the spectral responses of their targets, in this case, sweet corn plants. These vegetation indices are related to plant development (NDVI) and

photosynthetic pigmentation (GNDVI) and were chosen for their relationship with the bands, which are operated in the MAPIR camera, reducing the choice of indices to be used and their sensitivity to a series of parameters that can be evaluated. NDVI and GNDVI indices were used (Table 3).

TABLE 3 Vegetation indices used for calculations of SPAD Index, chlorophylls *a*, *b* and total.

Vegetation Indexes	Formulas	Reference
NDVI	$\frac{(NIR - R)}{(NIR + R)}$	Rouse et al. (1974)
GNDVI	$\frac{(NIR - G)}{(NIR + G)}$	Gitelson, Kaufman and Merzlyak (1996)

Normalized difference vegetation index (NDVI). Green Normalized Difference Vegetation Index (GNDVI). The letter R refers to the length range of the red band (590–670 nm), and G refers to the green band (500–590 nm).

The correlation coefficient and root mean squared error (RMSE) were used as parameters and data as percentages to validate the regression model. The following formula was used to calculate the RMSE percentage: $(RMSE \times 100 \times n) / \sum (X_i)$. Where RMSE= value obtained from the root mean square error, n= number of variables, and X_i = value measured in the field.

2.11 Supervised Classification

Supervised classification was used to verify the accuracy of the model in correcting for the presence or absence of Si and AMF in the plants using multispectral images. The neural network tool (multilayer perceptron), with a cross-validation technique (leave-one-out), relates bands and indices. Among the 32 values measured in the field, 31 were used for training and 1 for validation until all values were used for validation, resulting in a confusion matrix for each variable (Figure 4).

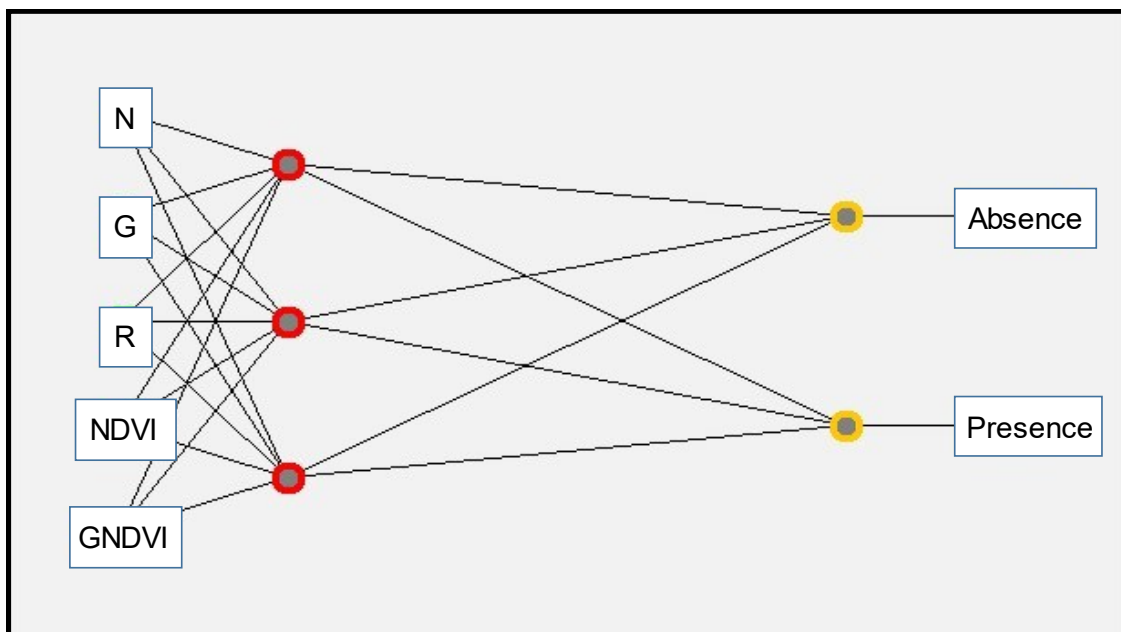


FIGURE 4 Schematic of neural networks for the classification of absence and presence of Si and arbuscular mycorrhizal fungi using the cross-validation technique (leave-one-out).

2.12 Statistical analysis

Data were subjected to analysis of variance and, when significant differences were found, the Scott- Knott test was applied at a 5% error probability level. Standard errors were calculated for all the means. All statistical procedures were performed using the SAS software, version 9.3 (SAS, 1996).

3 RESULTS

3.1 Agronomic evaluations

The dry mass of leaves, stems, tassels, and total had a significant effect only in the control treatment (CT), corresponding to 100% of water replacement in the soil. Drought stress (WT), which corresponds to 40% of irrigation water replacement, caused reductions of 57%, 35%, 34%, and 41% in the dry mass of stems, leaves, tassels, and total plants, respectively. AMF had no significant effect on the dry mass of sweet corn plants (Figure 5A-F).

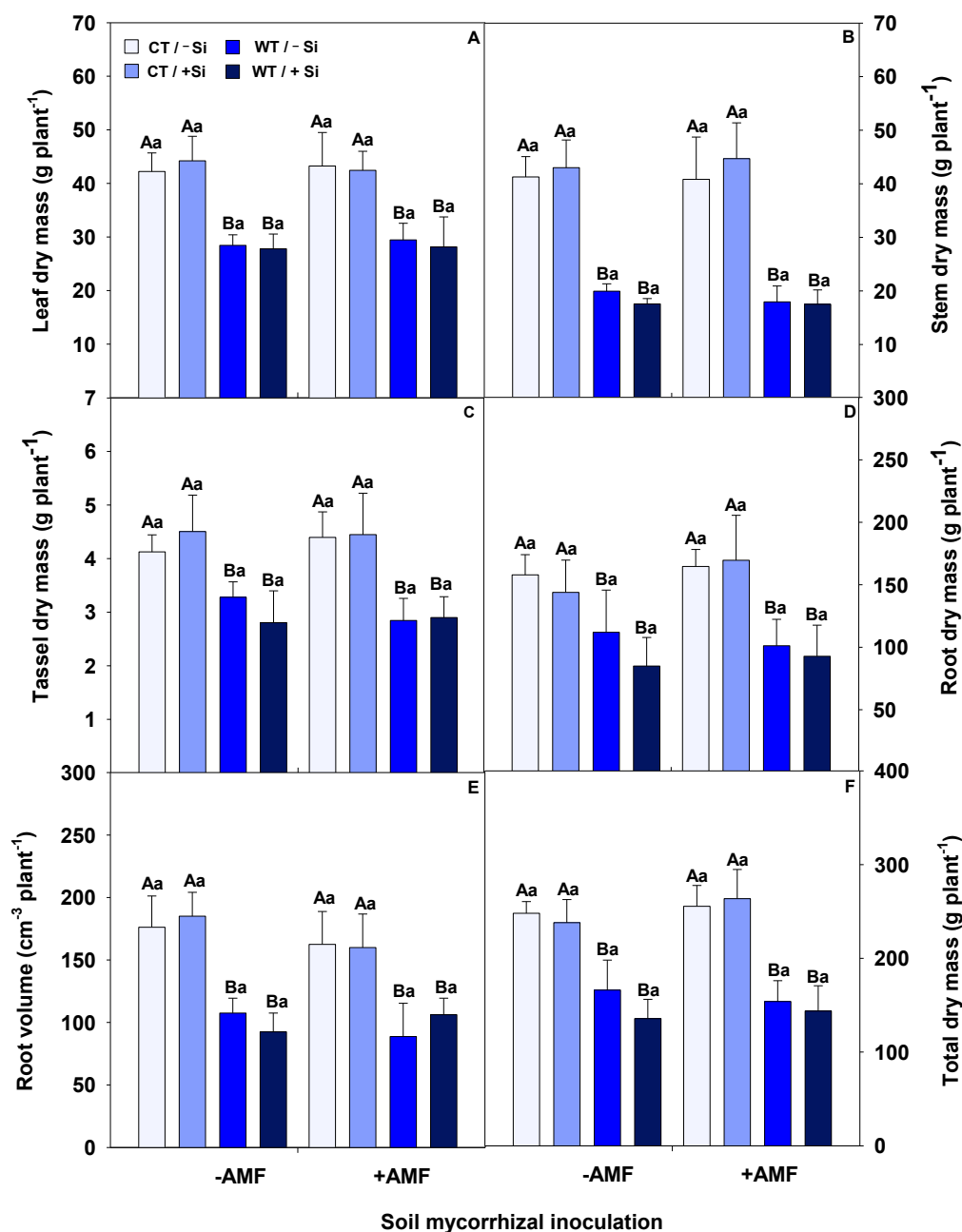


FIGURE 5 Effect of water replacement levels, AMF inoculation and Si application on leaf dry mass (A), stem dry mass (B), tassel dry mass (C), root dry mass (D), volume root (E) and total dry mass (leaf dry mass + stem dry mass + tassel dry mass + root dry mass) (F) as a function of CT (control = 100% irrigation water replacement) , WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

Root dry mass was affected by the WT. All treatments showed a weight reduction of 65–74 g, except for the treatments without AMF and with Si. The presence of AMF increased root dry mass by 28.3% in CT and the presence of Si (Figure. 5D).

Corn root volume showed a significant effect only in WT. WT reduced root production by volume from 33.6% to 50%. The presence of AMF had no significant effect on root volume, even in the presence or absence of Si (Figure 5E and 6).

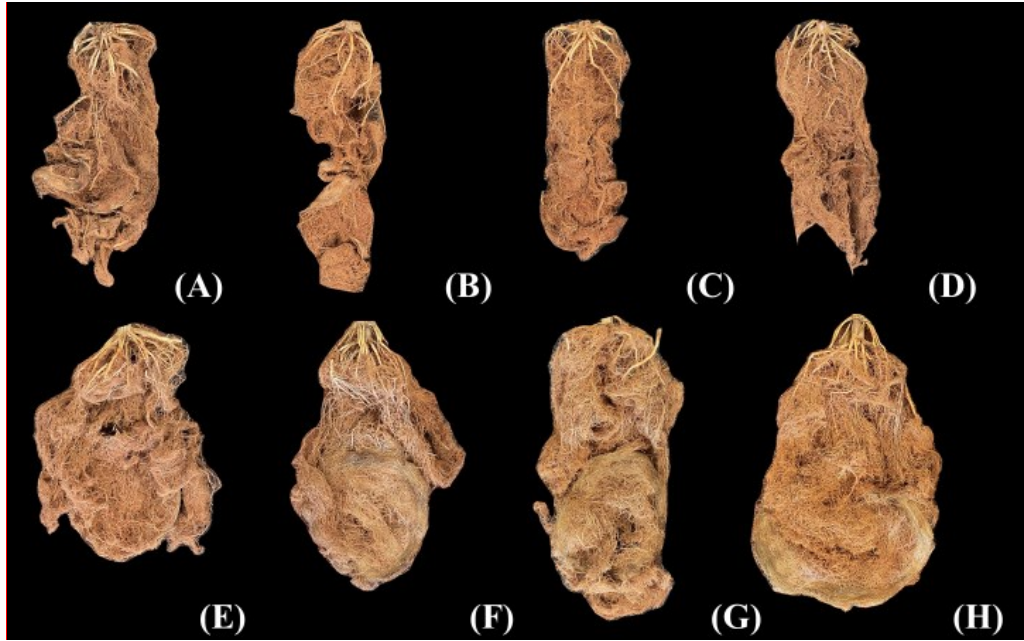


FIGURE 6 Effect of water replacement levels, AMF inoculation and Si application on the root system of sweet corn plants: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

The height of the plants was reduced by 30 cm in the WT compared to that in the CT. In the presence of AMF, no significant effects were observed in the presence or absence of Si. (Figure 7A). In the absence of AMF, corn ears did not develop WT, either in the presence or absence of Si. In the presence of AMF, the plants in WT had the height of their spikes reduced by approximately 14%. The same treatments did not differ in the presence or absence of AMF (Figure 7B).

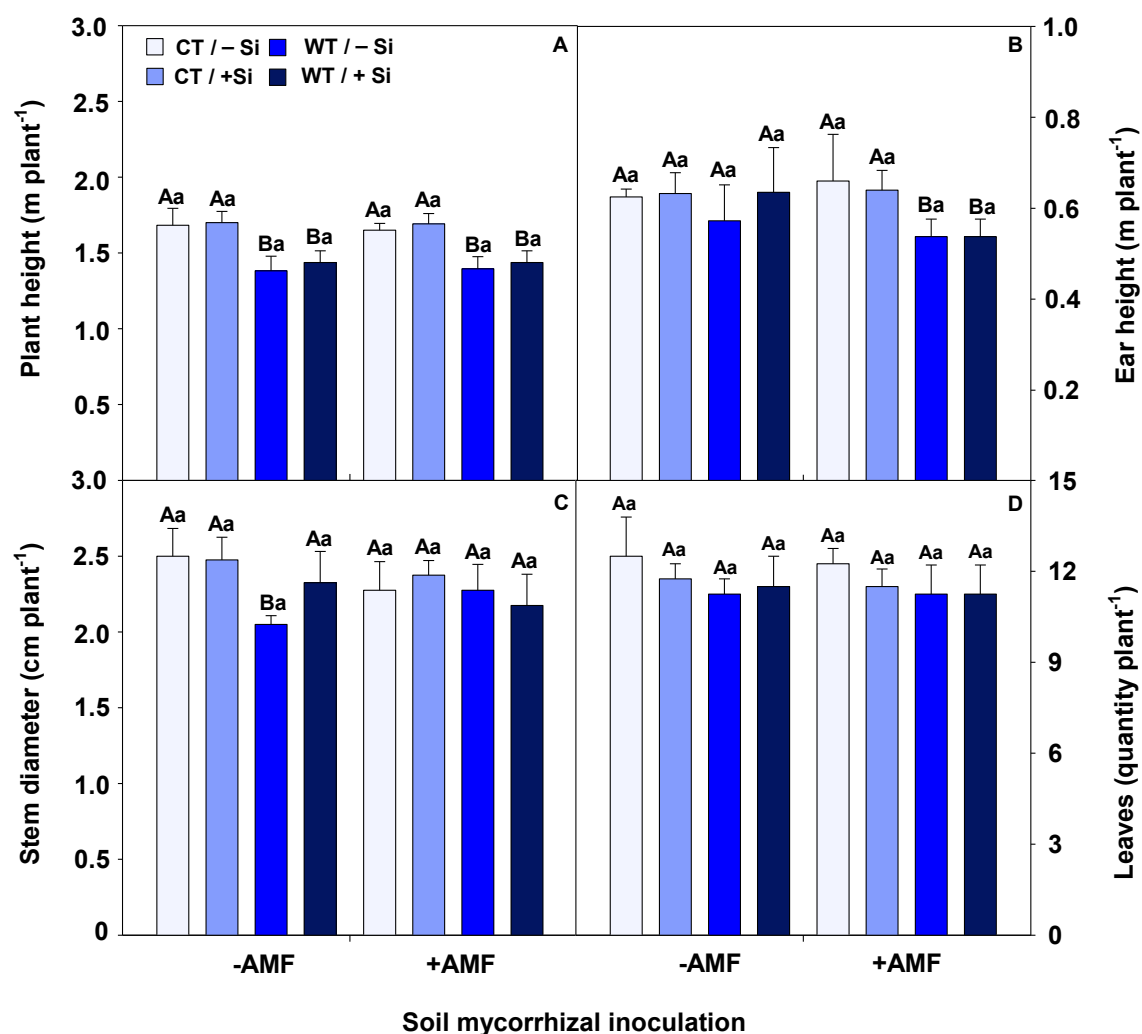


FIGURE 7 Effect of water replacement levels, AMF inoculation and Si application on plant height (A), spike height (B), stem diameter (C) and number of leaves (D) as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott-Knott test ($p < 0.05$).

The stem diameter had no significant influence on the presence of AMF in the same treatments. Analyzing the effect of WT, only the treatment in the absence of AMF and absence of Si that presented smaller stem diameter under drought stress, with a reduction of 9.7% compared to CT. The indices of other treatments were not affected by

the amount of water replacement (Figure 7C). The WT and the presence of AMF had no significant effects on the number of leaves (Figure 7D).

The soil temperatures before irrigation were statistically the same for the treatments as a function of the variation in the irrigation factor and the presence of AMF. However, the soil temperature after irrigation resulted in a lower temperature for the treatment in the absence of AMF and Si in CT, 25.54°C, and the other treatments did not have their temperatures affected by CT (Figure 8, Figure 9, and Figure 10). When comparing the temperatures of all treatments, a reduction in soil temperature was noted with irrigation.

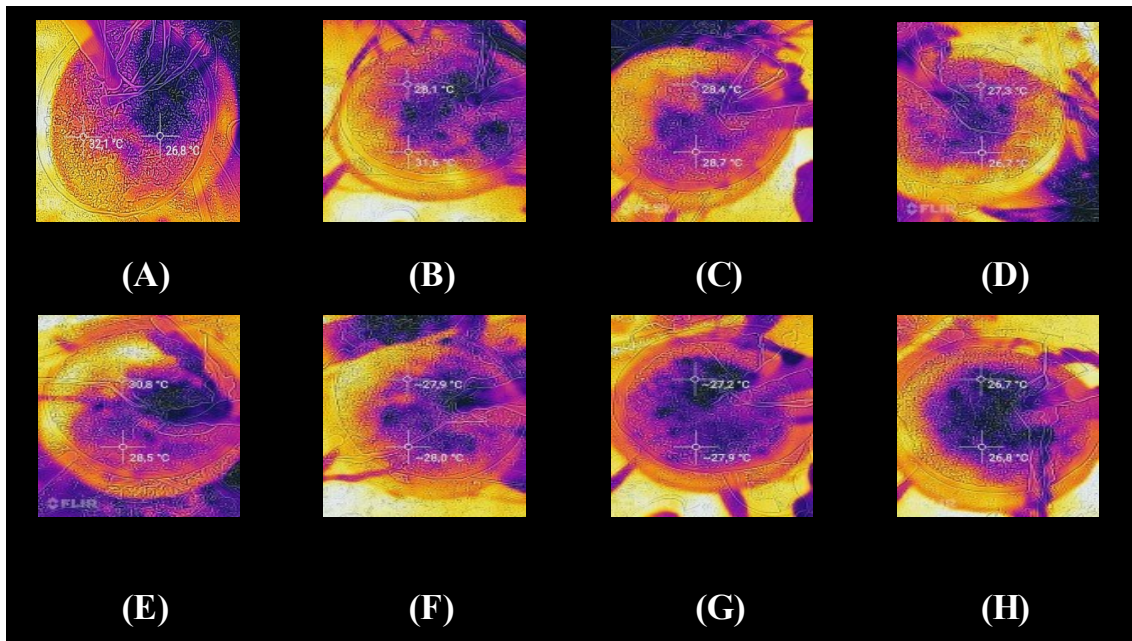


FIGURE 8 Effect of water replacement levels, AMF inoculation and Si application on soil temperature before irrigation in sweet corn. WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

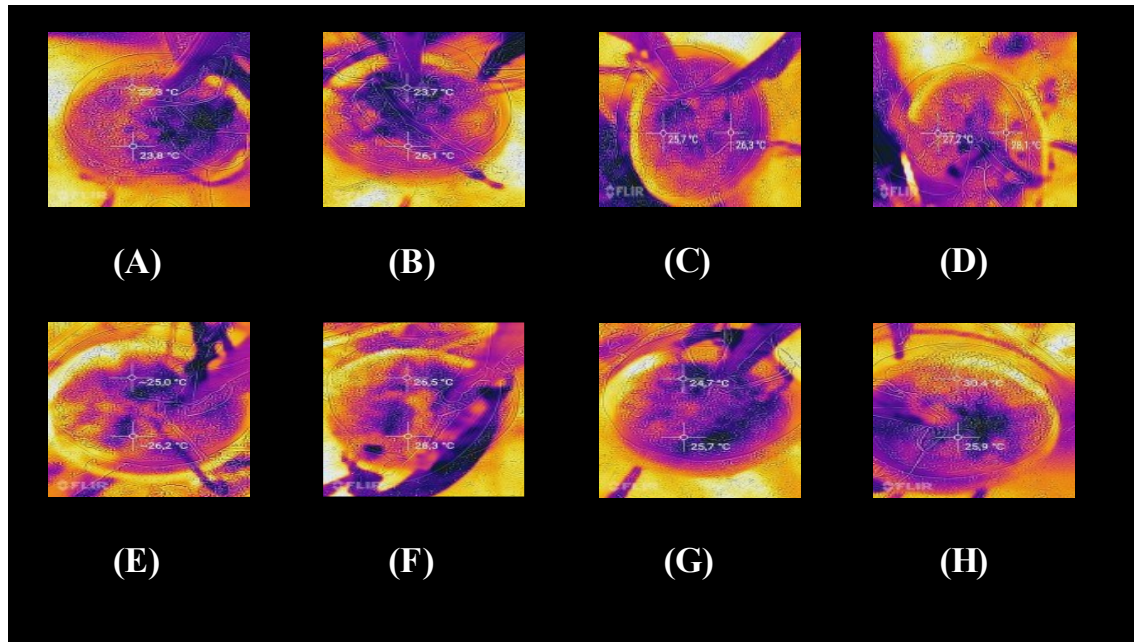


FIGURE 9 Effect of water replacement levels, AMF inoculation and Si application on soil temperature after irrigation in sweet corn: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

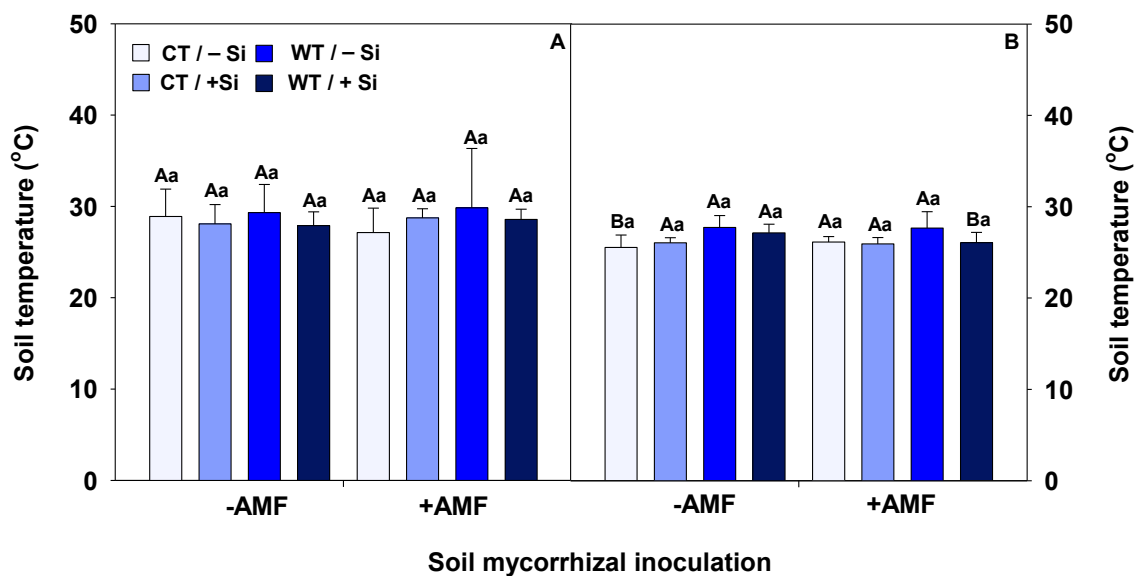


FIGURE 10 Effect of water replacement levels, AMF inoculation and Si application on soil temperature before (A) and after irrigation (B) as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in

relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

3.2 Analysis of Silicon and Phosphorus

The Si content in the soil was not affected by the WT and was statistically equal within each treatment. Analyzing the Si contents in the same treatments, in the absence of AMF, the only treatment that had the content affected was the treatment in WT, in the absence of Si, with a reduction of 17.7%. The other treatments did not affect the Si content in the presence of AMF (Figure 11).

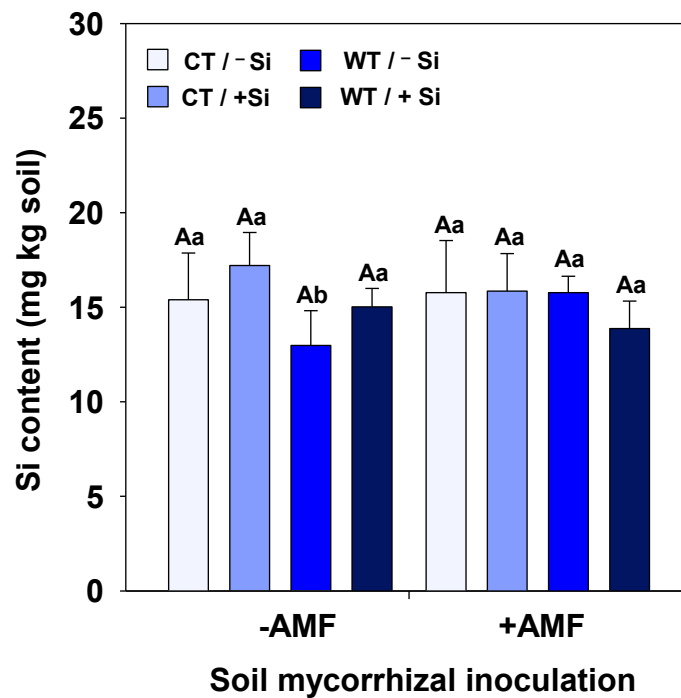


FIGURE 11 Effect of levels of water replacement, AMF inoculation and Si application on Si content in the soil as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

In the same way as Si, the P levels in the leaf had their values affected by WT, which showed reductions in the amount of P from 39.3% to 66.1% in the absence and

presence of Si. The presence of AMF had a significant effect only on CT in the absence of Si, which showed a 30% reduction in P content; and the other treatments did not differ from the presence of AMF (Figure 12A).

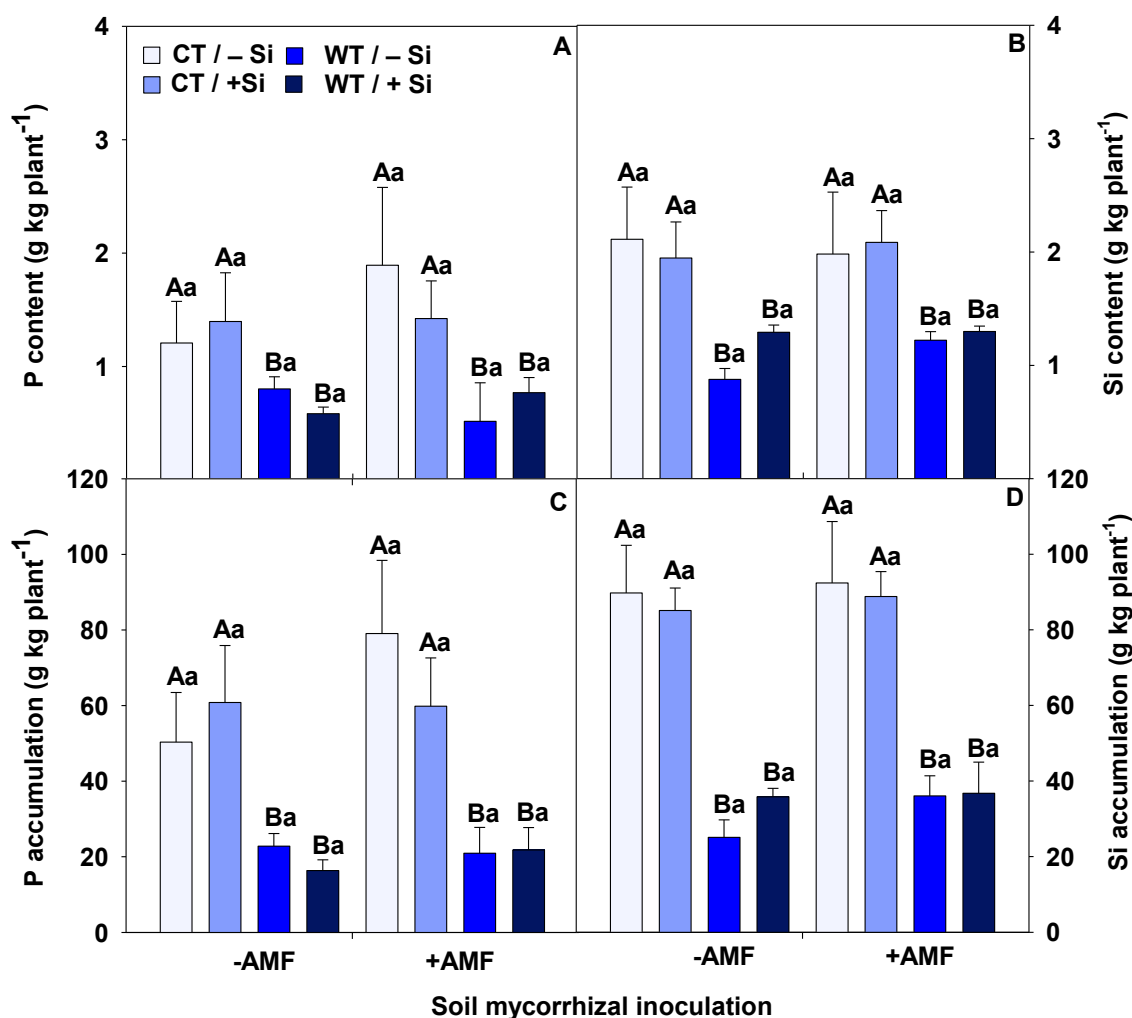


FIGURE 12 Effect of water replacement levels, AMF inoculation and Si application on P and Si content (A and B) and accumulation (C and D) in sweet corn plants as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

Leaf Si content was not influenced by AMF, and all treatments in the presence of AMF were equal to the same treatments in its absence. However, treatments submitted to

WT resulted in lower Si content in the leaves, with variations from 33% to 59% (Figure 12B).

3.3 Mycorrhizal colonization

AMF colonization showed statistically equal percentages for all plants, comparing the same treatments, both in CT and WT, with the exception of the treatment in the absence of AMF and absence of Si in CT, where there was a reduction in colonization by 37, 6%. Comparing the same treatment, in the presence of AMF, there was an increase in colonization, corresponding to 65% (Figure 13). Uninoculated treatments showed colonization by native soil fungi.

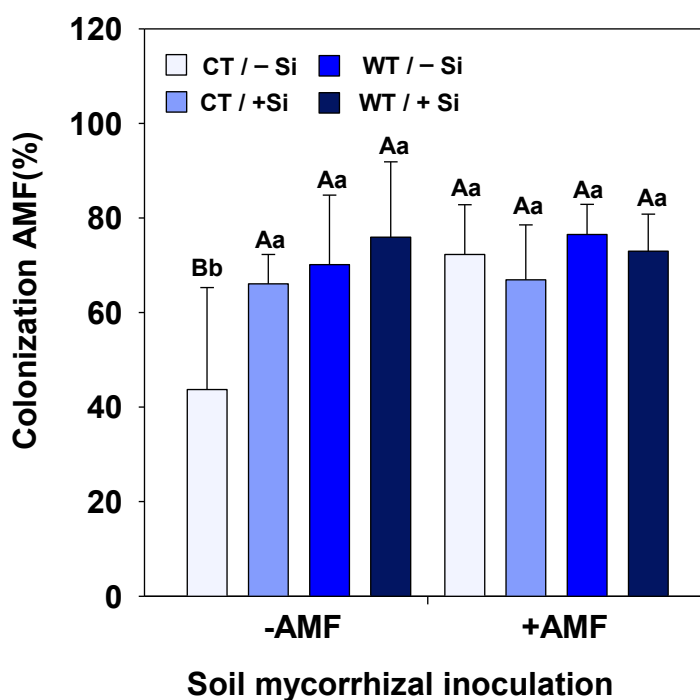


FIGURE 13 Effect of water replacement levels, AMF inoculation and Si application on AMF colonization in sweet corn as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

3.4 Plant yield results

The length of the ears was affected by the WT in the absence and presence of AMF, with differences of approximately 8 cm. AMF had no significant effect in each treatment, resulting in equal ear lengths (Figure 14A).

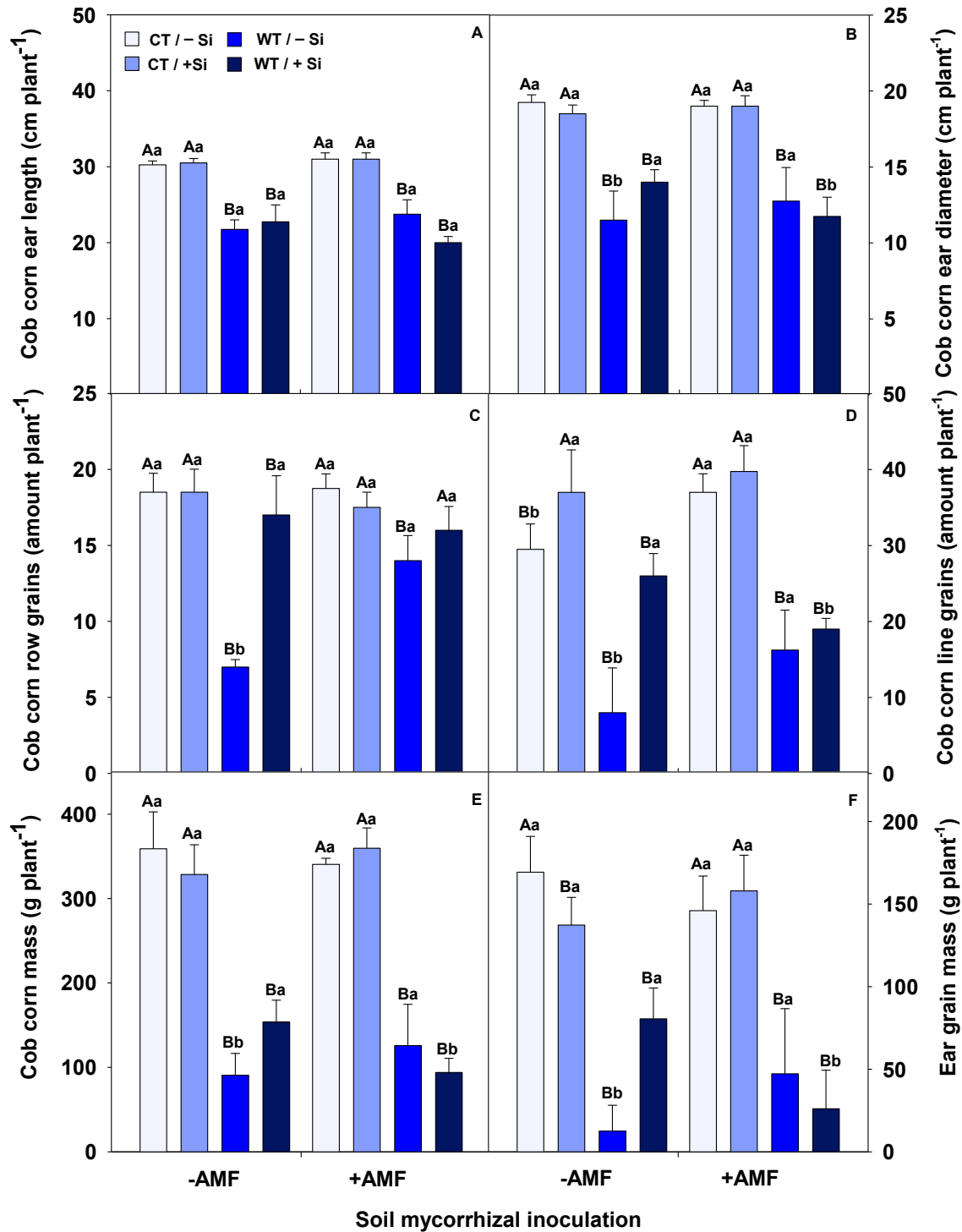


FIGURE 14 Effect of water replacement levels, AMF inoculation and Si application on corn ear length (A), corn ear diameter (B), corn ear grain rows (C), number of grains per

corn row (D), corn ear mass (E) and ear grain mass (F) in the sweet corn crop as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

Treatment submitted to WT resulted in smaller ear diameters, with variations from 18 to 40%. The diameter was influenced by the presence of AMF in the WT treatments, where, in the absence of Si, the presence of AMF showed a difference of more than 19.7%, whereas in the presence of Si, the presence of AMF resulted in a decrease in the 16.2% yield (Figure 14B).

The number of rows was affected by WT in the treatments absence of Si, absence of AMF, and presence of Si and presence of AMF, with a reduction of 81% and 54.3%, respectively. The number of rows was influenced by the presence of AMF in the WT treatments. In the absence of Si, the presence of AMF showed a difference of more than 75%, whereas in the presence of Si, the presence of AMF resulted in a yield decrease of 52.9% (Figure 14C).

The number of grains per ear row was affected by WT, with differences of 56% to 76% in all treatments, with the exception of the treatment in the absence of AMF and presence of Si. Analyzing the effect of the presence of AMF on the number of grains per row of ears, only the treatment subjected to WT in the presence of Si had the number of grains reduced by 63.5%; the other treatments were not influenced by AMFs (Figure 14D and 15).

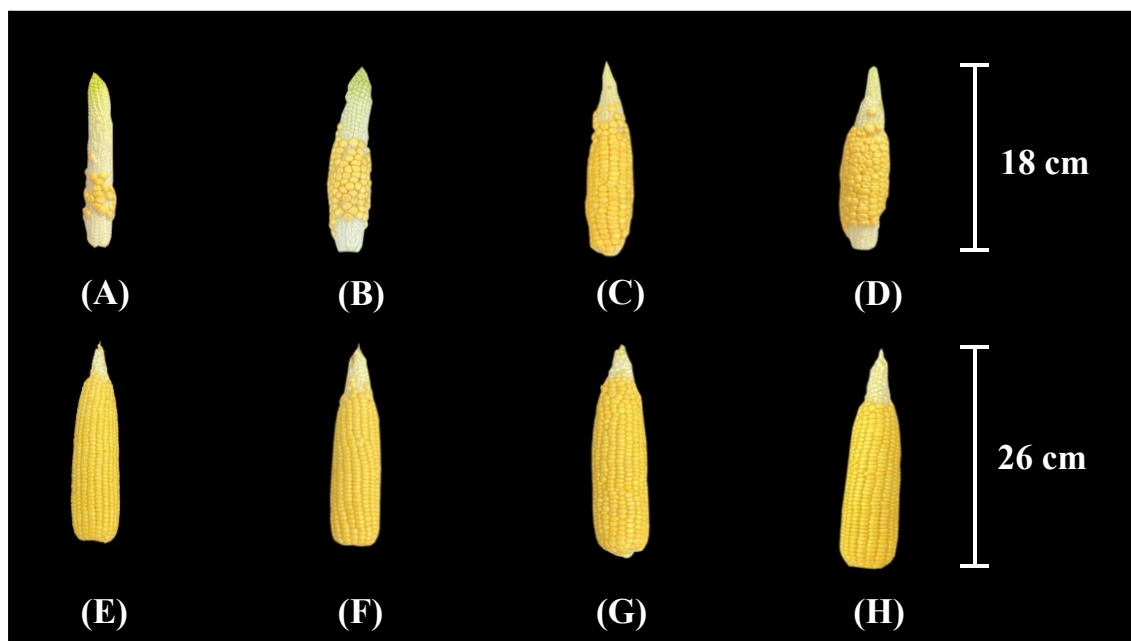


FIGURE 15 Effect of irrigation levels, AMF inoculation and Si application on sweet corn ears: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

The ear mass was affected by the WT in all treatments. In ears, the presence of AMF affected only the weight of the treatment with the presence of Si in WT, with a reduction of 38.9%, not influencing the other treatments (Figure 14E). Plants subjected to the WT showed lower grain mass, with differences greater than 100 g. The presence of AMF had a significant effect on WT in the presence of Si; the presence of AMF resulted in a reduction in grain weight by 67.6% (Figure 14F).

The size of ears with straw and without straw was altered in WT, which reduced the size of ears with straw around 25.5%. In ear size without straw, the reduction was approximately 20.2%. The presence of AMF within each treatment was significant only in the size of the ear with the straw, in the treatment in WT, in the presence of Si, reducing the size by 12%. The other treatments did not differ in the presence of AMF in terms of ear size with straw, just as the presence of AMF had no significant effect on ear size without straw (Figure 16A-B).

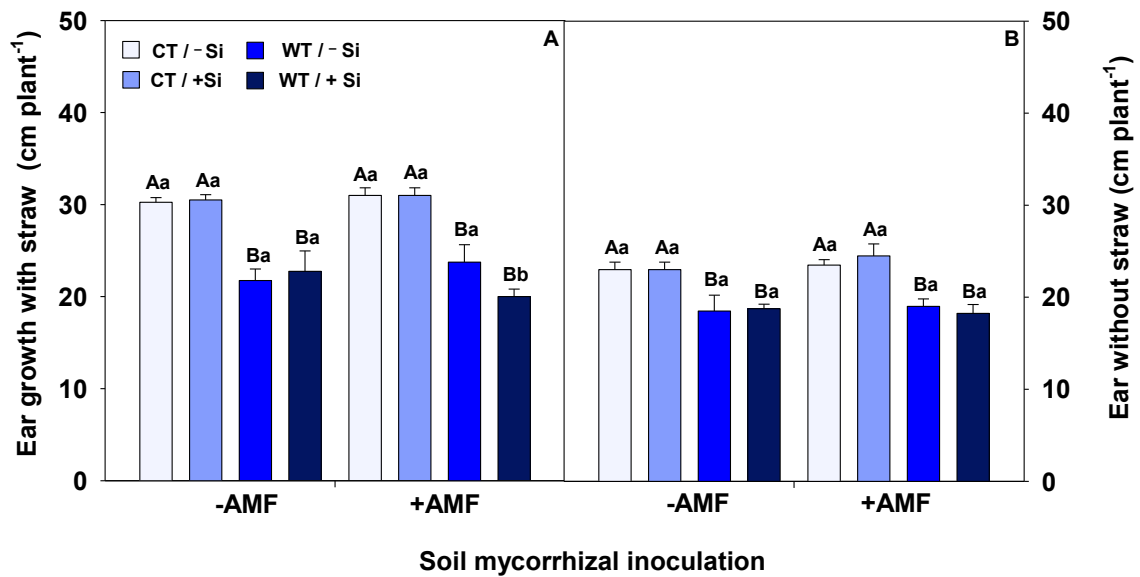


FIGURE 16 Effect of water replacement levels, AMF inoculation and Si application on ear growth with straw (A) and ear without straw (B) in sweet corn as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

The percentage of industrial yield was affected by WT in the treatments of absence of AMF, absence of Si, and presence of AMF, and presence of Si, with a reduction of 74.6% and 47.56%, respectively. Industrial yield was influenced by the presence of AMF in WT plants. Since in the absence of Si, the presence of AMF showed a difference of more than 59.7%, and in the presence of Si, the presence of AMF resulted in a decrease in the yield of 49.4% (Figure 17A).

Soluble solids were not influenced by the presence of AMF, considering the same treatments, as all were statistically equal. Still using the same agronomic parameters, the WT treatments showed a higher °Brix than the CT by approximately 10%, with the exception of the treatment in the presence of AMF and absence of Si, which did not have its soluble solid content affected by WT (Figure 17B).

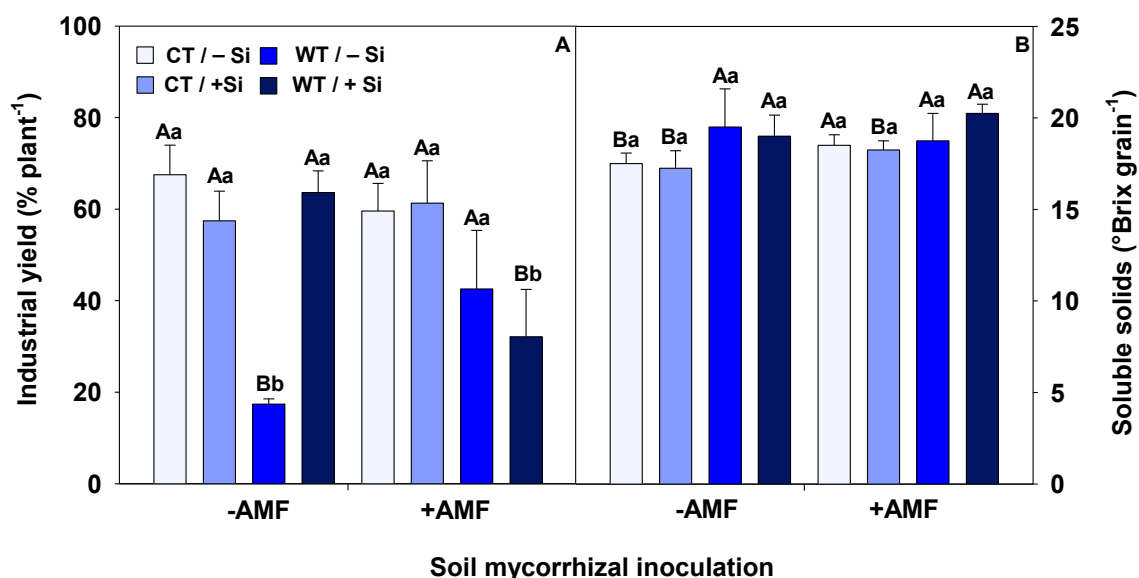


FIGURE 17 Effect of water replacement levels, AMF inoculation and Si application on industrial yield (A) and corn grain soluble solids (B) in the sweet corn crop as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

3.5 Physiological measures

Evaluation using a photosynthesis analyzer generated data on the following parameters: photosynthesis (A), transpiration (E), internal carbon (Ci), and stomatal conductance (Gs). The rate of photosynthesis had little effect on WT, in which only the treatment with the presence of AMF and the presence of Si had its value reduced by 35%. The other treatments did not differ from CT. Photosynthesis was also affected by AMF only in the CT treatment in the presence of Si. In the absence of AMF, the rate was reduced by 21.6%. Other treatments did not differ in the presence of AMF (Figure 18A).

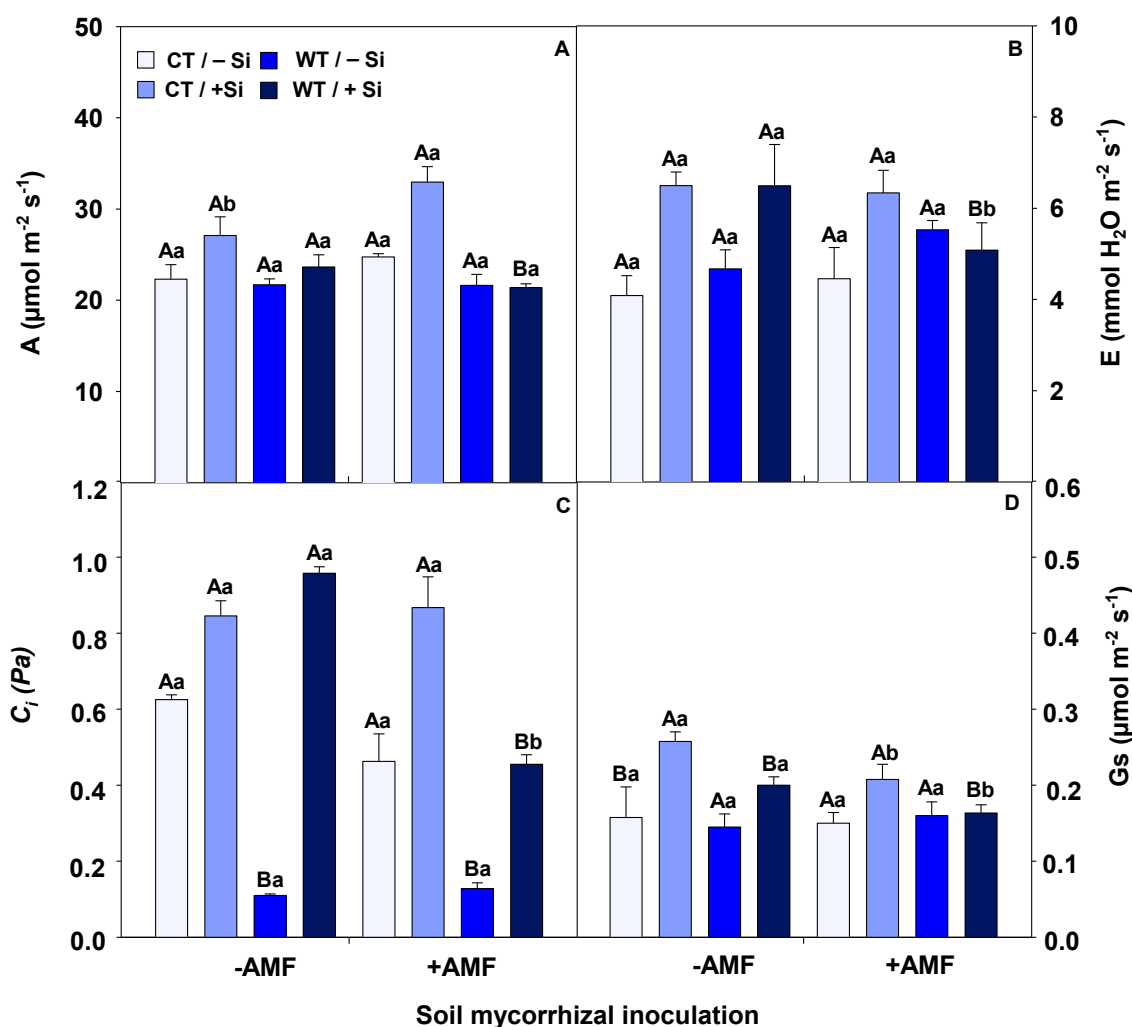


FIGURE 18 Effect of water replacement levels, AMF inoculation and Si application on A- photosynthesis (A), E- transpiration (B), C_i - internal carbon (C), G_s - stomatal conductance (D) in sweet corn as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

Regarding leaf transpiration, only the treatment with the presence of AMF and the presence of Si had a significant effect on WT, in which the transpiration rate was reduced by 19.8%. Likewise, only the treatment in WT and presence of Si had its rates reduced with the presence of AMF, where it was reduced by 22% (Figure 18B).

The internal carbon rate was affected the WT in the treatments absence of AMF, absence of Si, presence of AMF and absence of Si, and presence of AMF and presence of Si, which reduced by 82%, 72%, and 48%, respectively. The internal carbon value was also affected by the presence of AMF only in the WT treatment in the presence of Si; the presence of AMF decreased the C_i rate by 53% (Figure 18C).

Stomatal conductance significantly affected the WT; only the treatments with Si had reduced values. In the absence of AMF, there was a decrease of 23% and in the presence of AMF, the decrease was 24%. Only the CT treatment in the presence of Si had the stomatal conductance affected by the presence of AMF, with a reduction of 19%. The values of the other treatments increased in the presence of AMF (Figure 18D).

Plants subjected to WT in the presence of AMF had their chlorophyll *a* reduced by 24.5% and 38.4% for treatments in the absence and presence of Si, respectively. Comparing the same treatments in relation to the presence of AMF, only the plants in the CT treatment with the presence of Si had their chlorophyll *a* level affected. In the presence of AMF there was an increase of 26.7% (Figure 19A).

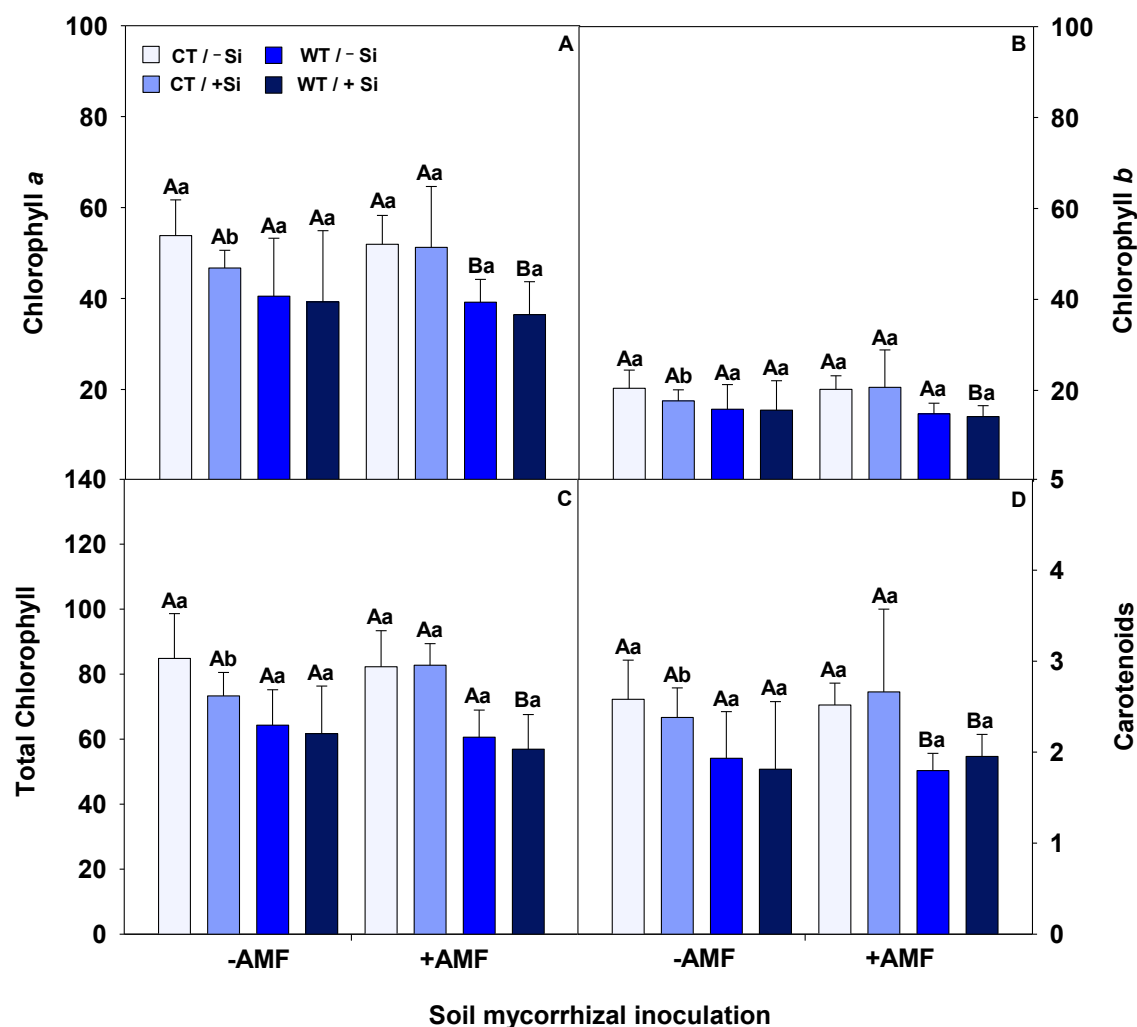


FIGURE 19 Effect of water replacement levels, AMF inoculation and Si application on chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C) and carotenoids (D) in sweet corn as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

In *b* chlorophyll analysis of the same treatments, comparing the CT and WT treatments, statistically equal results occurred, except for the treatment with the presence of AMF and the presence of Si in the WT, the chlorophyll content was reduced by 43.6%. Comparing the same treatments with each other and the presence of AMF, only the plants in the CT treatment in the presence of Si had their chlorophyll *b* content affected. In the presence of AMF, there was an increase of 42.7% (Figure 19B).

In the total chlorophyll analysis of the same treatments, comparing CT with WT, statistically equal results occurred, except in the treatment with the presence of AMF and the presence of Si, which in the WT had its chlorophyll content reduced by 38.9%. Comparing the same treatments with each other, in the presence of AMF, only the CT plants with the presence of Si had their total chlorophyll content altered; in the presence of AMF, there was an increase of 33.9% (Figure 19C).

Plants subjected to WT and with the presence of AMF had their carotenoid content reduced by 28.6% and 38.9% for treatments in the absence and presence of Si. Comparing the same treatments with each other, in relation to the presence of AMF, only the CT plants in the presence of Si had their carotenoid content affected; where the presence of AMF resulted in a 34% increase (Figure 19D).

In the water use efficiency, the treatments in WT, in the presence of AMF, there was a reduction in its levels, corresponding to 31% in the absence of Si and 18.6% in the presence of Si; the other treatments were not affected by WT. On the other hand, the presence of AMF was significant only in the CT treatment in the presence of Si. In the absence of AMF, the efficiency was reduced by almost 20%, whereas the fungus did not affect the other treatments (Figure 20A).

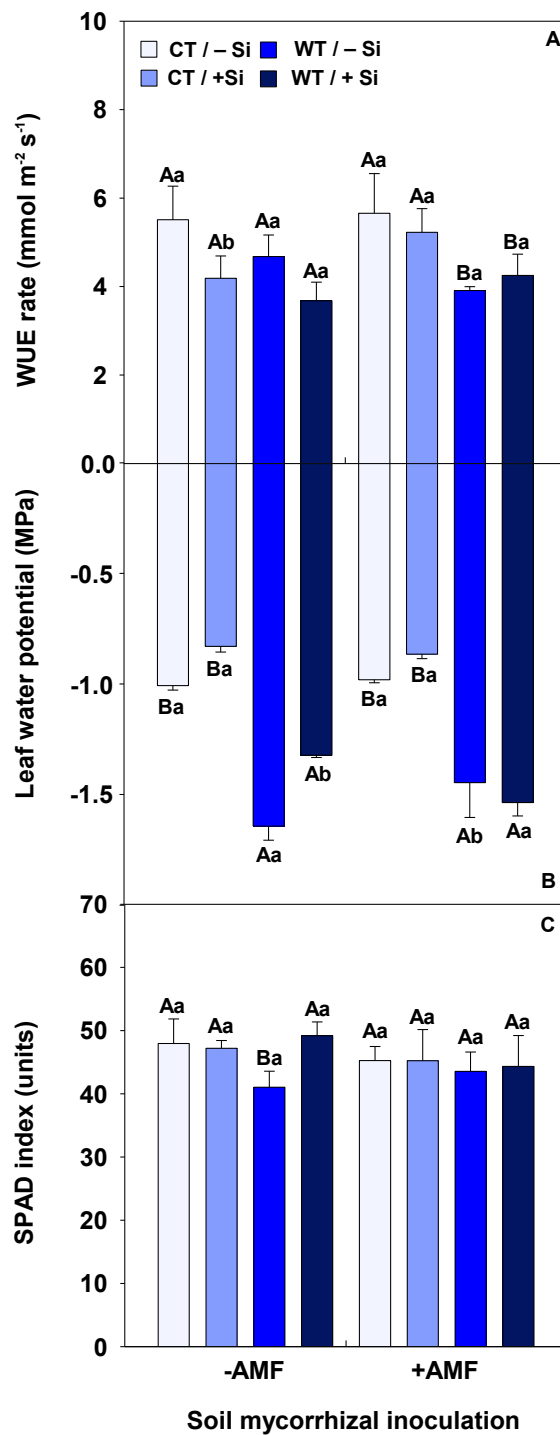


FIGURE 20 Effect of water replacement levels, AMF inoculation and Si application on the Water Use Efficiency Rate (A), leaf water potential in corn leaf (B) and SPAD index (C) in sweet corn as a function of CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si) in relation to +AMF (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn. Means at the same fluid replacement level, followed by different lowercase letters, differ by the Scott- Knott test ($p < 0.05$).

The xylem water potential was higher in the WT treatment. The treatment that had the greatest difference in potentials in relation to WT was the treatment with the presence of AMF and the presence of Si, with a difference of 43.7%. AMF had a significant effect on the WT only treatments. In the absence of Si, the presence of AMF reduced the potential by 12.1%, whereas in the presence of Si, the presence of AMF resulted in a greater potential, with a difference of 14%. The CT treatments were unaffected by AMF (Figure 20B).

The SPAD index had no significant effect on the presence of AMF under the same treatments. Analyzing the effect on WT, only the treatment in the absence of AMF and absence of Si showed the lowest SPAD index in WT, with a reduction of 14.4% compared to CT. The other treatments did not have indices affected by the WT (Figure 20C).

3.6 Multispectral imaging and supervised classification

3.6.1 Multispectral imaging

Using multispectral images of corn leaves, agronomic parameters were estimated using a simple linear regression model. The SPAD index, chlorophyll *a*, chlorophyll *b* and total chlorophyll were estimated using the MAPIR bands and vegetation indices. Carotenoids were tested, but could not be estimated using this method (Figure 21).

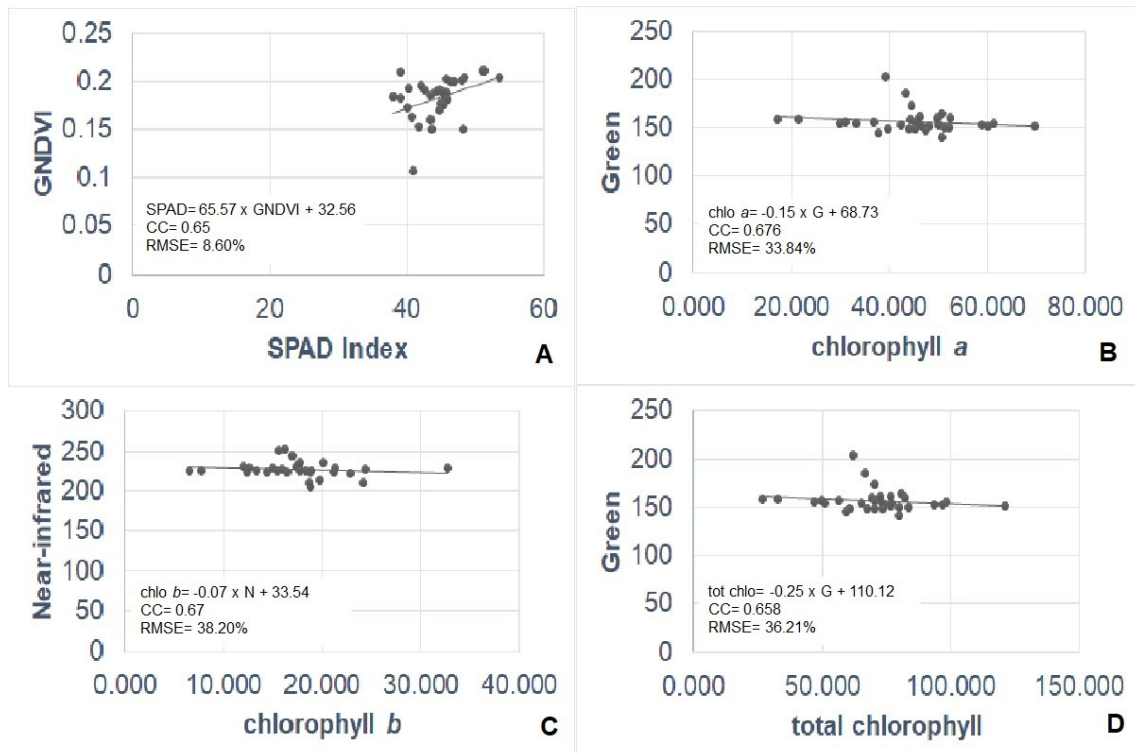


FIGURE 21 Regression models generated to estimate agronomic parameters: SPAD index (A), chlorophyll *a* (B), chlorophyll *b* (C) and total chlorophyll (D), using MAPIR bands (G-green and N-near-infrared) and vegetation indices (GNDVI).

The Simple Linear Regression model was able to estimate the agronomic parameters SPAD Index, chlorophyll *a*, chlorophyll *b* and total chlorophyll as 91.40%, 66.16%, 61.80%, and 63.79%, respectively, indicating good performance. Lower RMSE values indicated that the model was suitable for estimating the evaluated parameters.

Analyzing the values of the correlation coefficients between the variables measured and estimated by the model, which vary from -1 to 1, the closer to one, the greater the correlation between the two variables. Using this model, correlation coefficient values of 0.65 were calculated, that is, 65% of correlation between the predicted and estimated variables for the agronomic parameters SPAD Index and total chlorophyll, and 0.67 for the parameters chlorophyll *a* and *b*, showing 67% correlation between the variables.

As the photographs of the leaves were taken, the agronomic parameters that could be estimated were related to leaf measurements. The indices and multispectral bands used in the model to estimate the agronomic data were related to plant development and photosynthetic pigmentation.

The following images were generated using Envi Classic Software: Through them one can see the color differences varying with the variation of the evaluated values, namely, SPAD index (Figure 22), chlorophyll *a* (Figure 23), chlorophyll *b* (Figure 24) and total chlorophyll (Figure 25) for all treatments. This variation occurs according to the following color scale, in which the values of the analyzed parameters increase from left to right. The color scale used in the software was GRN-RED-BLU-WHT, where the scale increases from left to right.

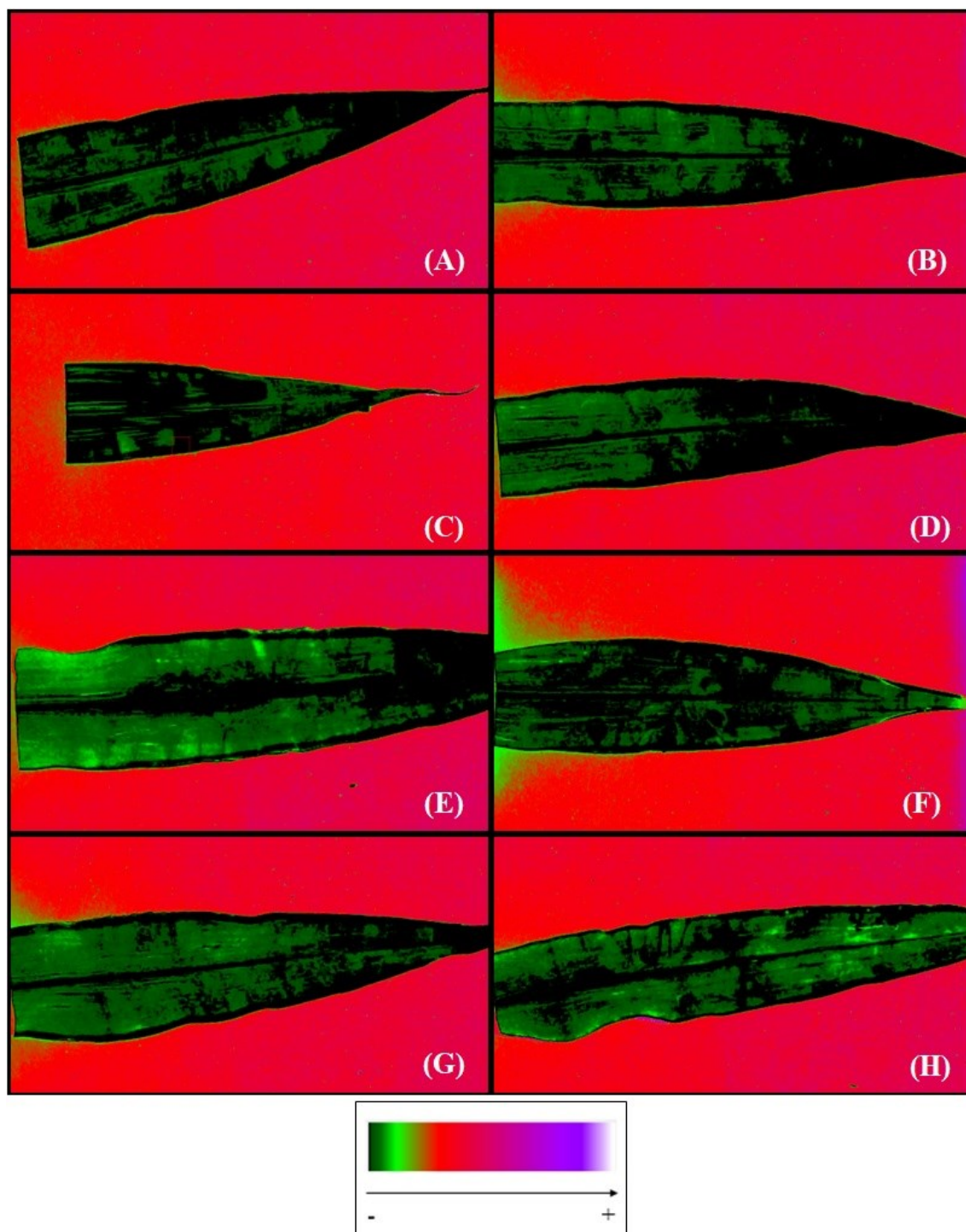


FIGURE 22 Multispectral images of sweet corn leaves collected by a MAPIR camera using the SPAD index: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

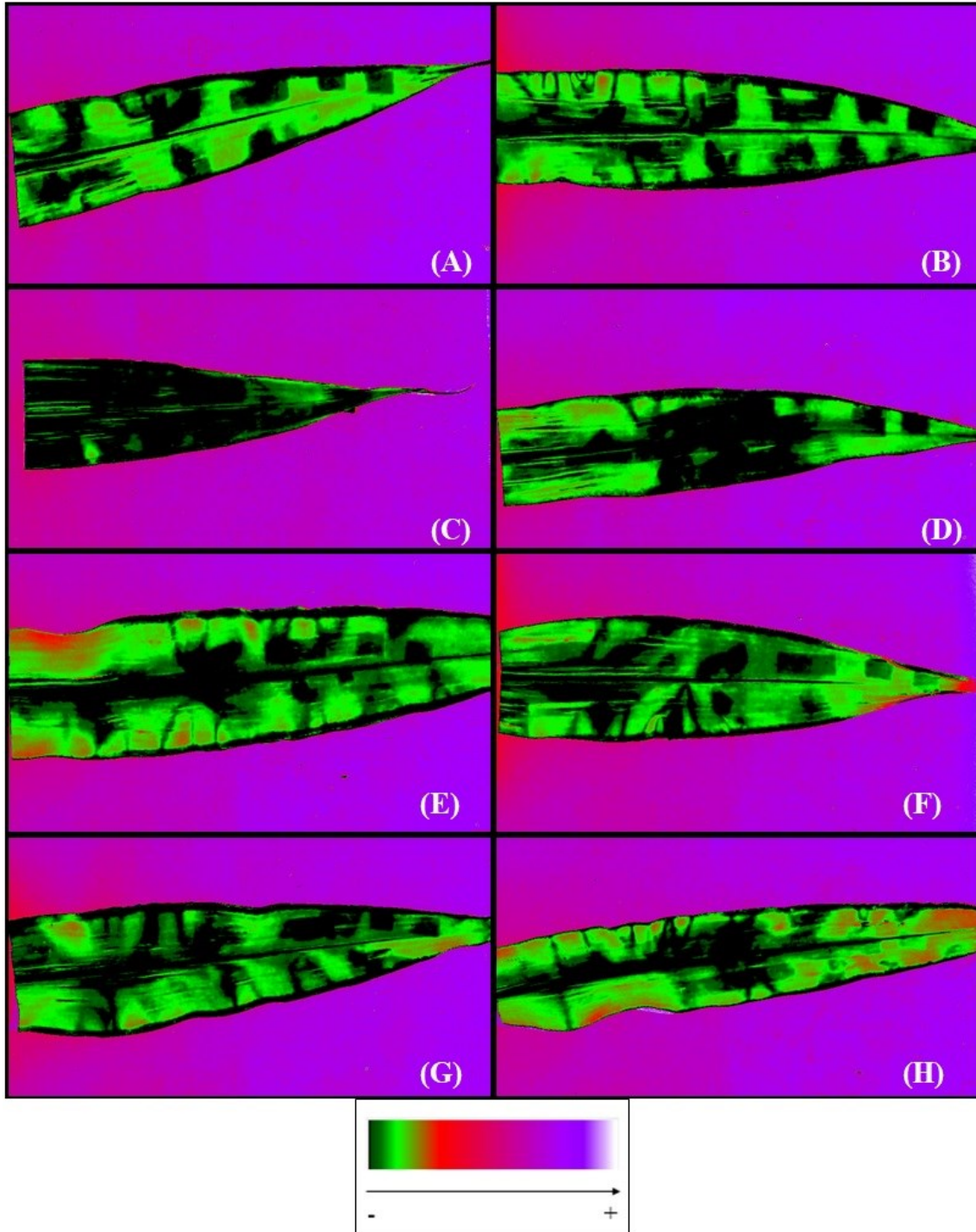


FIGURE 23 Multispectral images of sweet corn leaves collected by MAPIR camera from chlorophyll *a*: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

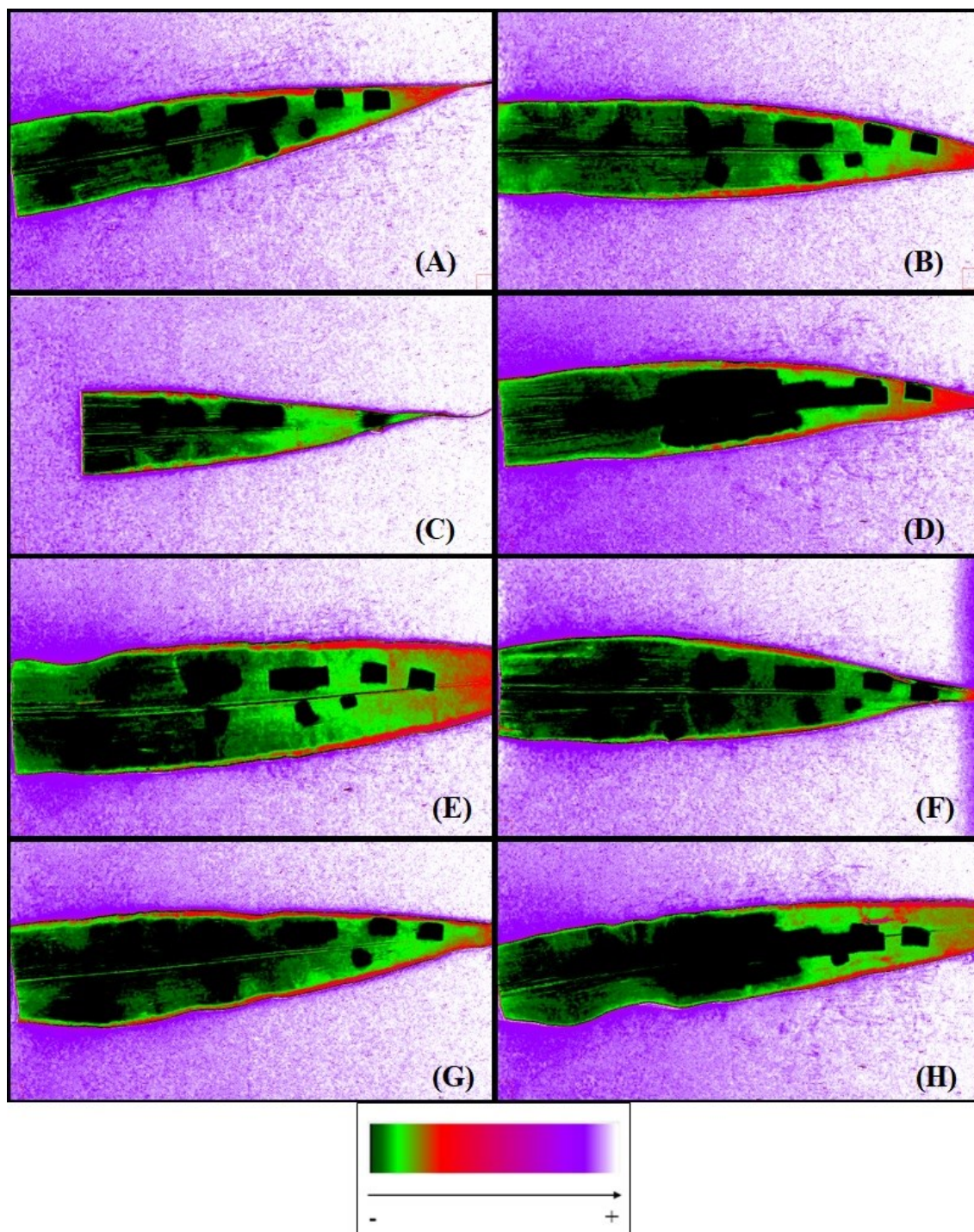


FIGURE 24 Multispectral images of sweet corn leaves collected by MAPIR camera from chlorophyll *b*: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

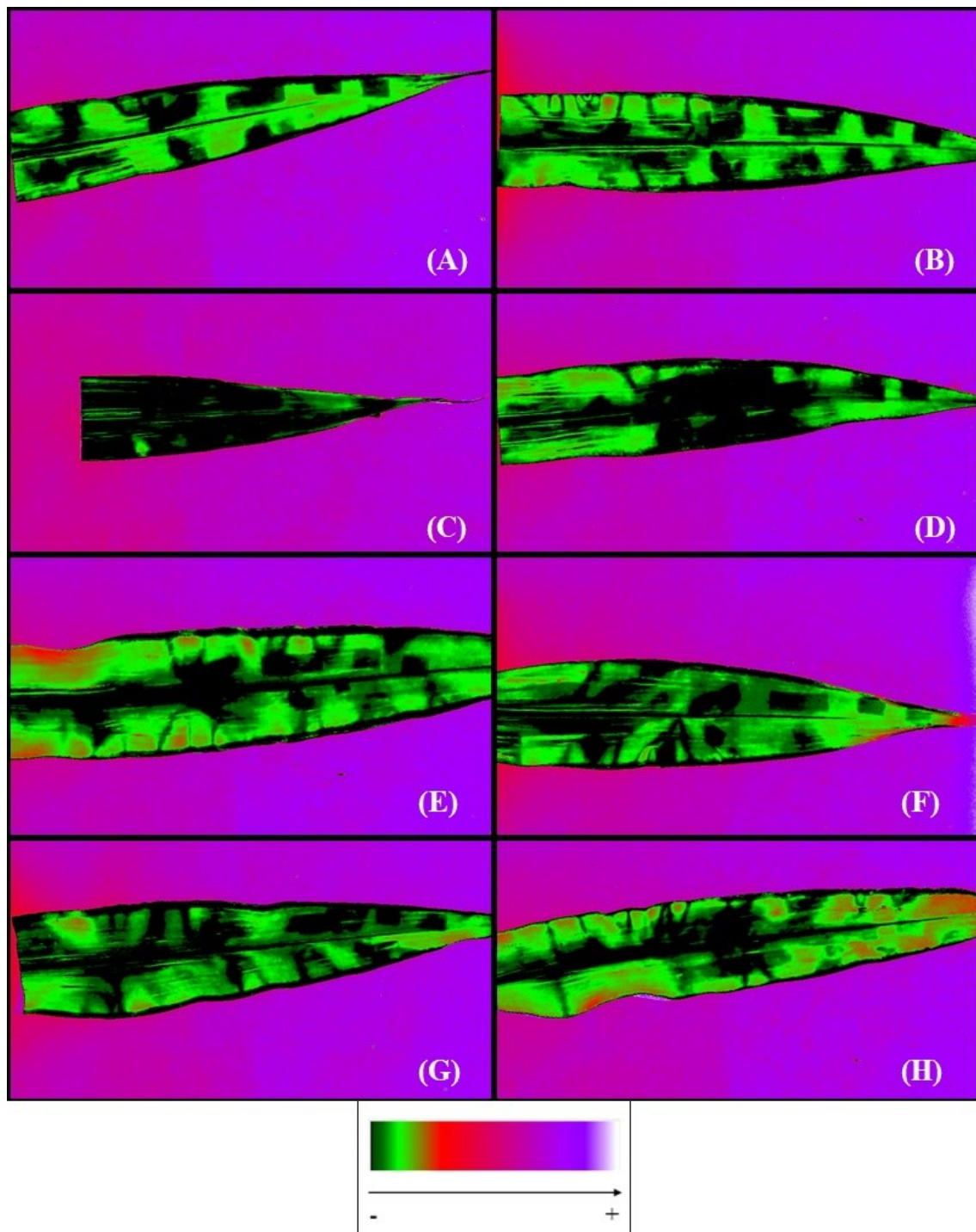


FIGURE 25 Multispectral images of sweet corn leaves collected by MAPIR camera from total chlorophyll: WT/-Si/-AMF (A); WT/-Si/+AMF (B); WT/+Si/-AMF (C); WT/+Si/+AMF (D); CT/-Si/-AMF (E); CT/-Si/+AMF (F); CT/+Si/-AMF (G); CT/+Si/+AMF (H). CT (control = 100% irrigation water replacement), WT (drought stress = 40% irrigation water replacement) standardizing to irrigate tension of -40KPa, -Si (absence of Si), +Si (presence Si), in +AMF ratio (presence of arbuscular mycorrhizal fungus) and -AMF (absence of the arbuscular mycorrhizal fungus) in sweet corn.

As shown in figures 22 to 25, the CT treatments had higher levels of the analyzed pigments than the WT treatments. This finding can be analyzed by the greater variation of colors within the scale used, whereas in the CT, there is a greater variation in the mapping of sweet corn leaves.

3.6.2 Supervised Classification

The leave-one-out cross-validation technique was used to classify sweet corn leaves in the presence or absence of Si and AMF through multispectral images. Methods based on using Neural Networks can be created to achieve these classifications. Confusion matrices were generated to understand the algorithm's performance in classifying leaves. The confusion matrix for the Si classification was 60% accurate, while the confusion matrix for the AMF classification was 56% accurate.

TABLE 4. Shows the Si confusion matrix.

Silicon Confusion Matrix

a	b	
12	4	A
9	7	B

TABLE 5. AMF confusion matrix.

Confusion matrix of arbuscular mycorrhizal fungi

a	b	
11	5	A
9	7	B

Where, a=Absence; b=Presence. Analyzing the matrix by the lines, it is correctly classified in the columns in which they coincide with the line. The different column represents what the algorithm got wrong in sorting.

In the Si confusion matrix in the absence classification (a), the algorithm was correct 12 and wrong 4 for the treatments, whereas in the presence classification (b), the algorithm was correct 7 and wrong 9 for the treatments tested. In the AMF confusion matrix in the absence classification (a), the algorithm had 11 correct answers and 5 errors

for the treatments, whereas in the presence classification (b), the algorithm had corrected 7 and 9 errors for the treatments tested. Absence ranking is more accurate because the tested factors generate subtle shades of color, making it more difficult for the algorithm to rank them.

4 DISCUSSION

The present research is part of a group that examines the effects that the association of AMF and Si has on plants under drought stress, especially in sweet corn. The presence of Si improved ear development, insertion height, and the SPAD index. The presence of AMF improved root volume, stem diameter, number of leaves, number of rows, and soluble solids. Plants under drought stress show reduced root and shoot growth and development.

In the present study, drought stress considerably affected plant development in terms of height, maturation, and yield. These results are significant regarding the effects of drought stress on root biomass. Lack of water inhibited root growth and development, and the plants were inoculated with a bacterial isolate. The effects of drought are reduced by reactions generated in the rhizosphere, which facilitate the metabolism of plants under stress (WANG et al., 2022).

The dry mass of the roots and shoots of sweet corn was significantly reduced by drought stress. In a similar study (MATOS et al., 2012), there was a reduction in the dry mass of corn plants under drought stress conditions; as the stress increased, the dry mass decreased. Furthermore, AMF inoculation resulted in greater root mass. This is due to the ability of fungal hyphae to aid in the absorption of water and nutrients, and to the functional group of the host in which AMF acts (IDRIS et al., 2022).

In another study (ISLAM et al., 2023), the combination of Si and AMF affected the dry mass of maize roots and shoots under saline stress conditions. Under these conditions, stress generates a reduction in water potential in the roots, decreases root water conductivity and cell permeability, which makes cell division and expansion difficult (ABDOLI; GHASSEMI-GOLEZANI; ALIZADEH-SALTEH, 2023), differing from the results of the study under discussion.

The drought affected the height of the sweet corn plants and the bean plants, where the differences were clear from the second day on. In tomato plants, both height and dry

mass were affected by a decrease in water replacement (50% replacement) (SANTIAGO et al., 2022). Metabolic responses to stress have been verified, in which metabolites are released in root exudates, altering the biochemistry of the rhizosphere and making plants better adapt to the imposed drought (ROSA et al., 2023).

The results showed a reduction in ear height in treatments where Si was applied, and when there was no fungal inoculation, plant height did not differ in the presence of Si. In another study of maize plants, the presence of Si caused a significant increase in plant height (PRABHA et al., 2022).

Research with *Bidens Pilosa* L. (SHEN et al., 2022) showed that AMF inoculation improved the accumulation of nutrients such as N and P. Sweet corn plants that received exogenous Si application resulted in greater P uptake, in addition to Furthermore, a positive regulation of the expression of P transporter genes, as well as a greater exudation of organic acids (NAIK et al., 2022).

Contrary to the results of the present study, in *Populus x canadensis*, the plants where the *Rhizophagus irregular* AMF species was inoculated showed a higher percentage of colonization, mainly in plants under drought stress (ZHANG et al., 2022).

In maize, it was verified that inoculation resulted in greater AMF colonization in the roots (SANTANA et al., 2023). In a survey of mesquite *Prosopis juliflora* (Sw) DC, the soil not inoculated with AMF showed a good percentage of inoculation, which is because the soil already has native AMF (AGUIAR et al., 2004), going against the results of significant colonization by native fungi.

The treatments that received silicon application had positive results in the number of rows of plants that were under drought stress, and in the number of grains per row. The presence of silicon was better at all levels of water replacement and AMF inoculation. In research on different sources of Si, the presence of the nutrients increased ear weight, grain weight and weight of 100 grains by 28.7%, 40.8%, and 26.5%, respectively (XIAO et al., 2023). It was also found that Si and AMF alone had significant effects on productivity and the number of ears per plant; however, the interaction between these factors was not significant (ISLAM et al., 2023).

The industrial yield is an important indicator of sweet corn cultivation. The results showed the effects of drought stress on industrial yield. In another study (NOEIM; SOLEYMANI, 2022), drought stress was found to affect the number of grains per corn, weight of 100 grains and grain yield. This is explained by the influence of drought on the

number and quality of pollen grains, affecting pollination and grain fertilization, in addition to influencing the redistribution of photoassimilates from the stem and grain storage, affecting weight and consequently productivity (GHASSEMI; FARZANEH; MOHARRAMNEJAD, 2020). Mung beans, tested with salicylic acid (HASAM; RASUL, 2022), and sweet corn, affected by P stress (KOAY; LUM, 2021), showed that lack of water plays an important role in productivity.

Photosynthetic measures affected AMF inoculation, in which the treatments under drought conditions had less variation in the control values than when there was no inoculation. Based on the results, the tests were conducted using the *Alhagi sparsifolia* Shap. (AILI et al., 2023), a type of legume, showed significant effects of AMF on photosynthetic indices (chlorophyll a, b and total chlorophyll). Furthermore, in another study with Ecolly (*Vitis vinifera* L.), evidence was obtained for the significant effect of drought stress on these same photosynthetic pigments, reducing their values (YE; WANG; LI, 2022).

Research carried out on tobacco subjected to drought stress and inoculated with AMF (BEGUM et al., 2021), rates of photosynthesis, intracellular CO₂ and transpiration decreased with drought; however, plants under stress conditions and inoculated with AMF showed an increase in photosynthetic rates. This relationship is due to stomatal and non-stomatal limitations, where increased drought stress induces stomatal closure, restricting CO₂ uptake. As stress increases, transpiration rates and stomatal conductance decrease due to closure. of the stomata. (WIJEWARDANA; HENRY; REDDY, 2017).

In research with cauliflower, three water replacement rates were tested: 40, 70 and 100 percent, and it was found that at the highest water replacement rate, the SPAD index was a lower value compared to the replacement rate of 40% (WENNECK, 2021).

The present study used multispectral images to estimate regression models that measured photosynthetic indices such as SPAD, chlorophyll *a*, chlorophyll *b*, and total chlorophyll through MAPIR bands and vegetation indices. In a similar study on corn, with a multispectral camera attached to a drone and using a multiple linear regression model, it was possible to estimate the SPAD using the NDVI and GNDVI vegetation indices (ZHAO et al., 2023). Using a portable object spectrometer, spectral data from *Sabina vulgaris* were measured, and it was possible to estimate the chlorophyll content through the NDVI and RVI indices using the red and near-infrared bands for their calculations (WANG et al., 2021b).

Because the images were taken with a MAPIR camera, where the number of bands was smaller, vegetation indices could not be used. The images were captured from the leaves; therefore, the agronomic parameters that could be estimated were restricted to information related to the leaves, thus limiting other parts of the plant, such as the ears. In the classification of Si and AMF using multispectral images, their absence was better classified than their presence. This can be explained by the subtle variations caused by the treatments in the plants and those captured by the sensors, which may go unnoticed by the human eye.

5 CONCLUSION

Drought stress affects root dry mass and volume, shoot dry mass, productivity, and nutrient uptake. The association between Si and AMF increased soluble solid content. The multispectral images obtained by the MAPIR camera allowed the estimation of the SPAD, chlorophyll *a*, *b* and total values by the GNDVI index and the green and near-infrared bands by a simple linear regression model, as well as the verification of the presence or absence of Si and AMF. In summary, AMF inoculation, Si application, and the combination of these factors mitigated the effects of drought stress on sweet corn plants, and multispectral images allowed the estimation of photosynthetic pigment levels.

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