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Integrated effects of silicon supply and water availability on the growth of contrasting common bean genotypes regarding water deficit tolerance: evaluation of root system, gas exchange, water use efficiency, and carbon isotope discrimination (δ ^{13}C ‰)

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ABSTRACT

This study investigated the role of silicon as a mitigator of water stress in two distinct genotypes of common bean (*Phaseolus vulgaris* L.) plants, BAT 477 and IAC carioca 80SH, known respectively as tolerant and susceptible to water deficit stress. Water stress is a significant constraint in agriculture, impacting crop productivity and yield. Silicon has been reported to enhance plant tolerance to various abiotic stresses, including water deficit. However, its effectiveness in non-accumulator plants, such as common bean, remains unclear. To clarify the role of silicon in non-accumulator plants, three studies were conducted. To achieve this goal, the objectives were to evaluate the uptake and kinetics parameters of silicon by common beans and to determine the impact of silicon on water use efficiency and physiological responses of the plants under water stress conditions. The methodology involved assessing silicon uptake through kinetic parameters such as I_{max}, K_m, and C_{min}, as well as analyzing the accumulation pattern of silicon during several phenological stages of the genotypes analyzed. The susceptible genotype had higher Si levels and Si accumulation in plants, most of this element was allocated in the leaves, also, showed an increase in dry plant mass. The tolerant genotype did not accumulate more Si compared to the susceptible genotype. Furthermore, water use efficiency was analyzed through physiological parameters related to gas exchange, relative water content, and carbon isotope discrimination (δ^{13}C ‰), serving as indicators of physiological responses to water stress. The findings of this research contributed to a better understanding of the role of silicon in mitigating water stress in common bean, a non-accumulator plant. However, this study also revealed the need for further extensive foundational research and field studies to assess and test the behavior of silicon in different scenarios, with a particular focus on non-accumulator plants, which have received less attention in previous studies.

Keywords: Photosynthetic parameters. Non-accumulator plants. Kinetics. Mitigating water stress. Plant tolerance.

RESUMO

Neste estudo o papel do silício, foi investigado, como um mitigador do estresse hídrico em dois genótipos distintos de plantas de feijão comum (*Phaseolus vulgaris* L.), BAT 477 e IAC carioca 80SH, conhecidos, respectivamente, como tolerante e suscetível ao estresse de déficit hídrico. As plantas estão sujeitas a períodos de estresse ambiental durante todo o seu ciclo de vida. Um desses fatores, a falta de água, pode influir negativamente no desenvolvimento e na produção da cultura, reduzindo área foliar e a taxa fotossintética. O silício é reconhecido por ser um elemento benéfico que auxilia na mitigação de vários estresses abióticos, como o déficit hídrico. No entanto, pouco se sabe sobre a sua eficácia em plantas não acumuladoras, como o feijoeiro. Para esclarecer o papel do silício em plantas não acumuladoras, foram conduzidos três estudos. Os objetivos foram avaliar a os parâmetros cinéticos de absorção de silício pelo feijoeiro, seu acúmulo e, ainda, determinar o impacto do silício na eficiência do uso da água e nas respostas fisiológicas das plantas sob condições de estresse hídrico. A metodologia envolveu a avaliação da absorção de silício por meio de parâmetros cinéticos como V_{max}, K_m e C_{min}, além da análise da marcha de absorção de silício durante determinadas fases fenológicas dos genótipos analisados. O genótipo suscetível apresentou níveis mais elevados e acúmulo de Si nas plantas, a maior parte desse elemento foi alocada nas folhas, incrementando a massa seca dessas plantas. O genótipo tolerante não acumulou mais Si em comparação com o genótipo suscetível. Além disso, a eficiência do uso da água foi analisada por meio de parâmetros fisiológicos relacionados à troca gasosa, conteúdo relativo de água e discriminação isotópica do carbono (δ^{13}C ‰), servindo como indicadores de respostas fisiológicas ao estresse hídrico. Os resultados desta pesquisa contribuíram para uma melhor compreensão do papel do silício na mitigação do estresse hídrico em feijão comum, uma planta não acumuladora. No entanto, este estudo também revelou a necessidade de mais pesquisas de base e estudos de campo para avaliar e testar o comportamento do silício em diferentes cenários, com um foco particular em plantas não acumuladoras, devido a lacuna de informação que ainda é latente em estudos sobre os benefícios do silício.

Palavras chave: Parâmetros fotossintéticos. Plantas não acumuladoras. Cinética. Mitigação do estresse hídrico. Plantas resilientes.

FIGURE LIST

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SUMMARY

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1   INTRODUCTION

1.1   Climate change and silicon as an ecologically green fertilizer

With the growing awareness that the world is experiencing climate change, it has become imperative to transform our approach to food cultivation, industrial development, and the creation of smarter, energy-efficient cities. (IPCC, 2023). Researchers and scientists have been playing their roles as protagonists in the search for alternatives that are grounded in the three pillars of sustainability: economic, social, and environmental (Wang et al., 2021). Conventional agriculture is considered one of the main vectors in greenhouse gas (GHG) emissions, accounting for approximately one-third of global emissions (Crippa et al., 2021; Menegat et al., 2022), and therefore, measures need to be taken (Stetter & Sauer, 2022; IPCC, 2023). According to the Food and Agriculture Organization of the United Nations (FAO, 2017, 2018), climate change is among the greatest challenges that agriculture currently faces in terms of mitigation and adaptation, requiring innovative approaches to mitigate its adverse effects.

As presented, climate change represents a serious problem at a global level, affecting all the population worldwide, including plants and animals. The concern for negative effects, as well as those that are still unknown, has led to a global race in search of alternatives to mitigate these effects and change the course of history. Legislators have been developing regulations aiming for the complete elimination of greenhouse gas emissions. Companies are developing methodologies and new alternatives to reduce carbon footprints, among other negative factors. Additionally, agriculture has been striving to develop new good agricultural practices that improve production in harmony with the increase of the practice of regenerative agriculture in a sustainable and circular economy.

The impact of climate change on the concentration of CO_2, which is currently above 400 μmol CO_2 mol^{-1} air, is predicted to double by the end of this century. Additionally, the average temperature is estimated to increase by 0.1–0.4 ℃ per decade (Vaughan et. al., 2018; Boris et. al., 2021). Climate change also has other effects, including uneven precipitation distribution, leading to prolonged drought periods followed by heavy rainfall, as well as exposing plants to temperature stress and increased ultraviolet (UV) radiation. The uneven distribution of water and the rising average temperature could facilitate the spread of various pests and plant diseases.

1.2   Silicon as an alternative source

Silicon (Si) is the second most abundant element in the Earth's crust and has shown promising potential as an alternative resource to mitigate the adverse effects of climate change on crop production. This element is predominantly found in the soil in the form of silicate or aluminum silicates. Despite its abundance, a significant portion of Si is not easily accessible to plants. Consequently, the application of Si fertilizers can enhance plant growth and improve their resistance to various stresses (Hassan et. al., 2018; Krishan et. al., 2020). However, the effectiveness of silicon fertilization may vary depending on soil and plant-specific factors, necessitating further research to identify the optimal conditions for Si application.

In the soil solution, Si exists as silicic acid ($Si[OH]_4$) at concentrations ranging from 0.1 to 2.0 mM (pH<9), it is part of minerals such as clay, feldspar, granite, quartz, and sand (Epstein, 1994). This element can be easily absorbed by plant roots in the form of monomeric or monosilicic acid (H_4SiO_4) through diffusion, mass flow, or membrane transporters, after absorption, it is transported via xylem. Due to negligible or very low redistribution, i.e. redistribution and limited mobility, periodic application of Si is needed. In leaves, it is deposited as silica (SiO_2H_2O) beneath the cuticle, reducing plant transpiration, Si concentration in plants varies significantly, typically accounting for 0.1% to 10.0% of dry mass (Liang et al., 2007), the amount is related to the plant's capacity to accumulate Si. Surprisingly, Si content in plants is equivalent to, or even higher than, major nutrients such as nitrogen (N), phosphorus (P), and potassium (K) obtained from fertilizers (Epstein and Bloom, 2005).

Although Si is not considered a nutrient for plants, many authors classify it as beneficial due to its potential role as a physical barrier, in metabolic, physiological parameters, enhancing plant tolerance under different abiotic and biotic stresses (Meena et al., 2014; Hassan et. al., 2018; Zargar et. al., 2019). Beneficial elements are elements that stimulate growth but are not essential or are only essential for certain plant species or under specific conditions. This definition applies to sodium (Na), silicon (Si), selenium (Se), and cobalt (Co) (Pilon-Smits et. al., 2009). Nevertheless, it is anticipated that Si's essentiality for higher plants will eventually be acknowledged, aligning with the definition of essentiality proposed by Epstein and Bloom (2005). Additionally, Si is noncorrosive and environmentally friendly, making Si fertilizers a high-quality choice for ecologically sustainable agriculture.

1.3   Silicon as a mitigation element to drought

Water stress is one of the major environmental constraints limiting plant growth and agricultural productivity. Drought induces a range of physiological and biochemical processes in plants that can reduce crop yields up to 70%, resulting in serious economic losses.

The beneficial effect of Si on plant water status has been extensively studied in various plant species subjected to drought stress, the improvements by Si application are wide. For instance: rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L) and sunflower (*Helianthus annuus* L) (Bokor et. alt., 2021). In general, Si application was found to increase plant growth, yield, water use efficiency and photosynthetic rate.

1.4   Common bean and water stress

The water demand of the common bean plant is influenced by several factors such as sowing time and location, variety, edaphoclimatic conditions, and developmental stage (Moreira et al., 1996). The amount of water required by the crop to achieve maximum productivity varies between 300 and 500 mm (Doorenbos and Kassam, 1979). Due to its low capacity for recovery after water deficiency and underdeveloped root system, the common bean plant is considered sensitive to water stress (Guimarães, 1996).

The lack of water can negatively influence the development and production of the crop, reducing leaf area (Hernández et al., 2004) and photosynthetic rate (Chaves et al., 2009). Water deficit can lead to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system (Monakhova and Chernyad'ev, 2002), thus impairing biomass development and production.

Increasing plant tolerance to abiotic stresses and improving the quality of agricultural products while protecting the environment are challenges of modern agriculture (Lavres et al., 2018). Achieving sustainability in these factors requires decision-making and the use of technologies, in the form of best agricultural practices, which are developed based on scientific knowledge.

The phase of the common bean most vulnerable to water deficiency extends from the beginning to the full bloom. However, the intensity of the damage caused by water deficiency will depend on the duration, intensity, frequency, and timing of occurrence. The effects of drought according to the phenological stage of the common bean (Oliveira et al., 2018):

a.   V0: The occurrence of water deficiency before germination will cause the seeds to rot instead of germinating. The respiration of the cells consumes the accumulated reserves in the seed, making them susceptible to soil-borne diseases and preventing germination.

b.   V1: If it occurs at this stage, the seedlings (recently germinated plants) may fail to emerge from the soil, thus reducing the plant population.

c.   V2 toV4: Drought at these stages indirectly affects grain productivity due to the reduction in leaf size and number, which are responsible for plant and root growth. A plant with a well-developed root system will have greater survival capacity during dry periods and a higher ability to absorb nutrients from the soil.

d.   R5 to R6: If drought occurs at these stages, it will cause flower abortion and drop, reducing the number of pods per plant and compromising grain productivity.

e.   R7 to R8: If the period of water deficiency occurs at these stages, it will lead to the shedding of newly formed pods and adversely affect seed formation, resulting in a reduction in mass and consequently grain productivity.

1.5   Indicators of physiological responses to water stress

The use of Si to alleviate biotic and abiotic stresses is already well established (Chen et al., 1969; Ma et al., 2016; Debona et al., 2017; Frew et al., 2018; Bokor et al., 2021) and has been garnering interest among researchers to conduct further studies aiming to elucidate the role of this beneficial element. Due to its associated benefits and various indirect effects, it stands out for water stress (Gong and Chen, 2012) or mineral toxicity (Kim et al., 2016). It also strengthens plant defenses against diseases (Polanco et al., 2014) and pest attacks (Epstein and Bloom, 2006), enhances the photosynthetic rate, and influences crop growth (Epstein and Bloom, 2006; Kim et al., 2012; Mateos-Naranjo et al., 2015). Si is known to be

the only element that provides resistance to numerous stresses in plants without causing damage when accumulated in excess (Ma et al., 2002).

Although the mechanisms underlying the beneficial effects of Si are still largely unknown, the application of external Si may optimize the absorption, transformation, and transfer of the light energy by under drought stress. In other words, the promotion of photosynthetic rate and increased activity of photosynthetic enzymes (Shen et al., 2010; Hosseini et al., 2017). Additionally, silicon is believed to be important in reducing evaporation and controlling stomatal conductance when it is taken up and deposited in the leaf cuticle, leading to a decrease in transpiration (Murata and Takahashi, 1991; Gao et al., 2006; Chen et al., 2018). Consequently, these mechanisms contribute to improving drought tolerance in plants.

The carbon found in atmospheric CO_2 comprises approximately 1.1149% of the heavier isotope ^{13}C and 98.9% of the isotope ^{12}C. During the process of carbon fixation, plants demonstrate a preference for the lighter isotope, resulting in a more negative carbon isotope composition in plant material compared to atmospheric CO_2. For instance, in C3 species, the carbon isotope composition of plant material is primarily influenced by discrimination during carboxylation, a crucial step carried out by the rate-limiting enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase). These processes are influenced by various factors, including stomatal regulation of leaf gas exchange and the ratio of substomatal (C_i) to ambient (C_a) CO_2 concentrations (Farquhar et al., 1989).

Additionally, discrimination occurs during the diffusion of CO_2 from the atmosphere to the chloroplast (Farquhar et al., 1982). The discrimination factor associated with Rubisco is approximately 29–30 per mil (‰), while discrimination during CO_2 diffusion in the air and the liquid phase is around 4.4 and 1.8‰, respectively. Farquhar et al. (1982) defined carbon isotope discrimination as $\Delta = Rair/Rp - 1$, where Rair and Rp represent the $^{13}C/^{12}C$ ratio in the atmosphere and the photosynthetic product, respectively.

Changes in $\delta^{13}C$ can arise from alterations in carboxylation efficiency, stomatal conductance (g_s), or both. The isotopic composition of carbon ($\delta^{13}C$) is based on the isotopic ratio of $^{13}C/^{12}C$, which is lower in plant tissue than in the atmosphere. This is a result of isotopic fractionation during photosynthesis and the isotopic composition of carbon in biomass, primarily determined by water availability and stomatal limitation (Bowling, 2002).

Therefore, the determination of carbon generally refers to the measurement of the amount of carbon in a sample. On the other hand, carbon isotope composition ($\delta^{13}C$) refers to the ratio of the stable isotopes of carbon, specifically the ratio of ^{13}C to ^{12}C, in a sample

relative to a standard. This measurement provides information on the source of the carbon and the metabolic pathways that the carbon has gone through in the organism. For example, the $\delta^{13}C$ value of a plant can provide information about the photosynthetic pathway it uses, which can in turn indicate its water-use efficiency and response to environmental conditions.

In this context, with the aim of elucidating the role of Si in non-accumulator plants and under water deficit, the present study addressed these multidisciplinary questions using tools such as Si accumulation and kinetics parameters, photosynthetic parameter, water use efficiency and isotopic carbon composition. To achieve this, the common bean (*Phaseolus vulgaris* L.) crop was selected due to its short production cycle, making it a model plant.

1.6   Hypothesis

This study raised the following hypothesis:

i)   Water deficit susceptible bean plants benefit more from Si supplementation under water stress conditions compared to tolerant material.

ii)   Water deficit tolerant material is more efficient in terms of intrinsic water use efficiency, due to higher Si absorption (kinetic attributes of absorption), greater carbon isotope discrimination (i.e., more negative δ 13C ‰ values), and higher Si accumulation in tissues.

1.7   Objectives

The general objective of this thesis was to assess new methodologies for nutritional management to reduce abiotic stresses, such as water stress. Thus, three experiments were designed to test the above-mentioned hypothesis.

1. Kinect analysis for silicon: Silicon absorption in two materials contrasting in terms of low soil water availability.
2. Silicon content and accumulation: Quantify the total amounts of silicon absorbed in these materials.
3. Water and silicon use efficiency: Determine the water and Si use efficiencies in bean genotypes exposed to water deficit.

The specific objectives of this thesis were:

a) Silicon absorption: Evaluate the kinetic parameters of silicon absorption (I_{max}, K_m and C_{min}).

b) Phase of highest accumulation: silicon uptake pattern in relation to phenological development stages in both genotypes.

c) Water use efficiency: Determine the photosynthetic gas exchange parameters and the efficiency of water use and relative water content.

d) Isotopic discrimination: Carbon isotopic discrimination ($\delta^{13}C$ ‰) in leaves.

1.8 Structure of the thesis

This thesis rises an introductory text followed by two chapters. First and second chapter comprises two manuscripts that will be submitted to the Journal of Science and Plant Nutrition, collection calling for papers: Silicon in Sustainable Agriculture. The first chapter will be submitted, with the title "Silicon uptake and kinetic parameters in two common bean genotypes: non-accumulator plants" and the second chapter with the title "Silicon's role in enhancing common bean (*Phaseolus vulgaris* L.) plant resilience: photosynthetic parameters and carbon isotope discrimination ($\delta^{13}C$ ‰)". The original texts were adapted to comply with institutional format requirements.

2   SILICON UPTAKE AND KINECT PARAMETERS IN TWO COMMON BEAN GENOTYPES: NON-ACCUMULATOR PLANTS

Abstract

Global warming and climate change are expected to increase with a significant impact on agricultural crops yields, such as common beans. Common bean is a species of recognized commercial and social value in Brazil, therefore, researchers and farmers should seek different strategies, such as varietal, fertilizer, water, and soil crop management in order to face these climatic change struggles. Silicon (Si) is a beneficial element for plants whose function is related to plant tolerance increase to abiotic and biotic stresses, such as water stress - osmotic and drought stresses. Drought susceptible genotype showed higher Si uptake than the water deficit tolerant genotype, whereas the tolerant genotype showed more uniform Si-distribution among the plant. Si content of the susceptible genotype were found, mainly, in the leaves. By comparing genotypes at the 2.0 mmol L^{-1} Si concentration, the I_{max} parameter was 37% higher at the tolerant genotype when compared to the susceptible genotype (kinetics parameters).

Keywords: beneficial element, alternative fertilizer, plant tolerance, abiotic stress, silicon.

2.1   Introduction

The global warming rates and climate changes are expected to increase within the coming years (Scheelbeek et al., 2018; IPCC, 2023). In the Center-South of Brazil, one of the most important common bean production regions, the annual precipitation is expected to decrease significantly by the year of 2050 - rainfall reduction mainly during the summer (Lobell and Gourdji, 2012; Scheelbeek et al., 2018; IPCC, 2023). Since common bean owns a high economic and social value in Brazil, management strategies - the use of drought-tolerant cultivars, the adoption of adequate fertilizer practices and improved water use efficiency (WUE) - must be adopted to circumvent weather events and enable the production of this crop in an economically viable way (Heinemann et al., 2017; Massignam et al., 2017).

To increase the plants tolerance to abiotic and biotic stresses and, simultaneously, to improve grain quality by the implementation of Good Agricultural Practices (GAP) is the

most challenging goal of modern agriculture (Lavres et al., 2018). Embrace technology is part of the path to construct sustainability and, also, it could be developed through scientific knowledge. In this context, the use of beneficial elements in agriculture, such as silicon (Si), may be a suitable alternative to mitigate abiotic stresses in cultivated plants of economic interest (Malavolta, 2006; Epstein and Bloom, 2006).

Silicon is considered a beneficial element for plant mineral nutrition (Stout and Arnon, 1939), being related to the increase of plants tolerance to abiotic and biotic disturbances, such as chewing insects attack and drought (Esptein, 1999; Gong and Chen, 2012). Silicon is the second most abundant element in the earth's crust, followed by oxygen (Epstein, 2009). However, tropical and subtropical soils tend to present around seven-fold less exchangeable Si in the soil solution when compared to the ones located in the Temperate Zone (Otsubo and Coutinho, 2001).

In soil solution, Si is found mainly as monosilicic acid (H_4SiO_4). After being absorbed by the plant roots, it is transported and deposited intra and/or extracellularly as insoluble silica ($SiO_2.nH_2O$) (Ma et al., 2011). In many species, a dense silica layer can be placed under the cuticle, which contributes to decrease water loss by reducing transpiration rate (Nwugo and Huerta, 2008). Additionally, this silica layer serves as a mechanical barrier to pathogen penetration and herbivore attack (Frew et al., 2016; Jeer et al., 2017).

Silicon is recognized as the only element which confers to plants the tolerance to several kinds of stresses. Beyond that, this element does not cause damage when accumulated in excess within the plant tissue (Ma et al., 2002). Some authors draw attention to the fact that silicon, a key element, should diminish the negative effects resulted of water deficit, since it can improve the activity of the antioxidant system (Ashraf, 2009; Ma et al., 2016) and increase the photosynthetic rate (Rahman et al., 2015; Kang et al., 2016).

It is well established that Si should be used as a mitigator element of abiotic and biotic stresses (Debona et al., 2017). Nevertheless, there is no information about Si effects on improving the WUE in common beans, which is a silicon non-accumulating plant. Therefore, there is still a lack of information regarding Si absorption mechanisms in common bean cultivars which are tolerant and resistant to water deficit (Ma et al., 2002; Richmond and Sussman, 2003).

In this context, our work aims to elucidate Si absorption and its kinetics by drought tolerant and susceptible common bean cultivars, to fill the lack of information on the use of Si in non-accumulating plants.

2.2 Materials and Methods

In order to obtain Si-uptake in common beans (*Phaseolus vulgaris* L.), two experiments were conducted in a greenhouse located at the Center for Nuclear Energy in Agriculture (CENA/USP), in Piracicaba/SP. Both experiments were conducted in the hydroponic system: (i) silicon absorption march and (ii) silicon absorption kinetics. This system contains an air pump to maintain the roots well-aerated. Cultivation assay: a couple of distinct genotypes of common beans were set to germinate in vermiculite until the primary leaves were completely expanded (V2 - Fernández et al., 1986). These seeds are distinguished by their behavior when exposed in diverse watering regimes. Selected genotypes were BAT 477 and IAC-carioca 80 SH, tolerant and susceptible to water deficiency, respectively. Secondly, the seedlings were transferred to plastic pots to grow in nutrient solution, containing 12 N; 1.0 P; 4.0 K; 4.75 Ca; 1.25 Mg; 1.25 S in mmol L^{-1}, and 46.27 B; 0.31 Cu; 17.92 Fe; 9.23 Mn; 0.54 Mo; 3.07 Zn; in µmol L^{-1} (adapted from Hoagland and Arnon, 1950). The Si-source selected was sodium silicate (Na_2SiO_3), as well as with the other elements, the nutrient solution was renewed every seven days. The nutrient solution pH and electrical conductivity (EC) were monitored weekly, pH was maintained between 5.5 - 6.0. The ambient temperature was maintained between 28° and 30° C since the greenhouse was equipped with a refrigeration system, the relative humidity of the air was 60 – 65 %.

2.2.1 Silicon absorption march

The seedlings (V2) were transferred to plastic pots containing 5 L of the nutrient solution. In order to acclimatize the plants to their new growth environment, the nutrient solution was diluted at 25% for the first week and then adjusted the strengths to achieve final concentration (100%). The Si absorption march was obtained through the plant-samples collected during the plant's life cycle. Silicon content in samples was determined by the method proposed by Elliot and Snyder (1991), adapted by Carneiro et al. (2007). Firstly, in order to draw the Si-uptake according to the phenological phases, five plant-samples were harvest throughout the complete cycle at: third open trifoliolate leaf (V4); pre-flowering (R5); formation of pods (R7); filling of pods (R8) and maturation (R9) (Fernández et al., 1986). Subsequently, biometric analyses were conducted, and total leaf was measured using the foliar area integrator (LI-3100C; Inc. Lincoln, USA).

2.2.2 Micropobe X-ray fluorescence spectroscopy (μ - XRF)

The first completely expanded trifoliolate leaf of each genotype was collected at the maturation phase (R9). Then the leaves were dried in a laboratory oven at 60ºC as exsiccated, until they reached constant mass. After dried, X-ray fluorescence line scans were performed at the bottom, middle, and tip of the central leaflet of each trifoliolate leaf. These three lines were evaluated to investigate Si accumulation tendencies along the leaf length.

The dried leaves were attached to Kapton® tapes fixed at the top of acrylic sample holders, which were built to load the samples into the Orbis PC X-ray spectrometer (Orbis PC, Edax). The X-rays, supplied by a rhodium anode, were shaped by a collimator to provide a *ca.* 2 mm wide beam. Operation conditions of tension and current were 30 kV and 400 μA, respectively. The measurements were performed from *ca.* 20 mm length lines composed of 16 scanned spots, with a dwell time of 30 seconds. The x-ray fluorescence photons emitted by the leaf tissue were detected by a 30 mm^2 silicon drift detector. The Si-Kα net intensity was obtained with a dead time smaller than 7%.

2.2.3 Silicon absorption kinetics

This analysis was carried out with plants at the stage of the third open trifoliolate leaf (V4). The assay followed the method proposed by Claassen and Barber (1974), which was described as a procedure to quantify the depletion of the element presented in the nutrient solution. The quantification is analyzed by the uptake of the element - in this case silicon-uptake – by the roots system over time (for 72 hours).

Firstly, the seedlings (V2) grown in vermiculite as described before, were transferred to plastic pots containing 0.4 L of nutrient solution with 25% of its ionic strength. The nutrient solution was complete, and the samples contained distinct Si-concentrations (Na$_2$SiO$_3$), during the subsequent days the ionic strength was adjusted until it achieves 100% (Reis and Lavres Junior, 2011; Rabêlo et al., 2017). To obtain a better response of Si-absorption kinetics by common beans, different concentrations were tested, (0.1; 0.5; 1.0; 1.5 e 2.0 mmol L^{-1}). The plants were arranged in a randomized block design, with four replications in a factorial arrangement 2 (genotypes) × 5 (Si-concentrations), totaling 40 experimental units.

Plants were grown until the third trifoliolate leaf was open (V4) thereafter, samples were kept 24 hours with Si-deprivation. The aim was to increase the absorption capacity of

this ion before the kinetics analysis began "low salt high carbohydrate" (Epstein and Hagen, 1952). After the deprivation period, treatments started, and Si-concentrations were established.

2.2.4 Kinetics parameters

The analysis is determined by three parameters: maximum influx (I_{max}), Michaelis-Menten constant (K_m) and minimum Si concentration in the solution, i.e. when the plant capability of absorption ceases (C_{min}). Thereby, kinetic analysis started when the plants were already 30 minutes in contact with the renewed nutrient solution. The procedure established was to collect 10 mL of the exhaust solution every 15 minutes during the first two hours. The total volume of nutrient solution was kept constant by adding deionized water. Secondly, the exhaust solution was collected every 30 minutes until the fourth hour since the assay began, and then, 60 minutes in the subsequent six hours. Finally, every 12 hours until the Si concentration remained constant - 72 hours (Reis and Lavres Junior, 2011).

2.2.5 Chemical analysis

Finally, dry mass of collected parts was carried out, after drying in an oven with forced-air circulation at 60 °C until constant mass. Chemical analysis was conducted after wet-digestion of leaf tissue for Kjeldahl-N, and for the other elements the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used.

After all, Si content in the samples was determined by the method proposed by Elliot and Snyder (1991), adapted by Carneiro et al. (2007). The plants were harvested and dried in an oven with forced-air circulation at 60 °C until constant mass. For sample preparation, 0.1 g of dry mass was weighted and placed inside of plastic tubes. To proceed, 2 ml of hydrogen peroxide (H_2O_2 – 30% v/v) and 3 ml of sodium hydroxide (NaOH – 25 mol L^{-1}) were added to start the wet-digestion. The samples were placed inside a hot bath ($\pm$ 90 C°) for 1 h and then transferred to the autoclave digester for 1 h at 123 °C and 0.15 MPa. Thereafter, to achieve the total volume of 20 ml, water was added to the samples, and subsequently 1 ml of the resultant solution was transferred to a new plastic tube. Furthermore, the final volume was water adjusted to 20 ml, and finally added 1 ml of chloridric acid (1:1 v/v) and 2 ml of molybdic acid ammonium (5%). After all, the yellowish color was formed, and the results were read at the absorbance of 410 nm.

The apparent recovery efficiency (RE) was estimated to identify if there was a difference between genotypes in using the nutrients applied during the culture cycle. This nutrient use efficiency was defined by the difference in nutrient uptake in all parts of the plants to the quantity of nutrient applied.

$$RE\ (\%) = \frac{nutrient\ accumulation}{nutrient\ applied} \qquad (1)$$

2.2.6 Statistical Analysis

Data were submitted to analysis of variance (ANOVA) at 5% probability. When ANOVA presented significant results, means were compared using Tukey's test. The analyzes were made using Sisvar statistical software (Ferreira, 2011). The results were expressed as mean ± standard error of the mean (SEM).

2.3   Results and Discussion

2.3.1   Silicon absorption march

The tolerant genotype (Figure 2.1b) showed more uniform Si-distribution along the plant, conversely the Si content from the susceptible genotype (Figure 2.1a) were found mainly in the leaves. Leaves from the susceptible genotype presented 77% higher Si content than the tolerant genotype, during the formation of the pods (R7 = 51 days after planting). The Si absorption march of the susceptible genotype (IAC carioca 80SH) was 27% higher when compared to the plants from the tolerant genotype (BAT 477) to water deficit (Figure 1).

The Si content in leaves, at the susceptible genotype, was 114% greater than in the roots, which was the next location where Si was placed. Regarding the uniform distribution in the tolerant genotype, this cultivar presented 58% higher content of Si in leaves than in the roots (Figure 2.1).
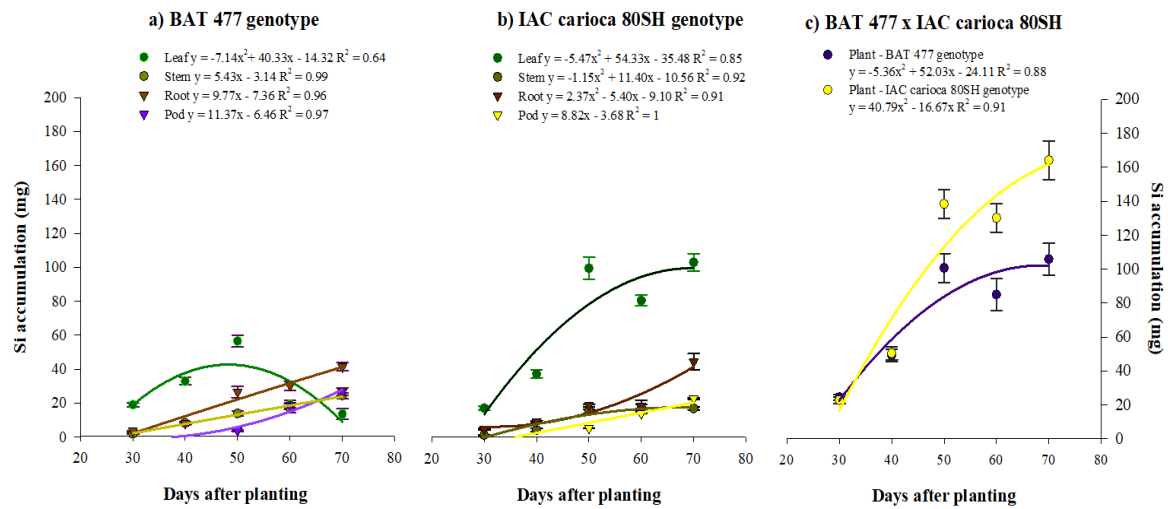
Figure 2.1. Silicon absorption march of common bean plants. a) Si accumulation (mg) in leaf, stem, root and pod at common bean cv. BAT 477 (tolerant) genotype as function of the phenological stage.
b) Si accumulation (mg) in leaf, stem, root and pod at common bean cv. IAC carioca 80SH (susceptible) genotype in function of the phenological stage. c) Si accumulation (mg) in plants. Bars represent the standard error of the mean (n = 4).

The plant dry matter (Figure 2.2) resulting from the susceptible genotype was 53% higher when compared to the tolerant genotype. The same pattern between the genotypes was observed at the leaf dry matter result, however the susceptible genotype accumulated more. As expected, the leaf dry matter decreases near the end of the culture cycle in both genotypes. Additionally, the total number of trifoliolate leaves and the foliar area index (Figure 2.3) corroborated the former results, which were 22% and 33% greater, for the susceptible genotype, 50 days after planting.

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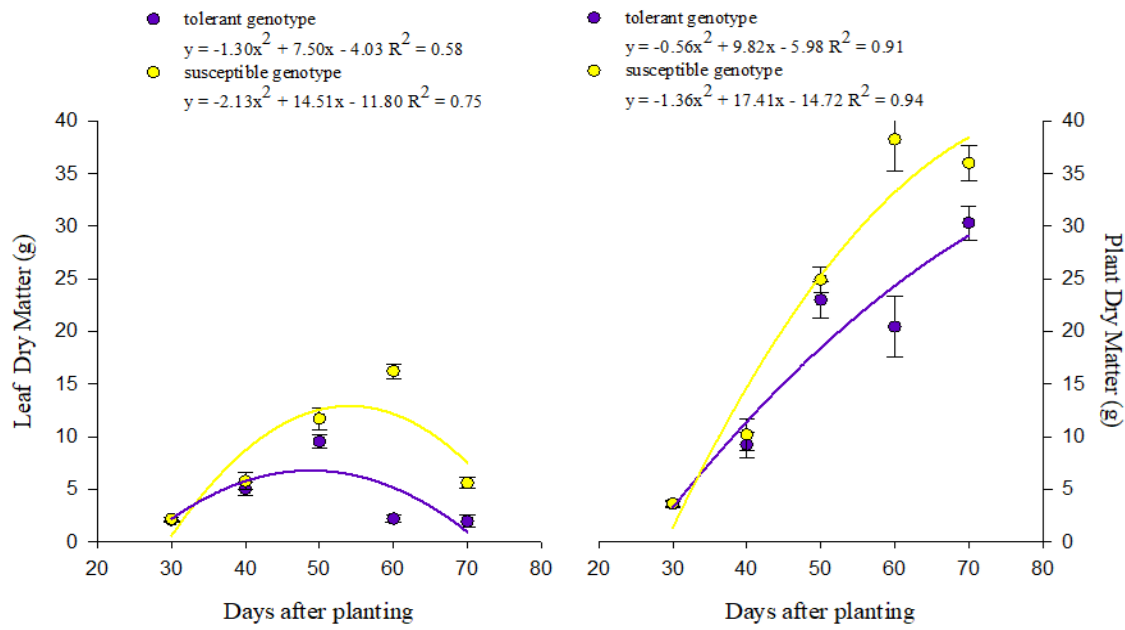
Figure 2.2. Leaf dry matter and plant dry matter by distinct common bean genotypes. Bars represent the standard error of the mean (n = 4). Different letters are different statistically, by the test Tukey at 5%.
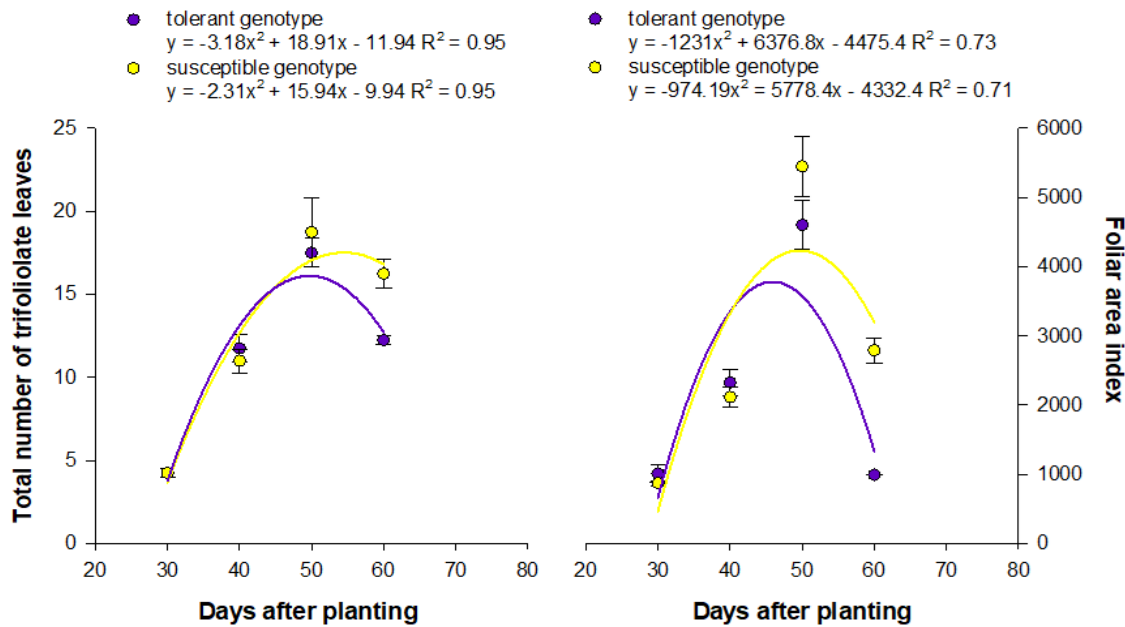
Figure 2.3. Total number of trifoliolate leaves and foliar area index by distinct common bean genotypes. Bars represent the standard error of the mean (n = 4). Different letters are different statistically, by the test Tukey at 5%.

2.3.2 Chemical analysis: absorption march

The Pearson's correlation among the variables performed for the absorption march of the distinct genotypes were highlighted (Figure 2.4a tolerant genotype and Figure 2.4b susceptible genotype). As can be observed, the correlation between the potassium (K) concentration and the Si leaf concentration revealed that there is a close and positive relationship between these elements (Figure 2.4a). Magnesium (Mg), sulfur (S) and boron (B) concentration and the Si root and pod concentration such as, Mg and zinc (Zn) concentration and the Si root concentration revealed that there is a close and positive relationship between these elements (Figure 2.4a). Turning to the correlation between phosphorus (P) and Si pod concentration showed that there is a strong and negative relationship (Figure 2.4a).

As for the correlation between the calcium (Ca), manganese (Mn) and Zn and the Si root concentration revealed that there is a strong and positive relationship. Regarding the strong and negative correlation between P and Si pod concentration, and potassium (K) and Si stem concentration, these results revealed that there is a close and antagonist relationship between these elements (Figure 2.4b).
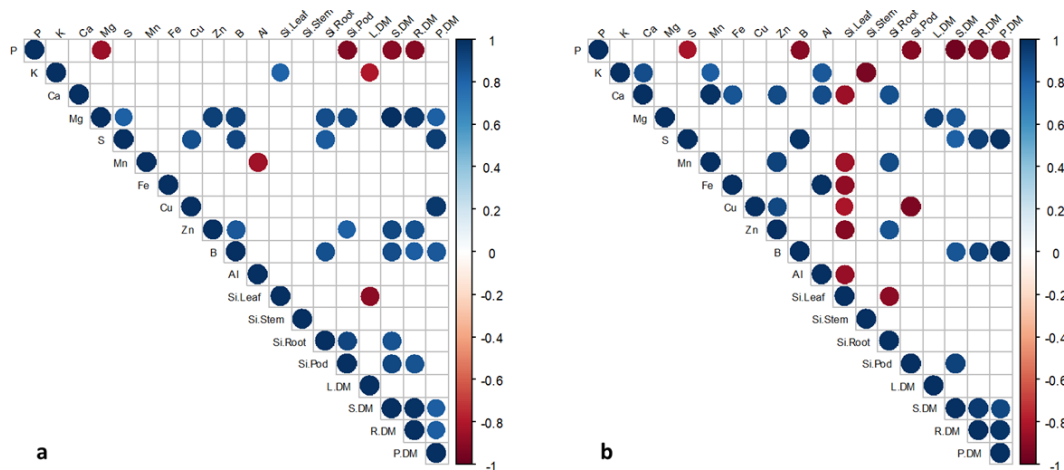
Figure 2.4. Pearson's correlation among macro and micronutrients (P, K, Ca, Mg, S, Mn, Fe, Cu, Zn, B and Al), Si leaf concentration (Si.Leaf), Si stem concentration (Si.Stem), Si root concentration (Si.Root), Si pod dry mass (Si.Pod), leaf dry mass (L.DM), stem dry mass (S.DM), root dry mass (R.DM), pod dry mass (P.DM) by distinct common bean genotype - tolerant to water deficit (BAT 477 genotype) (a) and susceptible to water deficit (IAC carioca 80SH genotype) (b). Legends represent a positive correlation when blue circles are presented and a negative correlation when red circles are presented. Larger circles and painted with stronger colors such as, dark blue demonstrate a stronger positive correlation as well as light blue demonstrate a weaker positive correlation.

The results of the apparent recovery efficiency (RE) presented at the heatmap demonstrate a pattern between genotypes, regardless of nutrient accumulation (Figure 2.5). It is worthy to highlight that the IAC carioca 80SH (susceptible genotype) showed higher efficiency to use the silicon supply, more than 10% of RE, which corroborates with greater Si accumulation and dry mass.
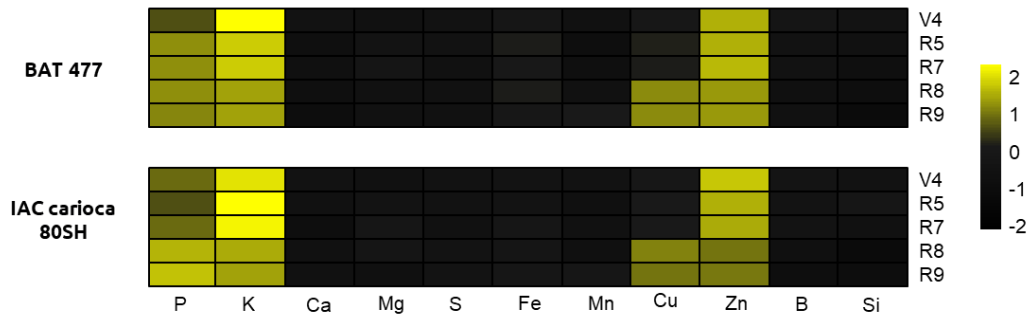
Figure 2.5. Heatmap of apparent recovery efficiency (RE%) of macro and micronutrients by distinct common bean genotypes as function of the phenological stage. Legends represent the Z-scores demonstrating the sample mean of each period (V4: third open trifoliolate leaf, R5: pre-flowering, R7: formation of pods, R8: filling of pods, R9: maturation).

2.3.3 Micropobe X-ray fluorescence spectroscopy (μ - XRF)

The silicon distribution along the leaf length was investigated through x-ray fluorescence (XRF) analysis. Therefore, three lines were scanned at each leaf, being the first line performed at the bottom, the second at the center, and the third at the top of the leaf.

Data show no clear differences regarding the Si distribution along the length nor any pattern along the width of the leaves, regardless of the genotype (Figure 2.6). A high horizontally variability can be found for both genotypes along the line scans. Si net intensities averages 113,61±34,34 and 140,46±58,50 cps (counts per second) for the tolerant and susceptible genotypes, respectively. These values suggest a slightly higher Si accumulation in the leaves of the water deficit susceptible genotype.
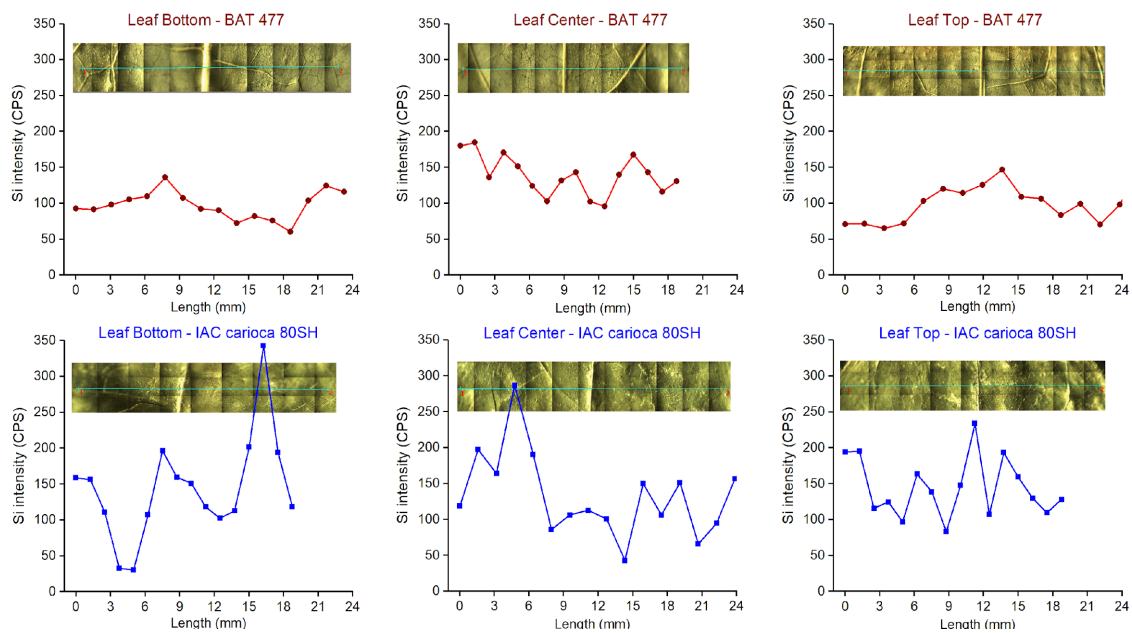
Figure 2.6: Silicon (Kα) fluorescence intensity at the adaxial face of common bean leaf (physiological maturation - R9) by X-ray fluorescence microanalysis (μ-XRF). The measurements were made in transversal lines of the bottom, center and top of the leaf. Legend: Leaf Bottom - BAT 477 = part near to the petiole (tolerant to water deficit); Leaf Center - BAT 477 = central part of the leaf; Leaf Top - BAT 477 = the tip of the leaf. Leaf Bottom - IAC carioca 80SH = part near to the petiole (susceptible to water deficit); Leaf Center - IAC carioca 80SH = central part of the leaf; Leaf Top - IAC carioca 80SH = the tip of the leaf.

2.3.4   Silicon absorption kinetics

The Si depletion in the nutrient solution had better accommodation at the potential model (Figure 2.7 and 2.8). Regardless of the genotype and the distinct Si concentration exposure, the Si presented in the nutrient solution was reduced to half after 6 h of the beginning of the experiment. The Si concentration presented in the solution remained constant after 48 h of exposure of the common bean plants to the different Si concentrations, the C_{min} value was estimated at that moment.

The Si depletion in the nutrient solution from the lowest concentration - 0.1 mmol L^{-1} – showed a pattern between genotypes, where both C_{min} values were 0.070 and 0.066 to tolerant and susceptible genotypes, respectively (Figure 2.7a-d). Furthermore, the tolerant genotype (Figure 2.7b-c) presented higher ability to seek the Si element in the nutrient solution even when the concentration was lower (0.5 and 1.0 mmol L^{-1}), which presented 81% and 117% higher capability than the susceptible genotype (Figure 2.7e-f).

Conversely, the standard once kept until the medium concentration, is now changed to a greater ability to seek the Si by the susceptible genotype (Figure 2.8a-c). The C_{min} in the nutrient solution containing 1.5 mmol L^{-1} of Si was 246% (Figure 2.8c-d) higher when compared to the tolerant genotype.
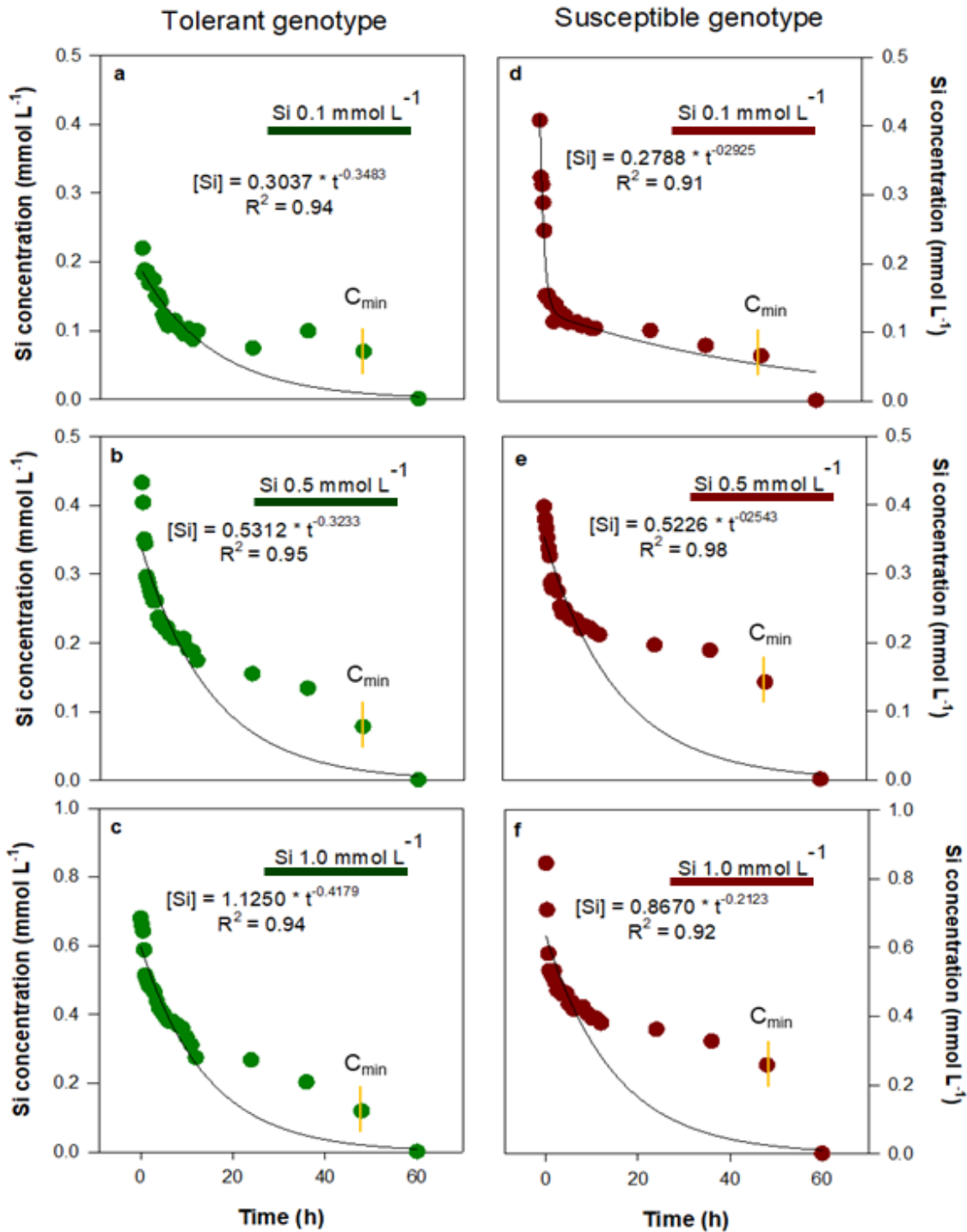
Figure 2.7. Silicon depletion curves for distinct common bean genotypes as function of different Si concentrated solutions (a. 0.1 mmol L^{-1}, b. 0.5 mmol L^{-1}, c. 1.0 mmol L^{-1} - tolerant genotype; d. 0.1 mmol L^{-1}, e. 0.5 mmol L^{-1}, f. 1.0 mmol L^{-1} - susceptible genotype) and time of collection of the nutrient solution aliquots.
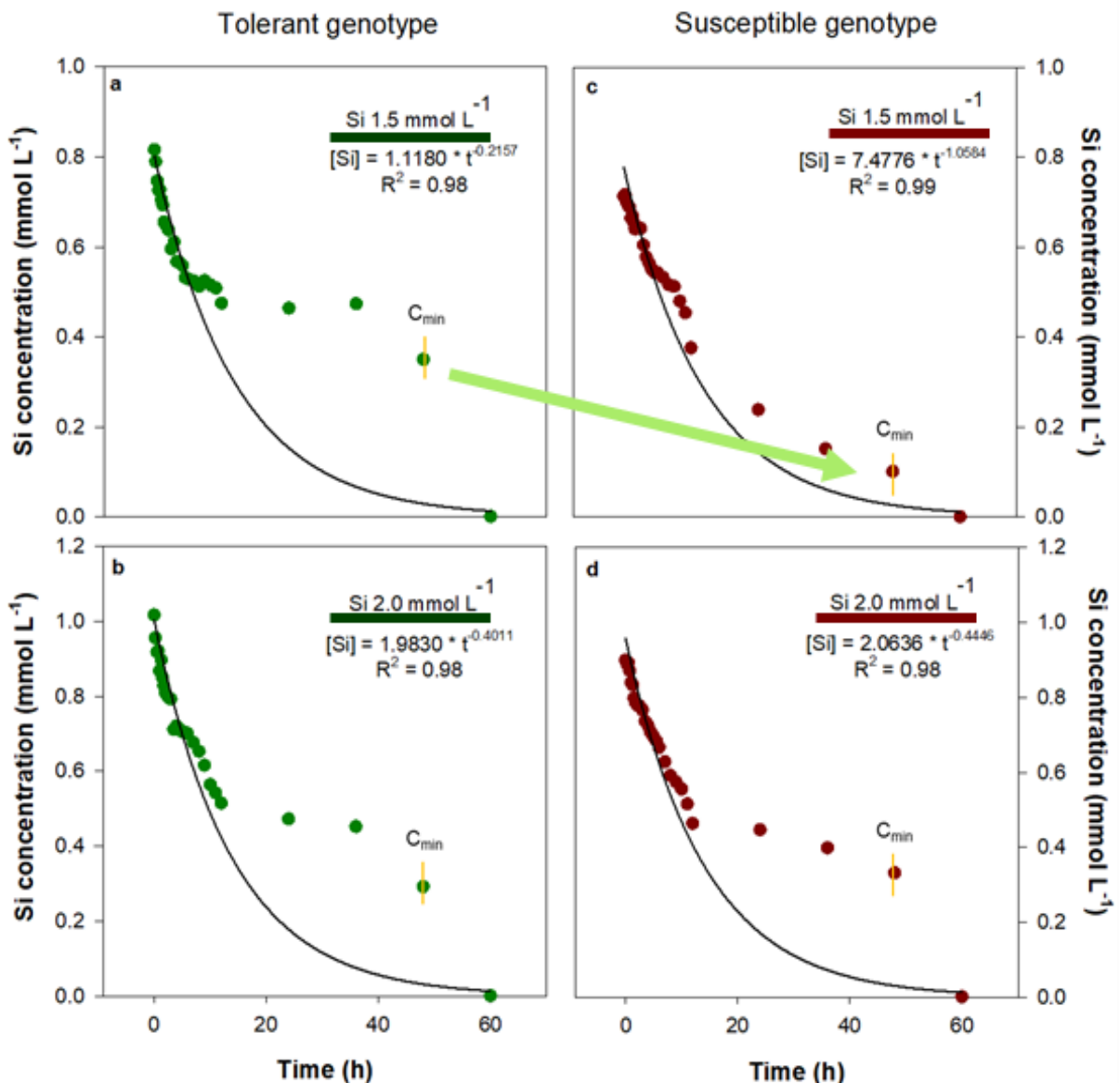
Figure 2.8. Silicon depletion curves for distinct common bean genotypes as function of different Si concentrated solutions (a. 1.5 mmol L⁻¹, b. 2.0 mmol L⁻¹ - tolerant genotype; c. 1.5 mmol L⁻¹, d. 2.0 mmol L⁻¹ - susceptible genotype) and time of collection of the nutrient solution aliquots.

2.3.5 Kinetics parameters

The highest I_{max} was observed in the tolerant genotype at the 1.5 mmol L⁻¹ Si concentration. The tolerant genotype (BAT 477) presented higher I_{max} at the 1.5 mmol L⁻¹ Si concentration, while the higher I_{max} was observed at the 2.0 mmol L⁻¹ Si concentration to the susceptible genotype (IAC carioca 80SH). Comparing genotypes, at the 2.0 mmol L⁻¹ Si concentration, the I_{max} parameter was 37% higher at the tolerant genotype when compared to the susceptible genotype.

Furthermore, the absorptive capacity (α) revealed a slighted difference of pattern where the higher absorptive capacity for both genotypes were found at the 1.5 mmol L^{-1} Si concentration. Notably, the α presented was 60% greater to the tolerant genotype than the susceptible genotype.

Table 2.1. Kinetic parameters I_{max}, K_m, C_{min} and absorptive capacity ($\alpha = I_{max}/K_m$) estimated from the Si absorption by distinct common bean genotypes supplied with Si in five crescent concentration.

Genotype	Si	Imax	Km	Cmin	α
	mmol L^{-1}	µmol g^{-1} FW h^{-1}	µmol L^{-1}	µmol L^{-1}	-
BAT 477 Genotype	0.1	0.0196 b	0.1400 c	0.0697 b	0.1400 c
	0.5	0.0693 b	0.2900 c	0.0784 b	0.2390 c
	1.0	0.0717 b	0.4500 b	0.1190 b	0.1593 c
	1.5	0.7450 a	0.6200 a	0.3497 a	1.2016 a
	2.0	0.5070 b	0.6400 a	0.2914 a	0.7922 b
IAC carioca 80SH Genotype	0.1	0.1118 c	0.2000 b	0.0657 c	0.5590 ab
	0.5	0.0480 c	0.2900 b	0.1423 b	0.1655 c
	1.0	0,2412 b	0.6100 a	0.2586 a	0.3954 b
	1.5	0.2180 b	0.2900 b	0.1010 b	0.7517 a
	2.0	0.3700 a	0.5600 a	0.3318 a	0.6607 a

2.3.6 Chemical analysis: kinetics

Silicon leaf content (Figure 2.7a) in the susceptible genotype was 42% higher than the Si content in the tolerant genotype presented at the 2.0 mmol L^{-1} Si concentration. Furthermore, the results presented by Si plant content (Figure 2.7b) demonstrated 45% and 37% higher Si content at the Si 1.5 and 2.0 mmol L^{-1} concentration, respectively, in the susceptible genotype, when compared to the tolerant genotype.

Moreover, the stem leaf content (Figure 2.7d) in the susceptible genotype was 52% higher than the Si content in the tolerant genotype presented at the 1.5 mmol L^{-1} Si concentration. On the contrary, the stem leaf content (Figure 2.7d) in the tolerant genotype was 75% higher than the Si content in the susceptible genotype presented at the 0.5 mmol L^{-1} Si concentration.
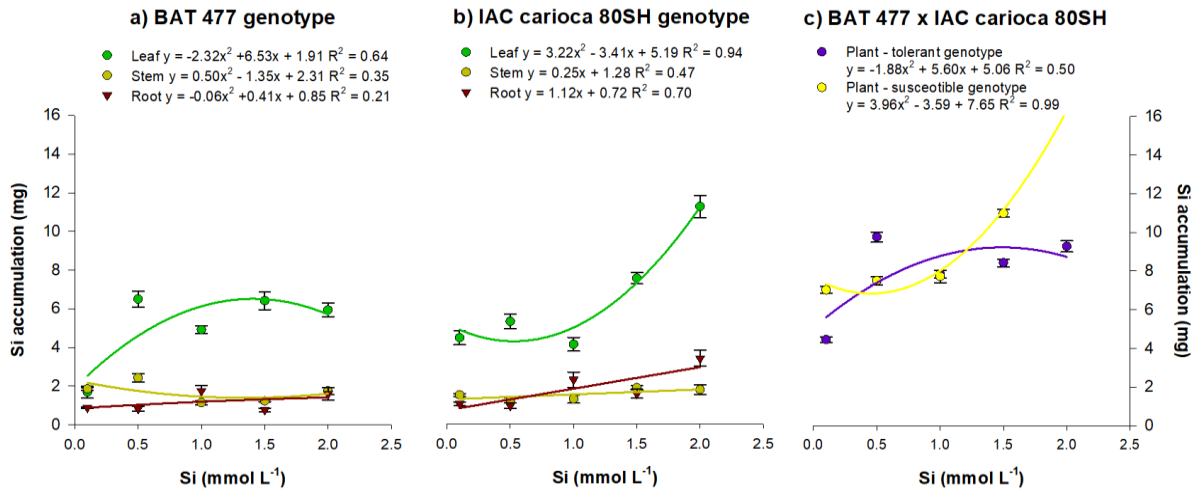
Figure 2.9. Silicon accumulation in common bean plants as a function of the Si concentration in the nutrient solution. a) Si accumulation (mg) in leaf, stem and root at common bean BAT 477 genotype. b) Si accumulation (mg) in leaf, stem and root at common bean IAC carioca 80SH genotype. c) Si accumulation (mg) in plants. Bars represent the standard error of the mean (n = 4).

The Pearson's correlation among the variables performed for the Si kinetics absorption of the distinct genotypes were highlighted (Figure 8a tolerant genotype and Figure 8b susceptible genotype). As observed, the correlation between the P concentration and the Si stem concentration, and Fe concentration and the Si root concentration revealed that there is a lighter and negative relationship between these elements. In addition, the correlation between Mn and Si stem concentration showed that there is a stronger and negative relationship (Figure 8a).

As for the correlation between variables evaluated to the susceptible genotype, none of them showed close relationship to the Si concentration in the plant (Figure 8b).
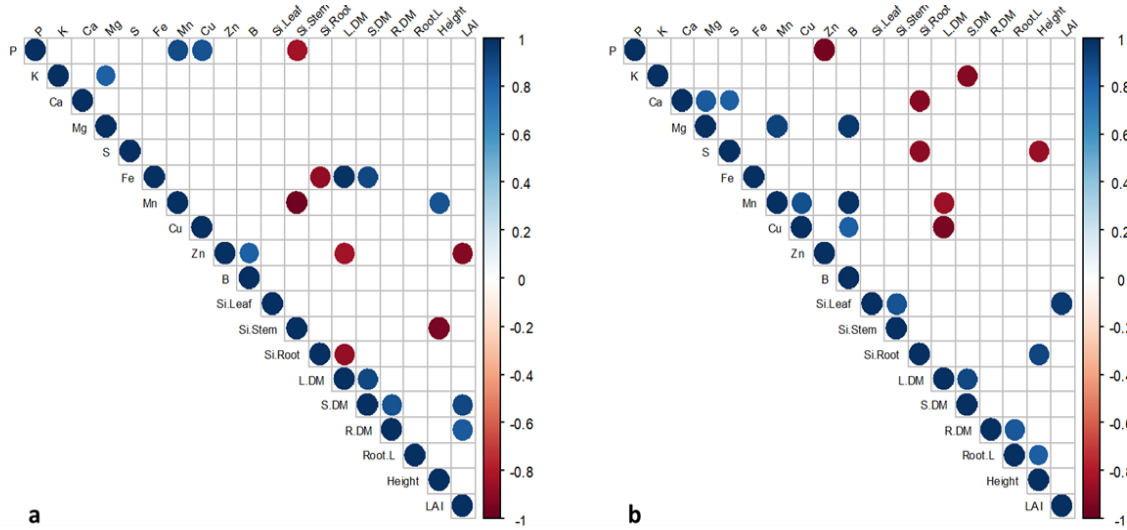
Figure 2.10. Pearson's correlation among macro and micronutrients (P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B), Si leaf concentration (Si.Leaf), Si stem concentration (Si.Stem), Si root concentration (Si.Root), leaf dry mass (L.DM), stem dry mass (S.DM), root dry mass (R.DM), root length (Root.L), plant height (Height) and area leaf index (IAF) by distinct common bean genotype - a. tolerant to water deficit (BAT 477 genotype) and b. susceptible to water deficit (IAC carioca 80SH genotype). Legends represent a positive correlation when blue circles are presented and a negative correlation when red circles are presented. Larger circles and painted with stronger colors such as, dark blue demonstrate a stronger positive correlation as well as light blue demonstrate a weaker positive correlation.
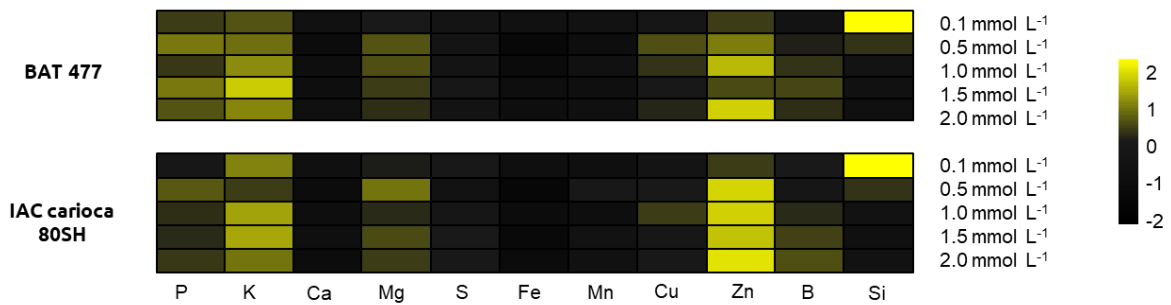

Figure 2.11. Heatmap of apparent recovery efficiency (RE%) of macro and micronutrients by distinct common bean genotypes as function of the Si concentration in the nutrient solution. Legends represent the Z-scores demonstrating the sample mean of each concentration (mmol L^{-1}).

Our results tend to clarify the path to elucidate Si beneficial effects in non-accumulating plants. Since the common bean crop has a significant economic and social impact in Brazil, in order to keep its higher yield, the use of beneficial elements is considered a Good Agriculture Practice (GAP) strategy.

Needless to say, the susceptible genotype's intensity in absorbing Si and allocating this element inside the leaves was higher than expected - susceptible genotypes presented 77% higher Si content than the tolerant genotype. In addition, this data shows that this non-accumulating plant has the capacity of absorbing this beneficial element which could be used as a tool to diminish the transpiration rate during a dry period (Nwugo and Huerta, 2008). It is well-known that in many regions worldwide, precipitation has become less uniform and more intense, also, because of the advent of increasing temperatures, evapotranspiration tends to get higher (Konapala et al., 2020).

Regardless of the genotype the beneficial element was absorbed and accumulated in its different parts, being a sustainable strategy to mitigate biotic and abiotic stresses, unconcerned of the crop's classification, considered as a Si accumulating plant or not. Broadly speaking, through the past few decades, the use of silicon as a stress mitigator has been studied, mainly, as a valid strategy to alleviate the harm that could affect grass plants, known as accumulator plants.

Nowadays, several studies have been developed with the purpose to improve the knowledge considering Si absorption and efficiency in non-accumulating plants. For instance, Si supplementation in soybean plants improved cell wall thickness of the nodules (Marschner et al., 1990; Kumar et al., 2017). Moreover, silicon is proven to mitigate stresses, such as salinity and water deficit, of *Sesbania aculeata* plants, by increasing the strengths of root nodulation and nitrogen fixation in the presence of this element. In addition, in this study, data showed that Si was absorbed and accumulated at higher rates when the plant was under stress and, on the contrary, less absorbed when in the absence of the stress (Kurdali et al., 2013). Also, in tomato (*Solanum lycopersicum* L.), under water deficit stress, silicon addition improved the energy redistribution in PSII reaction center, as well as the biomass of the seedlings (Zhang et al., 2018).

Throughout the Si absorption kinetics and its parameters experiment, in this work, each genotype showed distinct threshold and affinity. Regardless of the genotype evaluated, the Si depletion curves built throughout the 72 hours highlighted how quickly the element was absorbed. Taken together and bringing our results to light, the same observations were made, proving that the common bean also absorbed and accumulated Si all over its tissue.

Interestingly noted that even between varieties of the same non-accumulating species, Si uptake can be demonstrated in a different approach, hence efficiently absorbed, and distributed.

2.4   Conclusions

In a sustainable and agronomical point of view, silicon can be considered and wild used as a tool to diminish the transpiration rate, to help in the rigidity of the plant, to improve plant biomass and yield, and, also, to potentiate the defense enzymes-system of the plants. It is well-known that climate change directly affects our plantations and that sustainable strategies must be adopted in order to mitigate these negative impacts.

This study allowed us to draw some conclusions about the Si accumulation and kinectis parameters. Both genotypes were more efficient in utilizing Si when subjected to a lower dose (0.1 mmol L^{-1}), indicating a high-affinity system for both genotypes. At intermediate doses, the susceptible genotype indicated the activation of a Si exclusion system, which changed with increasing doses. When supplied with 2 mmol L^{-1}, the genotype started to increase the Si content.

Finally, to summarize through the perspective of the silicon absorption march the: (i) susceptible genotype: had higher Si levels and Si accumulation in plants, most of this element was allocated in the leaves, also showed an increase in dry plant mass; (ii) tolerant genotype: did not accumulate more Si compared to the susceptible genotype. From the perspective of kinetic analysis, the highlighted points were that: (i) both genotypes were more efficient in Si utilization when subjected to a lower dose of 0.1 mmol L^{-1}; (ii) at the lowest dose, the K_m was lower compared to the constant value at other doses, indicating a high-affinity system for both genotypes; (iii) at intermediate doses, the susceptible genotype indicates the activation of a Si exclusion system, which changes with increasing dose; (iv) supplied with 2 mmol L^{-1}, the genotype began to increase its Si content.

These data elucidate Si absorption and kinetics by drought tolerant and susceptible common bean varieties presenting the effectiveness of this useful element even in plants that are considered non-accumulating.

3   SILICON'S ROLE IN ENHANCING COMMON BEAN (*Phaseolus vulgaris* L.) PLANT RESILIENCE: PHOTOSYNTHETIC PARAMETERS AND CARBON ISOTOPE DISCRIMINATION ($\delta^{13}C$ ‰)

Abstract

The growing awareness of climate change has prompted a need to rethink our approach to food cultivation, industrial development, and the creation of sustainable cities (IPCC, 2023). The concentration of CO_2 in the atmosphere, currently exceeding 400 µmol CO_2 mol^{-1} air, is projected to double by the end of this century (Vaughan et al., 2018; Boris et al., 2021). To address these challenges, researchers and producers are focusing on developing new technologies and sustainable strategies. Climate change has disrupted precipitation patterns, leading to prolonged droughts and intense rainfall events. This irregular distribution of water, coupled with rising temperatures, can negatively impact in photosynthetic rates, water use efficiency, dry mass, and grain productivity. Silicon (Si) has emerged as a valuable element in enhancing plant tolerance to these adverse conditions by regulating stomatal conductance, thus reducing water loss through transpiration. In this study, the common bean (*Phaseolus vulgaris* L.), a non-accumulator plant sensitive to water stress, was selected as the model plant. The experiment induced drought during the V4 phenological stage and continued until pod formation (R7), affecting leaf size and number, root growth, and ultimately grain productivity. Parameters such as photosynthetic efficiency, water use efficiency, and carbon isotope discrimination ($\delta^{13}C$ ‰) were analyzed as physiological indicators of water stress response. This research contributes to the existing knowledge on Si's role in enhancing plant resilience to biotic stresses by studying these physiological responses, by non-accumulator plants.

Keywords: *Phaseolus vulgaris* L, silicon, water stress, fertilizer, leaf gas exchange.

3.1   Introduction

The awareness of climate change, that the world is experiencing, is growing. It has become imperative to transform our approach to food cultivation, industrial development, and the creation of smarter, energy-efficient cities. (IPCC, 2023). The impact of climate change on the concentration of CO_2, which is currently above 400 µmol CO_2 mol^{-1} air, is

predicted to double by the end of this century (Vaughan et al., 2018; Boris et al., 2021). Researchers and producers have been striving to develop new technologies focused on good agricultural practices that improve production in harmony with the increase of the practice of regenerative agriculture in a sustainable and circular economy. Afterall, higher yields can improve farmers' living income, which is one of the three pillars that sustainability is based on.

Climate change also affects precipitation distribution, leading to prolonged drought periods followed by heavy rainfall. This uneven distribution of water and the rising average temperature could facilitate the spread of various pests and plant diseases, besides affecting grain productivity, dry mass, photosynthetic rates, and water use efficiency. In addition, long periods of drought or depending on the intensity of the negative effects could damage the plant for the whole cycle.

Silicon (Si) has been found to improve the tolerance, it strengthens plant cell walls, enhances root development, and regulates stomatal conductance, reducing water loss through transpiration. This improved stress tolerance allows plants to better withstand adverse climatic conditions and maintain productivity even under challenging environments. This beneficial element is the second most abundant in the Earth's crust and is predominantly found in the soil in the form of silicate or aluminum silicates. Despite its abundance, a significant portion of Si is not easily accessible to plants.

This element can be easily absorbed by plant roots in the form of monomeric or monosilicic acid (H_4SiO_4) through diffusion, mass flow, or membrane transporters, after absorption, it is transported via xylem. Due to a very low redistribution, periodic application of Si is needed. In the leaves, it is deposited as silica (SiO_2H_2O) beneath the cuticle, reducing plant transpiration, Si concentration in plants varies significantly, among 0.1% to 10.0% of dry mass (Liang et al., 2007). Although Si is not considered a nutrient for plants, it is a beneficial element due to its potential role as a physical barrier, in metabolic and physiological parameters, enhancing plant tolerance under different abiotic and biotic stresses (Meena et al., 2014; Hassan et. al., 2018; Zargar et. al., 2019).

Drought induces a range of physiological and biochemical processes in plants that can reduce crop yields up to 70%, resulting in serious economic losses (IPCC, 2023). The common bean is considered sensitive to water stress due to its low capacity for recovery after water deficiency and underdeveloped root system (Doorenbos and Kassam, 1979; Guimarães, 1996). Water deficit can negatively and vastly influence the development of the

crop, reducing leaf area (Hernández et al., 2004) and photosynthetic rate (Chaves et al., 2009), consequently, reducing yield.

In this context, at this work the plant selected was the common bean (*Phaseolus vulgaris* L.), a non-accumulator plant and sensible to water stress, such as a drought condition, which was initiated at the V4 phenological stage. This is the stage where high intensity drought indirectly affects grain productivity due to the reduction in leaf size and number, which are responsible for plant and root growth. A plant with a well-developed root system will have greater survival capacity during dry periods and a higher ability to absorb nutrients from the soil. Additionally, the drought condition was kept until the phenological stage of pod formation (R7). Which could lead to the shedding of newly formed pods and adversely affect seed formation, resulting in a reduction in mass and, consequently, grain productivity.

The use of Si to alleviate biotic and abiotic stresses is already well established (Chen et al., 1969; Ma et al., 2016; Debona et al., 2017; Frew et al., 2018; Bokor et al., 2021) and has been garnering interest among researchers to conduct further studies aiming to elucidate the role of this beneficial element. The $\delta^{13}C$ value is influenced by isotopic effects during diffusion from the atmosphere to the chloroplasts and during carboxylation reactions. These processes are influenced by various factors, including stomatal regulation of leaf gas exchange and the ratio of substomatal (C_i) to ambient (C_a) CO_2 concentrations (Farquhar et al., 1989). Changes in $\delta^{13}C$ can arise from alterations in carboxylation efficiency, stomatal conductance (g_s), or both. The isotopic composition of carbon ($\delta^{13}C$) is based on the isotopic ratio of $^{13}C/^{12}C$, which is lower in plant tissue than in the atmosphere (Farquhar et al., 1989).

To determine the impact of silicon on non-accumulator plants, such as common beans under water deficit, indicators of physiological responses to water stress were analyzed. Whereby, photosynthetic parameters, water use efficiency and carbon isotope discrimination ($\delta^{13}C$ ‰).

3.2 Material and Methods
3.2.1 Local

Common bean (*Phaseolus vulgaris L.*) plants from two distinct genotypes: IAC carioca 80SH (water deficit susceptible), and BAT 477 (water deficit tolerant), were cultivated in 5L pots, filled with a Red-Yellow Latosol (Oxisol; Embrapa, 2006), in

greenhouse conditions in the Center for Nuclear Energy in Agriculture, Piracicaba-SP, Brazil.

3.2.2 Soil physical and chemical characteristics

The soil employed in this experiment was collected from the surface layer (0 – 20 cm) in the rural area of Itatinga-SP (22º 43' 31" S e 47º 38' 57" W). The soil was sandy and presented the following chemical characteristics: pH ($CaCl_2$) 4.2; organic matter: 5 g dm^{-3}, P (resin) 2 mg dm^{-3}, K 0.3 $mmol_c$ dm^{-3}, Ca^+ $mmol_c$ dm^{-3}, Mg^+ $mmol_c$ dm^{-3}, H + Al 25 $mmol_c$ dm^{-3}, Al 3 $mmol_c$ dm^{-3}, CTC 27 $mmol_c$ dm^{-3}, S 6 mg dm^{-3}. Soil correction was carried out 30 days prior to seeding, using calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) in a 3:1 proportion. The dose was determined aiming at achieving 60% of base saturation.

3.2.3 Treatments

The experiment consisted of a randomized blocks design under a triple factorial scheme, with 2 genotypes (IAC carioca 80SH and BAT 477) x 2 water regimes (water deficit and adequate water supply) x 2 silicon (Si) conditions (absent and adequate Si supply), and 7 repetitions, totalizing 8 treatments and 56 experimental units (Table 3.1).

Table 3.1. Treatments description

Treatment	Genotype	Si supply	Water supply
T1	IAC carioca 80SH	Adequate	Deficit
T2	IAC carioca 80SH	Adequate	Adequate
T3	IAC carioca 80SH	Absent	Deficit
T4	IAC carioca 80SH	Absent	Adequate
T5	BAT 477	Adequate	Deficit
T6	BAT 477	Adequate	Adequate
T7	BAT 477	Absent	Deficit
T8	BAT 477	Absent	Adequate

The cultivars were chosen to investigate the responses from a water deficit tolerant (BAT 477) and a water deficit susceptible one (IAC carioca 80SH). Adequate Si supply was determined as a soil Si concentration of 200 mg dm^{-3}, supplied with sodium silicate. The water deficit was induced by reducing the water supply to 40% of soil field capacity from the V4 phenological stage on.

3.2.4 Cultivation essay

Sodium silicate was incorporated into the soil 30 days after liming, aiming at achieving a Si concentration of 200 mg dm^{-3} in the Si adequate supply treatments. This referred Si concentration was determined according to the results obtained in the absorption march (chapter 1), as well as the values found by Mayumi et al. (2016) and Mayumi et al. (2018). After Si incorporation, five common bean seeds were placed in each pot for germination, however, eleven days later, thinning was made to maintain only two plants per pot.

All treatments were sustained under adequate irrigation (soil moisture at 80% of field capacity) until V4 phenological stage, then, water stress was initiated in treatments T1, T3, T5 and T7, by keeping soil moisture at 40% of field capacity until harvest. The relative soil water content was controlled during the experiment through weighting the pots daily and adjusting the weight to the wanting field capacity.

3.2.5 Photosynthetic parameters

Leaf gas exchanges were determined using a portable infrared gas analyzer (LI-6400XT, LI-COR, Inc. Lincoln NE, USA), on completely expanded mature trifoliums. Throughout the assay, there were two moments when the analysis was carried out: (i) first flower emission (R5) and (ii) pod formation (R7), both measurements were held in clear days among 09h30 am and 10h30 am. The parameters evaluated were, net CO_2 assimilation (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, μmol mol^{-1}), and instantaneous water-use efficiency – which was calculated as the ratio between A and E - ($iWUE$, μmol CO_2 mmol H_2O). The light absorption (0.84) (Schreiber et al., 1998) exposed to a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}. During the measurements, the ambient CO_2 concentration

(inside the chamber) was adjusted to 400 µmol mol^{-1}, vapor pressure deficit based on leaf temperature was 2.07 KPa and relative humidity was 50% (Schreiber et al., 1998; Lichtenthaler et al., 2007; Baker, 2008).

Table 3.2: Air temperature and humidity during the leaf gas exchange evaluations

	Air temperature (ºC)	Leaf temperature (ºC)	Air humidity (%)
R5	25.7	27.0	49.8
R7	30.9	30.0	57.9

Note: R5 and R7 is the description of the phenological stage where: first flower emission - R5; and during the pod formation - R7.

3.2.6 Biometrical analysis

At R8/R9 phenological stage, the plants were harvested and dried in an oven with forced-air circulation at 60ºC until reach constant mass. Then, the samples were weighted to determine plant dry mass.

3.2.7 Silicon and Nitrogen Chemical analysis

Si content was determined by the method proposed by Elliot and Snyder (1991), adapted by Carneiro et al. (2007). The common bean leaves were harvested at R8 stage, then, they were dried in an oven with forced-air circulation at 60 °C until constant mass. For sample preparation, 0.1 g of dry mass was weighted and placed inside of plastic tubes. To proceed, 2 ml of hydrogen peroxide (H_2O_2 – 30% v/v) and 3 ml of sodium hydroxide (NaOH – 25 mol L^{-1}) were added to start the wet-digestion. The samples were placed inside a hot bath (± 90 °C) for 1 h and then transferred to the autoclave digester for 1 h at 123 °C and 0.15 MPa. Thereafter, to achieve the total volume of 20 ml, water was added to the samples, and subsequently 1 ml of the resultant solution was transferred to a new plastic tube. Furthermore, the final volume was water adjusted to 20 ml, and finally added 1 ml of chloridric acid (1:1 v/v) and 2 ml of molybdic acid ammonium (5%). After all, the yellowish color was formed, and the results were read at the absorbance of 410 nm.

Leaves' samples were dried in an oven with forced-air circulation at 60 °C until constant mass. The material was analyzed for %N in automated mass spectrometer coupled to an ANCA-GSL N analyzer (Sercon Co.,UK). The total N concentrations were calculated according to the method of Barrie et al. (1995).

3.2.8 Isotopic composition of $\delta^{13}C$ in leaves tissue

The isotopic composition of carbon ($\delta^{13}C$ ‰) and carbon abundance was determined in the leaves of the common bean plants. The leaves were dried in an oven with forced-air circulation at 60 °C until constant mass therefore ground. The carbon abundance was performed using an ANCA-GSL mass spectrometer (Hydra 20-20 SERCON Co., Crewe, GBR) coupled with an automatic carbon analyzer, following the methodology described by Barrie and Prosser (1996). The $\delta^{13}C$ values were calculated using the equation (Farquhar, 1982):

$$\delta^{13}C \ (‰) = [(Rsample \ / \ Rstandard) - 1] \times 1000$$

Where R is the ratio of $^{13}C/^{12}C$, and the reference material used is the Vienna Pee Dee Belemnite (VPDB) with a value of 0.01117960 (Craig, 1957).

3.2.9 Statistical analysis

Data were submitted to analysis of variance (ANOVA) at 5% probability. When ANOVA presented significant results, means were compared using Tukey's test. The analyzes were made using Sisvar statistical software (Ferreira, 2011). The results were expressed as mean ± standard error of the mean (SEM).

3.3 Results and Discussion
3.3.1 Photosynthetic parameters

3.3.1.1 First flower emission (R5)
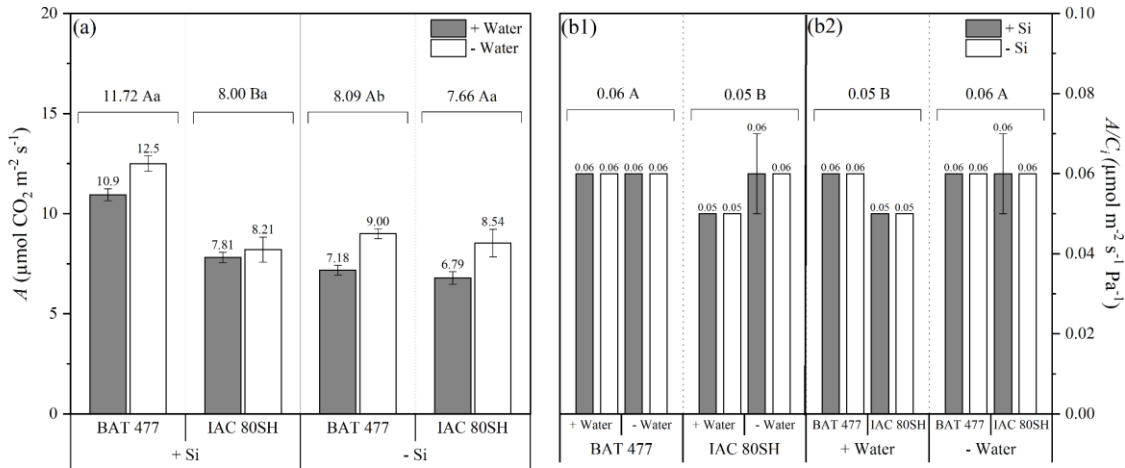
Figure 3.1: Net CO_2 assimilation (A) was evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. Means followed by the same letters are not significantly different between genotypes at the silicon level (uppercase letters) and silicon supply at the genotype level (lowercase letters), according to the Tukey test at a significance level of 5%. Apparent carboxylation efficiency (A/C_i) was also evaluated and means followed by the same letters are not significantly different regardless of genotype (b1) and water supply (b2). Bars represent the standard error of the mean (n = 3).

Our results on net CO_2 assimilation (A) by the leaves of common beans (Figure 3.1.a) were influenced by the genotype and external supply, while the water condition treatment did not have an impact on the results. The differences between genotypes were only highlighted when silicon (Si) was supplied. As shown, BAT 477 exhibited a 46.5% higher net CO_2 assimilation compared to IAC carioca 80SH under the same Si-fed treatment. Furthermore, when comparing the same genotype at different Si levels, BAT 477 demonstrated a 44.9% greater net CO_2 assimilation when Si was supplied compared to when it was not.

Examining the results of the apparent carboxylation efficiency (A/C_i) (Figure 3.1.b), which represents the ratio of net CO_2 assimilation to leaf intercellular CO_2 concentration, no interaction was observed among the three factors evaluated in this research. However, differences were found between genotype and water supply. When comparing the mean apparent carboxylation efficiency (Figure 3.1.b1) of BAT 477 and IAC carioca 80SH, regardless of water or Si supply, an increase in efficiency of 20% was observed in BAT 477. Furthermore, analyzing the results regarding water regime (Figure 3.1.b2), it can be

concluded that irrespective of Si supply or genotype characteristics, the A/C_i outcomes were 20% higher in the water deficit treatment compared to well-watered common beans.

It is imperative to recognize that the water-deficit regime treatment was initiated 10 days prior to this analysis. Therefore, it may be the reason behind this unexpected result. The literature highlights that a decrease in the ratio between A/C_i is expected when multiple factors are established, such as plants grown under water stress and its severity, the duration of the stress, the growth stage, and genetics. Water deficit induces various physiological disruptions, including a decrease in stomatal conductance and faces water stress often encounter a limitation in gas exchange due to stomatal closure, which regulates water loss but hampers carbon assimilation (Chaves et al., 2002; Chai et al., 2016).
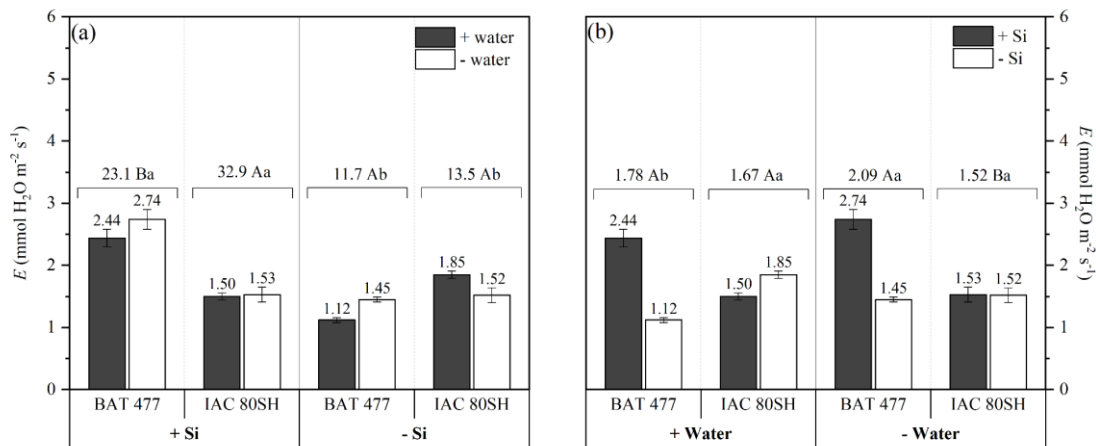
Figure 3.2: Transpiration (E) was evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. (a) Means followed by the same letters are not significantly different between genotypes at the silicon level (uppercase letters) and silicon supply at the genotype level (lowercase letters), according to the Tukey test at a significance level of 5%. (b) Means followed by the same letters are not significantly different between genotypes at the water level (uppercase letters) and water supply at the genotype level (lowercase letters), according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Transpiration (E) is another important photosynthetic parameter that should be evaluated, especially in research involving water stress. Our transpiration results (Figure 3.2.a) exhibited a similar pattern to the net CO_2 assimilation results (Figure 3.1.a), except for the BAT 477 genotype in the non-Si level. Firstly, when comparing the transpiration rate in the Si-fed level, BAT 477 showed a 102.3% higher rate compared to the results of the same genotype in the non-Si level. Secondly, when comparing the two genotypes in the Si-fed level, BAT 477 exhibited a 71.5% higher transpiration rate than IAC carioca 80SH. However, in the non-Si supply level, BAT 477 had a transpiration rate 31.3% lower than that of IAC carioca 80SH. Finally, when analyzing the different results of transpiration among genotypes

in the water level (Figure 3.2.b), it was observed that BAT 477 exhibited a 17.4% higher transpiration rate in the water deficit treatment compared to the well-watered treatment. Furthermore, when comparing the genotypes in the water deficit level, BAT 477 displayed a significantly higher transpiration rate of 37.5% compared to the rate of IAC carioca 80SH.
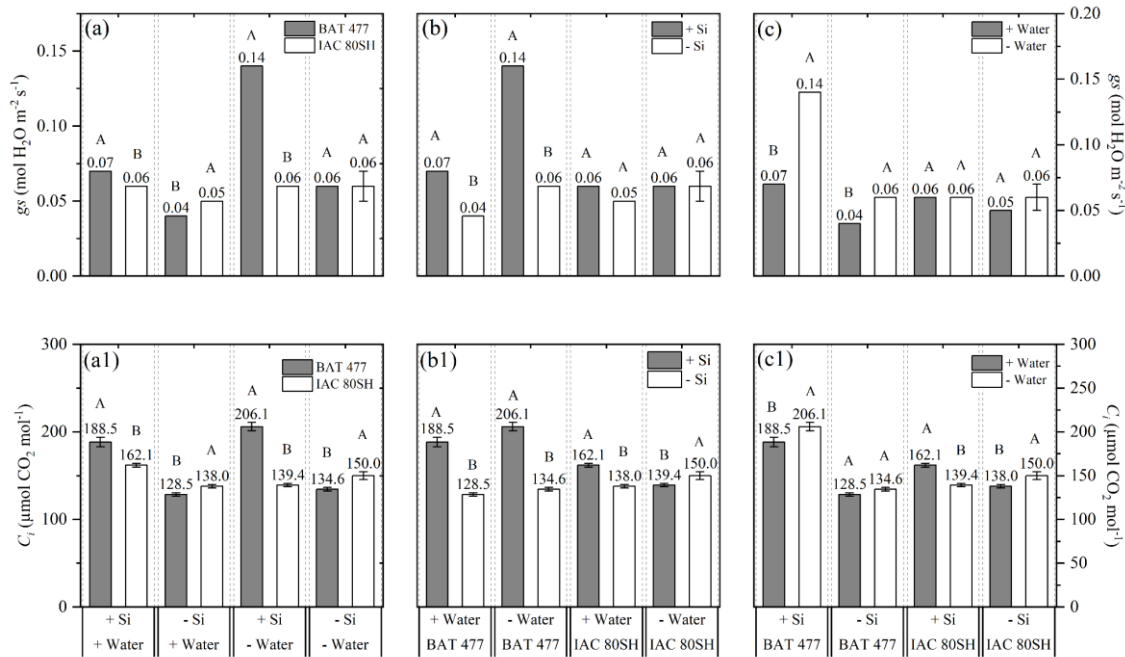
Figure 3.3: Stomatal conductance (g_s) and leaf intercellular CO_2 concentration (C_i) were measured in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, known for their tolerance and susceptibility to water deficit, respectively. Means followed by the same letters were not significantly different between (a) genotypes in each silicon and water supply level, (b) silicon in each water condition and genotypes, and (c) water regime in silicon supply and genotype levels, as determined by the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Our results on stomatal conductance (g_s) and leaf intercellular CO_2 concentration (C_i) showed a significant triple interaction, indicating that the combination of water, silicon, and genotype directly affects the behavior of common bean plants. In terms of stomatal conductance, BAT 477 exhibited exceptional performance in the Si-fed water deficit condition (Figure 3.3.a). For instance, its stomatal opening efficiency was 133.3% higher than that of IAC carioca 80SH, and the leaf intercellular CO_2 concentration was 47.8% higher (Figure 3.3.a1). When considering Si supply in the water deficit and genotype levels (Figure 3.3.b and b1), BAT 477 showed 133.3% and 53.1% higher efficiency in g_s and C_i, respectively, compared to BAT 477 without external Si supply in the water deficit condition. Finally, from the perspective of water condition, the BAT 477 plants supplied with Si (Figure

3.3.c and c1) exhibited 100% and 9.3% higher efficiency in g_s and C_i, respectively, compared to the same genotype grown under well-watered conditions.

Continuing with the BAT 477 genotype and Si-fed, when considering the well-watered condition (Figure 3.3.a and a1), it is noteworthy that there was a 16.7% increase in g_s and a 16.3% increase in C_i compared to the IAC carioca 80SH. Furthermore, in the Si-fed level under well-watered conditions (Figure 3.3.b and b1), the BAT 477 genotype showed a 75% increase in g_s and a 46.7% increase in C_i compared to the level without external Si supply.
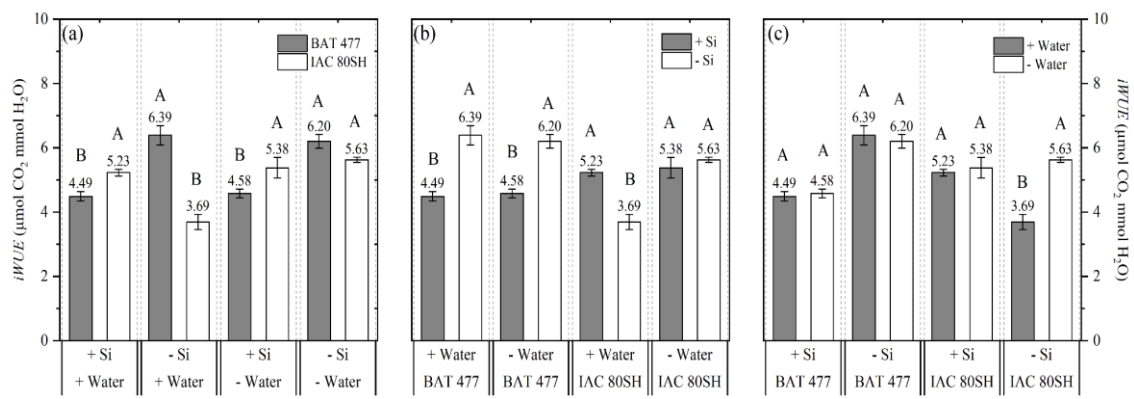
Figure 3.4: Instantaneous water use efficiency ($iWUE$) was evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, known for their tolerance and susceptibility to water deficit, respectively. No significant differences were found between genotypes in each silicon and water supply level (a), silicon in each water condition and genotypes (b), and water regime in silicon supply and genotype levels (c), as determined by the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Our results revealed a significant triple interaction between the ratio of net CO_2 assimilation (A) and transpiration (E), which is commonly referred to as Instantaneous water use efficiency ($iWUE$). Firstly, in the treatment without Si supply and in the well-watered level (Figure 3.4.a), BAT 477 exhibited a 73.2% higher efficiency in water use compared to IAC carioca 80SH. Conversely, IAC carioca 80SH showed better performance in $iWUE$ results in the Si-fed level within the well-watered condition and in the water deficit condition, with increases of 16.5% and 17.5% respectively, compared to BAT 477.

Secondly, considering the perspective of Si supply (Figure 3.4.b), BAT 477 exhibited a 42.3% higher $iWUE$ in the well-watered condition when supplied with Si compared to the treatment without external Si. Furthermore, in the absence of Si supply and under water deficit conditions, this genotype demonstrated a 35.4% increase in $iWUE$ compared

to the Si-fed treatment. On the other hand, IAC carioca 80SH showed greater efficiency, with a 41.7% increase in $_iWUE$, in the Si-fed and well-watered condition compared to the treatment without Si supply. Lastly, from the viewpoint of water conditions, IAC carioca 80SH in the non-Si-fed level exhibited a 52.6% higher $_iWUE$ in the water deficit treatment compared to the well-watered treatment.

3.3.1.2 Pod formation (R7)
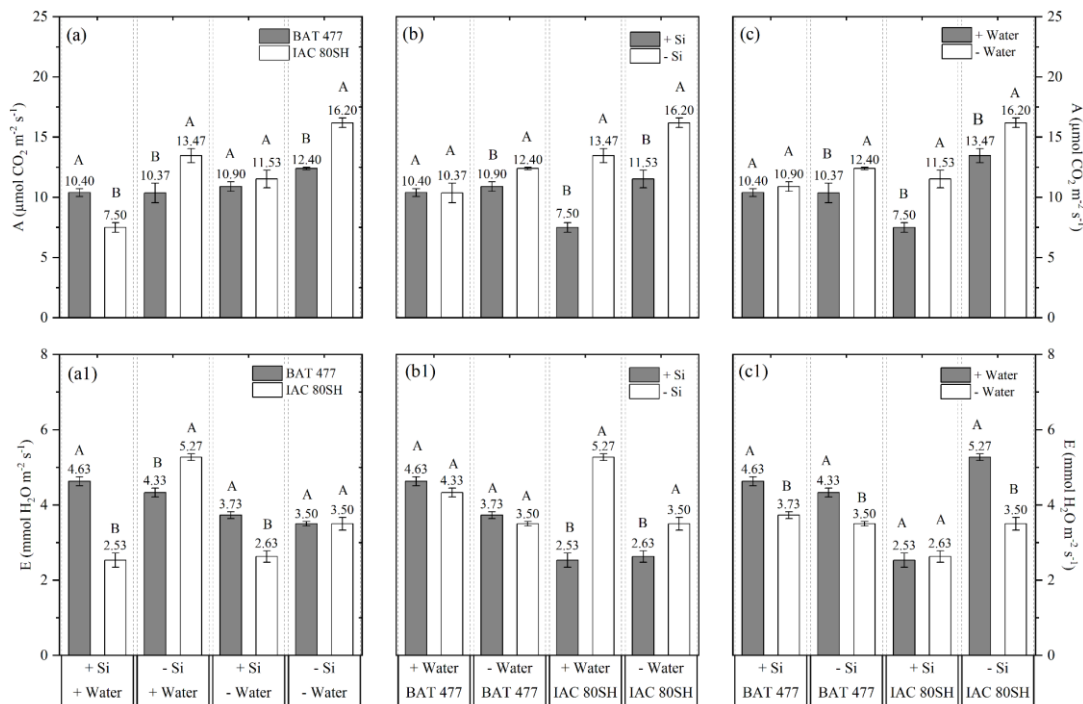
Figure 3.5: Net CO_2 assimilation (A) (a) and transpiration (E) by distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. Means followed by the same letters are not significantly different between (a) genotypes in each silicon and water supply level, (b) silicon supply in each water condition and genotypes, and (c) in water regime in silicon supply and genotype levels, as determined by the test Tukey at 5% significance. Bars represent the standard error of the mean (n = 3).

Our results showed a triple interaction between net CO_2 assimilation (A) and transpiration (E) rates (Figure 3.5). These results were obtained after a longer period of water deficit compared to the initial leaf exchange analysis at the R5 stage. Firstly, when evaluating the genotypes without Si supply under water deficit conditions (Figure 3.5a and a1), the IAC carioca 80SH exhibited a 30.6% higher net CO_2 assimilation rate than the BAT 477, while the transpiration rate did not show significant differences between the genotypes.

Moreover, in the well-watered level without Si supply, the IAC carioca 80SH displayed a 30.1% increase in net CO_2 assimilation and a 21.7% increase in transpiration rates compared to the BAT 477. Additionally, the IAC carioca 80SH demonstrated a higher instantaneous water use efficiency ($iWUE$) than the BAT 477 under water stress conditions and in the well-watered level. Specifically, the iWUE was 42.1% and 20.3% higher, respectively, regardless of Si supply. Furthermore, the IAC carioca 80SH showed a 64% higher efficiency compared to the well-watered regime of the IAC carioca 80SH, regardless of Si supply (Figure 3.6a).

On the contrary, when considering the perspective of the BAT 477 genotype, the net CO_2 assimilation and transpiration rates in the Si-fed and well-watered level were, respectively, 38.7% and 83% higher than those of the IAC carioca 80SH (Figure 3.5a and a1). Additionally, the instantaneous water use efficiency of the BAT 477 genotype under water deficit conditions was 43.3% higher than the response in the well-watered level (Figure 3.6a). It is important to note that the BAT 477 exhibited a higher transpiration rate, 41.8%, in the Si-fed and water deficit level compared to the IAC carioca 80SH. However, no difference in carbon assimilation was observed between the genotypes, indicating a lack of efficiency in net CO_2 assimilation of the BAT 477 genotype under water stress conditions, even with Si supply (Figure 3.5a and a1).

Secondly, when evaluating these rates from the perspective of Si supply under different water conditions and genotype levels, it can be confirmed that an increase in rates was observed when plants were treated without Si supply. For example, under water stress conditions, the BAT 477 exhibited 13.8% higher net CO_2 assimilation compared to the Si-fed plants. Similarly, under the same circumstances, the IAC carioca 80SH showed a performance in net CO_2 assimilation and transpiration that was 40.5% and 33.1% higher, respectively, when the same genotype was grown without Si supply. Furthermore, under the well-watered level and without Si supply, the IAC carioca 80SH demonstrated even higher rates of net CO_2 assimilation and transpiration, reaching 82.7% and 108.3%, respectively (Figure 3.5b and b1).

Finally, to analyze the results from the perspective of water conditions in the Si supply and genotype levels (Figure 3.5c and c1), a greater response in net CO_2 assimilation was observed in the water deficit condition in the IAC carioca 80SH, which showed a 20.3% increase, despite a decrease of 50.6% in the transpiration rate. It is expected to have a decrease in the rates of gas exchange as plants tend to close their stomata for longer periods to prevent water loss. It is worth noting that regardless of the genotype, there was an increase

of 65.7% in instantaneous water use efficiency (Figure 3.6b) in the water deficit plants compared to the well-watered condition. This result may indicate a mechanism of tolerance to water stress conditions, which can assist the plants during prolonged periods of stress (Soureshjani et al., 2019; França et al., 2020).

Likewise, in line with these results, plants under water stress also exhibited a decrease of 13.2% in instantaneous water use efficiency when subjected to Si supply compared to the level without Si (Figure 3.6b). This finding challenges our hypothesis, as Si is known for its ability to alleviate water stress (Chen et al., 1969; Ma et al., 2016; Debona et al., 2017; Soureshjani et al., 2019; França et al., 2020). BAT 477 under water deficit conditions showed a net CO_2 assimilation rate that was 19.6% higher than the well-watered condition in the absence of Si supply. Conversely, the same plants exhibited a 23.7% lower transpiration rate than the well-watered ones (Figure 3.5c and c1). Additionally, regardless of the genotype, plants grown under water deficit with Si supply displayed a higher instantaneous water use efficiency of 39.1% compared to the well-watered condition.
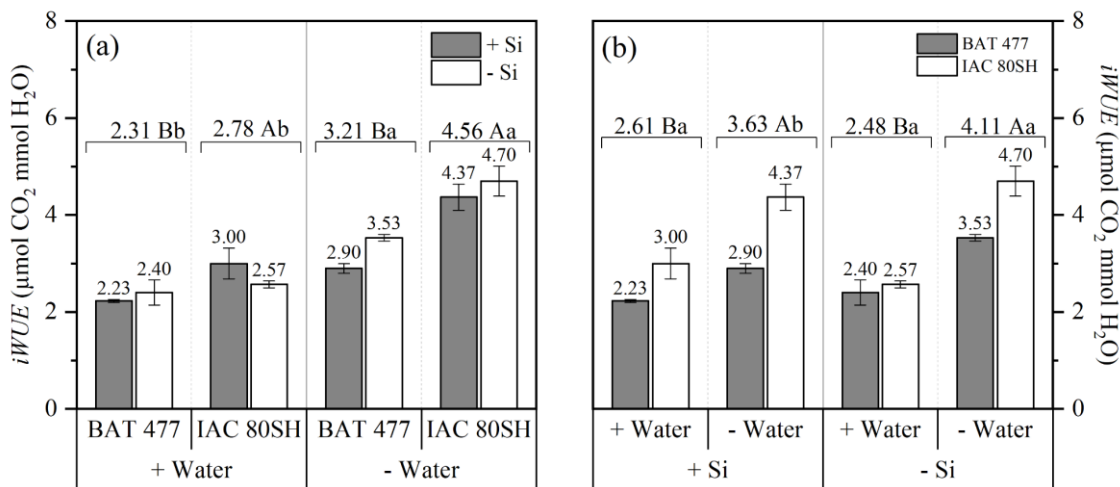
Figure 3.6: Instantaneous water use efficiency ($iWUE$) was assessed in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, which are respectively tolerant and susceptible to water deficit. (a) Means followed by the same letters are not significantly different: between genotypes in the water condition level (upper case letters) and water regime in the genotype level (lower case letters); and (b) between water conditions in the silicon level (upper case letters) and within the same water regime in the silicon level (lower case letters), as determined by the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Our results for stomatal conductance (g_s) and leaf intercellular CO_2 concentration (C_i) revealed a significant triple interaction among the factors investigated in this study. Analyzing the stomatal conductance results of plants that were exposed to the variables for a longer period, we observed a distinct pattern compared to the initial analyses. From the

perspective of BAT 477, plants grown under Si supply and in the well-watered level exhibited higher stomatal conductance and leaf intercellular CO_2 concentration (Figure 7a and a1), with values 340% and 7.1% higher than those of IAC carioca 80SH, respectively. Furthermore, there was a 69.2% increase in gs in the water condition level (Figure 3.7c). It is noteworthy that the results for BAT 477 showed a 214.3% increase in gs and a 52.1% increase in C_i (Figure 3.7a and a1) compared to the initial analysis of photosynthetic parameters (Figure 3.3a and a1) conducted in this research.

The BAT 477 genotype demonstrated a 24.9% higher efficiency in increasing the intercellular carbon concentration in the leaf (Figure 3.7a), even when grown under a water and Si-deficient treatment, compared to IAC carioca 80SH. Conversely, when comparing IAC carioca 80SH to itself, there was a 48.1% increase in efficiency when supplied with Si under water stress conditions compared to when grown without Si (Figure 3.7b).
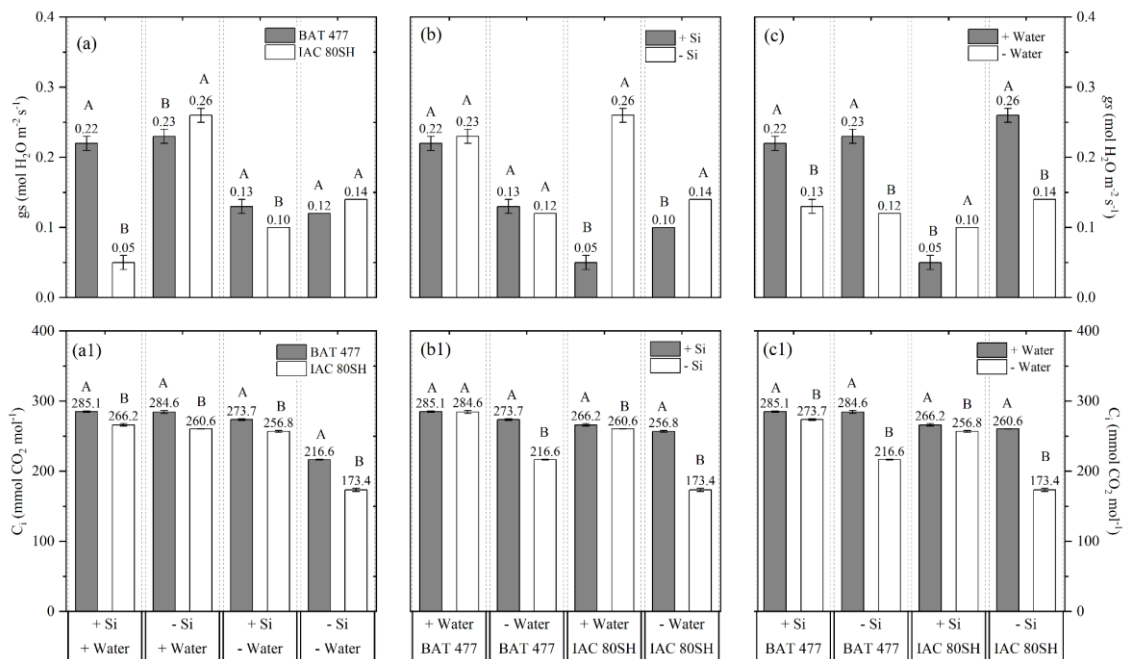
Figure 3.7: Stomatal conductance (g_s) and leaf intercellular CO_2 concentration (C_i) were evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, which are tolerant and susceptible to water deficit, respectively. Means followed by the same letters are not significantly different between: (a) genotypes in each silicon and water supply level, (b) silicon in each water condition and genotype level, and (c) water regime in silicon supply and genotype levels, according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

In addition, in the results of apparent carboxylation efficiency (A/C_i), BAT 477 exhibited 26.3% higher efficiency at the Si-fed level compared to the level without Si supply, regardless of the water condition (Figure 3.8a). Conversely, regardless of the Si supply in the well-watered level, BAT 477 results decreased by 38.9% compared to the average under

water deficit (Figure 3.8b). It is interesting to note that, regardless of the genotype, the A/C_i results of the plants grown under Si supply were 30.3% higher when these plants were under water stress conditions compared to well-watered conditions (Figure 3.8c). In contrast, plants grown in a well-watered regime without Si addition exhibited 36.4% higher A/C_i results than those grown with Si supply (Figure 3.8c).

Evaluating from the perspective of the treatment without Si addition, the genotype IAC carioca 80SH demonstrated 53.9% higher A/C_i efficiency compared to BAT 477. Furthermore, when comparing the IAC carioca 80SH genotype at the Si level, the plants treated without Si exhibited 97.4% higher A/C_i efficiency than those treated with Si (Figure 3.8a). The results followed similar patterns to the previous findings when evaluated at the water regime level in each genotype and under each Si condition (Figure 3.8b and c). In other words, the genotype IAC carioca 80SH, when subjected to water stress conditions, exhibited a 73.2% higher efficiency compared to those grown under normal water conditions. Additionally, it displayed a 42% higher efficiency than BAT 477 under water stress conditions (Figure 3.8b).
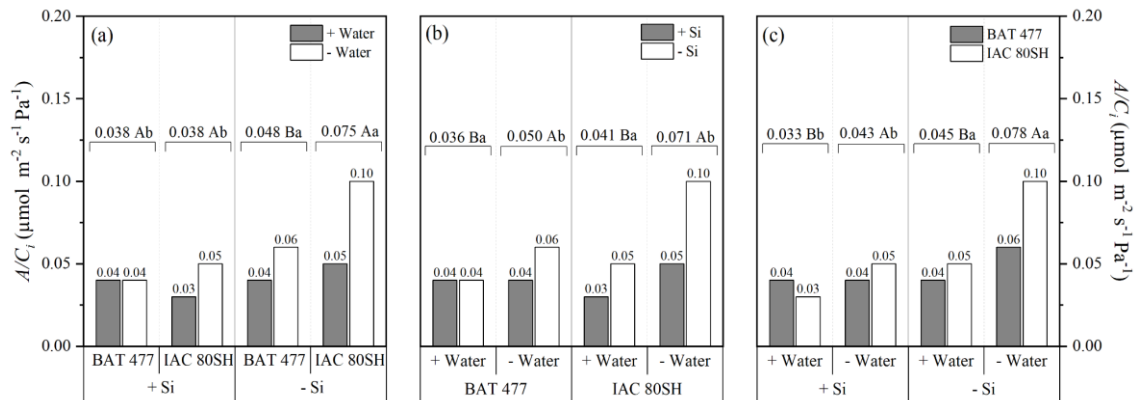
Figure 3.8: Apparent carboxylation efficiency - A/C_i was evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. (a) Means followed by the same uppercase letters are not significantly different between genotypes at the silicon level, while lowercase letters indicate no significant difference in silicon supply at the genotype level; (b) Uppercase letters indicate no significant difference between genotypes at the water condition level, while lowercase letters indicate no significant difference within the same water regime level for each genotype; and (c) Uppercase letters indicate no significant difference between water conditions at the silicon level, while lowercase letters indicate no significant difference within the same water regime level for silicon supply, according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

3.3.1.3 Silicon accumulation
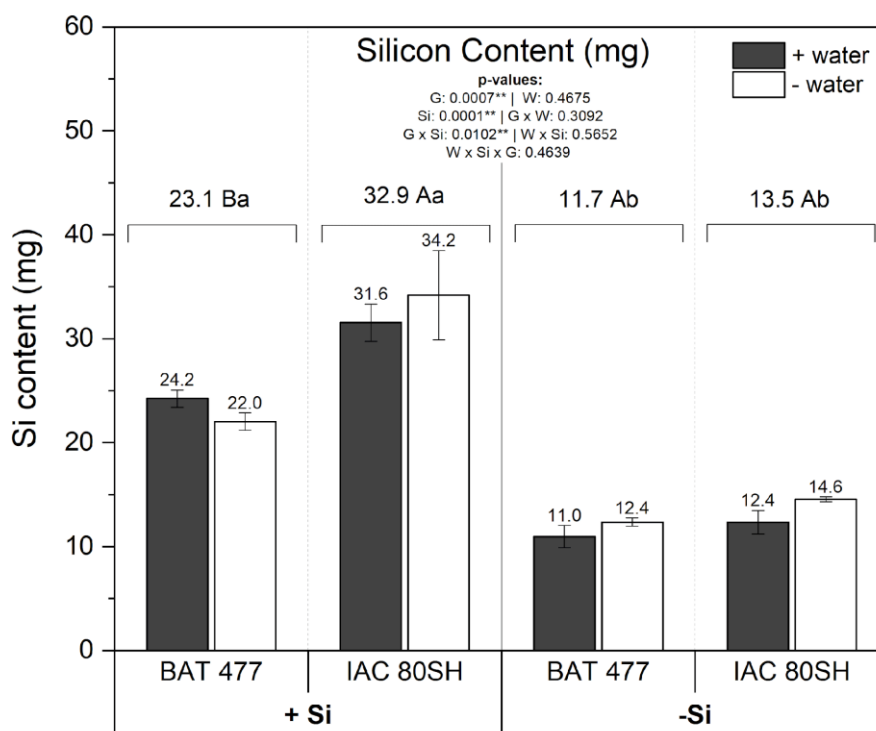
Figure 3.9: Silicon accumulation was assessed in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. Means followed by the same uppercase letters are not significantly different between genotypes at the silicon level, while lowercase letters indicate no significant difference in silicon supply at the genotype level, as determined by the Tukey test at a significance level of 5%. Error bars represent the standard error of the mean (n = 3). Subtitle for p-value: G = genotype;
W = water; and Si = silicon.

The variable water did not significantly influence Si accumulation. However, the variable genotype played a significant role in determining Si accumulation. For example, the IAC carioca 80SH genotype accumulated 42.1% more Si than the BAT 477 genotype in the Si-fed level. Furthermore, the mean Si accumulation in the IAC carioca 80SH genotype without external Si addition was 143.7% lower than the mean accumulation in the same genotype under Si-fed conditions, regardless of the water condition (Figure 3.9).

Finally, regardless of the common bean genotype or water supply regime, the average Si accumulation in the Si-fed treatment was 122.2% higher than the mean accumulation in plants without Si supply. It is worth highlighting that both genotypes were able to absorb and accumulate some silicon, even without external silicon input (Figure 3.9).
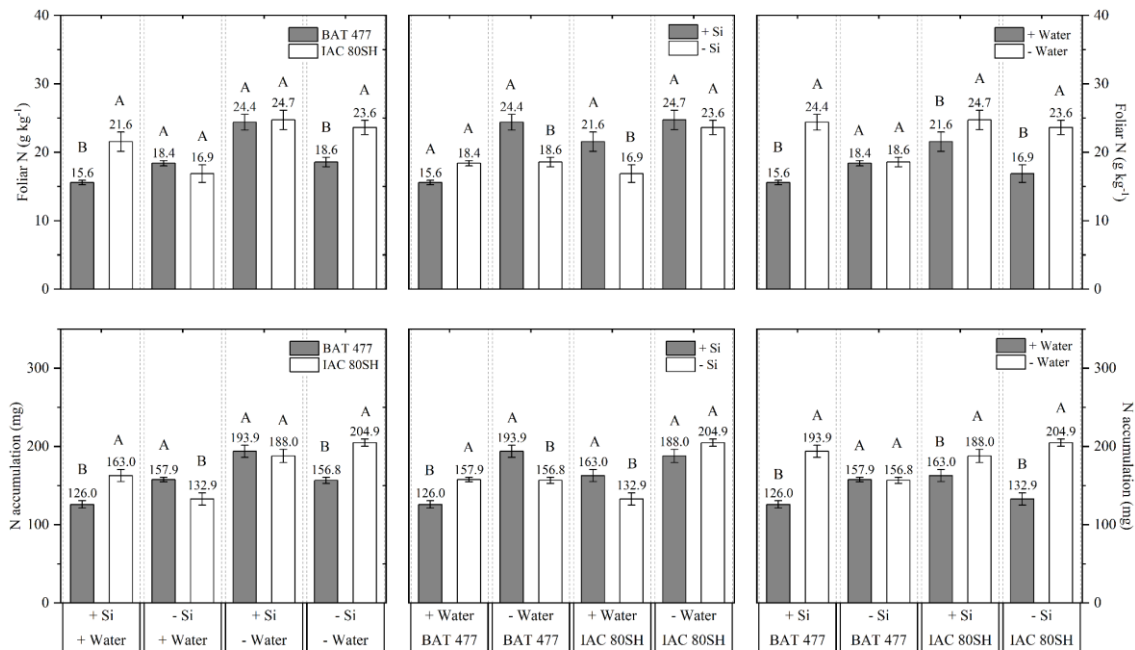
Figure 3.10: Foliar nitrogen (N) concentration and accumulation were evaluated in distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively, tolerant and susceptible to water deficit. Means followed by the same letters are not significantly different between: (a) genotypes in each silicon and water supply level, (b) silicon in each water condition and genotype level, and (c) water regime in silicon supply and genotype levels, according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Our results for N concentration and accumulation in leaves revealed a significant triple interaction among the factors investigated in this study, as well as a consistent pattern. The concentration and accumulation of N were, respectively, 38.5% and 29.4% higher in IAC carioca 80SH than in BAT 477 when under adequate water regime and in the presence of Si (Figure 3.10a and a1). When evaluated from the perspective of plants grown in the presence of Si (Figure 3.10b and b1) at the adequate water level, IAC carioca 80SH exhibited, respectively, 27.8% and 22.6% higher concentration and accumulation of N compared to the tolerant genotype.

On the other hand, when evaluated based on the presence or absence of water, the Si-fed IAC carioca 80SH exhibited higher N concentration and accumulation, respectively, by 14.4% and 15.3% under water deficit compared to the adequate water regime (Figure 3.10c e c1). Furthermore, it is worth noting and remembering that the Si accumulation in these same plants was 42.4% higher than that observed in BAT 477 (Figure 3.9). In other words, IAC carioca 80SH showed similar results for Si and N under water deficit. The literature suggests that plants under stress, in the presence of Si, tend to absorb more Si and exhibit a

behavior closer to normal due to the beneficial effects of this element (Ma et al., 2001; Zargar et al., 2019; Ramírez-Olivera et al., 2021).

The BAT 477 genotype plants grown under water stress conditions showed, respectively, 31.2% and 23.7% higher nitrogen concentration and accumulation when in the presence of silicon compared to without the external addition of the element (Figure 3.10b and b1). Furthermore, when evaluated according to the presence or absence of water, the genotype grown under Si-fed conditions exhibited, respectively, 56.4% and 53.9% higher nitrogen concentration and accumulation in plants under water deficit compared to well-watered plants (Figure 3.10c and c1).

However, a result that draws much attention is the higher nitrogen concentration and accumulation in IAC carioca 80SH under water stress conditions and without external Si addition. In the IAC carioca 80SH genotype, the nitrogen concentration and accumulation were higher, respectively, by 26.9% and 30.7% when the plants were under water deficit and without external Si presence compared to BAT 477 (Figure 3.10a and a1). A similar result is observed from the perspective of water conditions, where the IAC carioca 80SH genotype without Si presence exhibited 39.6% and 54.9% higher nitrogen concentration and accumulation when under water stress compared to well-irrigated conditions (Figure 3.10c and c1).
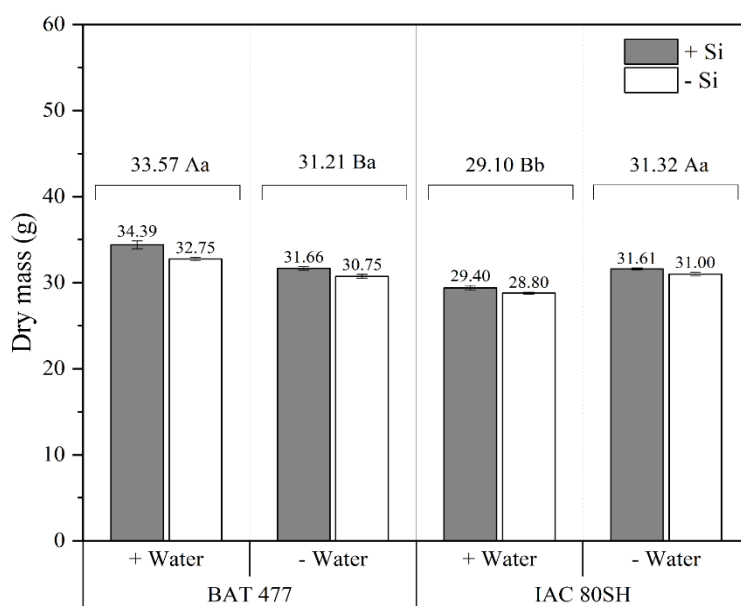
Figure 3.11: The total dry mass of distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively tolerant and susceptible to water deficit, was evaluated. Means followed by the same letters are not significantly different: by water condition at the genotype level (uppercase letters) and between

genotypes at the same water regime level (lowercase letters), according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 4).

The dry mass results showed a double interaction at the level of water regime and observed genotype, with the presence or absence of Si being irrelevant. However, it is possible to evaluate the performance of the genotypes under different water conditions, even by observing their photosynthetic expression and the accumulation of N and Si. The BAT 477 genotype plants grown under adequate water regime accumulated 7.6% more dry mass compared to plants grown under water stress. Additionally, when comparing BAT 477 to IAC carioca 80SH, both under an adequate water regime, BAT 477 accumulated 15.4% more dry mass. On the other hand, observing the performance of the IAC carioca 80SH genotype, the plants grown under water stress accumulated 7.6% more dry mass than the plants grown under adequate water regime (Figure 3.11).
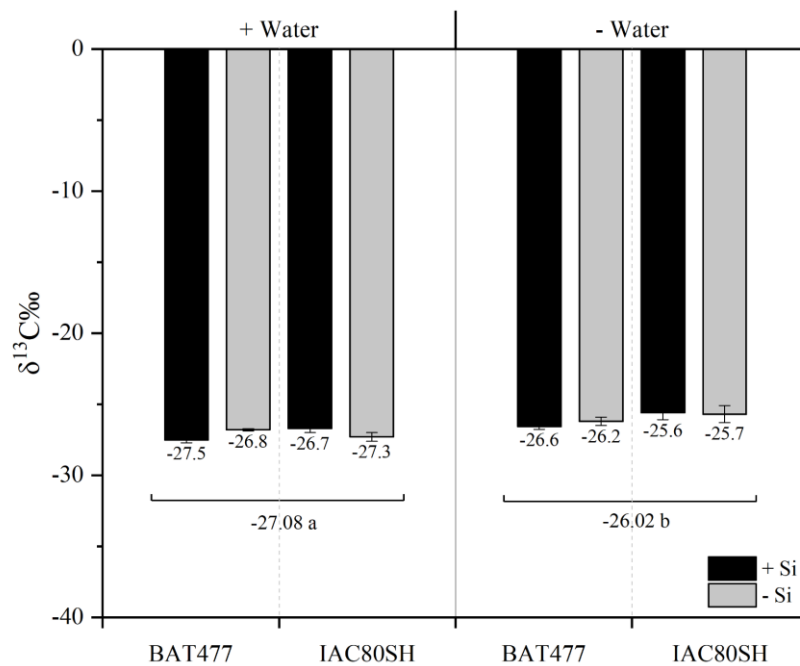
Figure 3.12: Leaf carbon $\delta^{13}C$ (‰) of distinct common bean genotypes, BAT 477 and IAC carioca 80SH, respectively tolerant and susceptible to water deficit, was evaluated. Means followed by the same letters are not significantly different, according to the Tukey test at a significance level of 5%. Bars represent the standard error of the mean (n = 3).

Regardless of the genotype or Si supply, the result for isotopic composition of carbon ($\delta^{13}C$ ‰) at the water deficit level was the highest 4.1% when compared to the well-watered condition (Figure 3.12). In situations of water stress or limited water availability, the uptake of CO_2 will be restricted, leading to a decrease in the leaf intercellular CO_2 concentration to

atmospheric carbon ratio and an increase in $\delta^{13}C$ in the plant. Carbon-13 is heavier than carbon-12, which is one of the reasons for its lower absorption by plants, 13C is present in the atmosphere at 1.1%, while 12C is at 98.9%, with isotopic discrimination being the ratio between 13C and 12C. Also, Rubisco is the most efficient enzyme in discriminating carbon; therefore, the more negative the 13C value, the greater the discrimination by photosynthetic carboxylation enzymes and the more efficient water usage. Therefore, this is an excellent parameter to assess the plant's response when subjected to water stress, particularly in evaluating the potential for greater tolerance to water scarcity (Farquhar and Richards, 1984; Mateus et al., 2021).

3.4   Conclusions

Notwithstanding, our research aimed to validate the use of silicon as a stress mitigator for water deficit in the non-accumulator plant, common bean. The results presented in this work showed that common bean plants can absorb and accumulate the beneficial element, silicon. The mean $\delta^{13}C$ ‰ values under normal water conditions exhibited a greater negative deviation compared to plants exposed to water stress, characterized by stomatal closure. The ^{13}C to ^{12}C ratio serves as a valuable tool for evaluating water stress levels and identifying stress-tolerant genotypes. In summary, plants that do not naturally accumulate silicon (Si) demonstrated an accumulation of this beneficial element within their tissues, predominantly in the leaves, when provided with Si supplementation. Our findings do not align with the existing literature regarding the impact of Si on enhancing photosynthesis and Water Use Efficiency (WUE).

Despite the results found in this study not being relevant for confirming the use of silicon in common beans as an element that improves water use efficiency ($_iWUE$) under water stress or increases the photosynthetic rate even when the plant is under stress, it is worth highlighting that, as mentioned during this research, other studies have found affirmative results, contrasting with our own findings. Nevertheless, we have discovered some positive insights that warrant further investigation.

4 FINAL REMARKS AND OUTLOOK

The utilization of silicon in agriculture as an alternative means to mitigate climate change holds significant promise. Its capacity to enhance stress tolerance, resist diseases and pests, improve nutrient uptake efficiency, and sequester carbon makes it a valuable tool in promoting sustainable and resilient agricultural systems. However, additional research is required to optimize silicon application methods, determine crop-specific responses, and evaluate its long-term effects on soil and ecosystem dynamics.

As the world faces the challenges of climate change and the need for sustainable agriculture, the integration of silicon into farming practices can contribute to building resilient food systems and mitigating the negative impacts of a changing climate. This study allowed us to draw some conclusions about the Si accumulation and kinectis parameters. both genotypes were more efficient in utilizing Si when subjected to a lower dose (0.1 mmol L^{-1}), indicating a high-affinity system for both genotypes. At intermediate doses, the susceptible genotype indicated the activation of a Si exclusion system, which changed with increasing doses. When supplied with 2 mmol L^{-1}, the genotype started to increase the Si content.

The susceptible genotype showed a higher silicon concentration and accumulation in plants, as well as an increase in dry mass. Most of the absorbed and accumulated silicon in the susceptible genotype was found in the leaves. The tolerant genotype did not accumulate more silicon and was not more efficient in fertilizer utilization.

Data from the study to validate the use of silicon as a stress mitigator for water deficit in the non-accumulator plant, common bean, highlighted the urgency and necessity for further research to elucidate Si pathways and its role as a mitigator in non-accumulator plants. Nevertheless, these findings provide valuable insights that warrant additional investigation.

REFERENCES

ASHRAF, M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. **Biotechnology Advances,** v. 27, p. 84–93, 2009.

AZEVEDO, R.A.; ALAS, R.M.; SMITH, R.J.; LEA, P.J. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-tipe and catalase-deficient mutant of barley. **Physiologia Plantarum**, v. 104, p. 280-292, 1998.

BAKER, N.R. Chlorophyll Fluorescence: A probe of photosynthesis in vivo. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 59, p. 89-113, 2008.

BARRIE, A.; PROSSER, S.J. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: BOUTTON, T.W.; YAMASAKI S-I. (Eds.). **Mass spectrometry of soil**. New York: Marcel Dekker, 1996. p. 1-46.

BARRS, H.D.; WEATHERLEY, P.E. A Re-Examination of the Relative Turgidity Techniques for Estimating Water Deficits in Leaves. **Aust. J. Biol. Sci.**, v. 15, p. 413-428, 1962. http://dx.doi.org/10.1071/BI9620413

BATAGLIA, O.C.; FU RLANI, A.M.C.; TEIXEIRA, J.P.F.; FURLANI, P.R.; GALLO, J.R. **Métodos de Análise Química de Plantas**. Campinas: IAC, 1983. 48 p. (Boletim Técnico, 78).

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Bioc.**, v. 72, p. 248-54, 1976.

BOKOR, B.; SANTOS, C.S.; KOSTOLÁNI, D.; MACHADO, J.; SILVA, M.N. da; CARVALHO, S.M.P.; VACULÍK, M.; VASCONCELOS, M.W. Mitigation of climate change and environmental hazards in plants: Potential role of the beneficial metalloid silicon, **Journal of Hazardous Materials**, v. 416, art. 126193, 2021. https://doi.org/10.1016/j.jhazmat.2021.126193.

BOWLING, D.R.; MCDOWELL, N.G.; BOND, B.J.; LAW, B.E.; EHLERINGER, J. 13C content of ecosystem respiration is linked to precipitation and vapor pressure deficit. **Oecologia**, v. 131, p. 113-124, 2002.

BOYER, J.S. Water deficits and photosynthesis. In: KOZLOWSKI, T.T. **Water deficits and plant growth.** New York: Academic Press, 1976. v. 4, p. 153-190.

CARNEIRO, J. M. T., ROSSETE, A. L. R. M., OLIVEIRA, G. S., BENDASSOLI, J. A. Versatile Flow Injection System for Spectrophotometric Determination of Silicon in Agronomic Samples. **Soil Science and Plant Analysis**, v. 38, p. 1411–1423, 2007. DOI: 10.1080/00103620701377245

CHAI, QIANG; GAN, YANTAI; ZHAO, CAI; XU, HUI-LIAN; WASKOM, REAGAN M.; NIU, YINING; SIDDIQUE, KADAMBOT H. M. Regulated deficit irrigation for crop production under drought stress. A review. **Agron. Sustain**. v. 36, 2016. https://doi.org/10.1007/s13593-015-0338-6

CHAVES, M.M.; FLEXAS, J.; PINHEIRO, C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. **Annals of Botany**, v. 4, p. 551-560, 2009.

CHAVES, M.M.; PEREIRA, J.S.; MAROCO, J.; RODRIGUES, M.L.; RICARDO, C.P.P.; OSÓRIO, M.L.; CARVALHO, I.; FARIA, T.; PINHEIRO, C. How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. **Annals of Botany**, v. 89, p. 907–916, 2002. https://doi.org/10.1093/aob/mcf105

CHEN, C.H.; LEWIN, J. Silicon as a nutrient element for *Equisetum arvense*. **Canadian Journal of Botany**, v, 47, p. 125-131, 1969.

CHEN, D.; WANG, S.; YIN, L.; DENG, X. How Does Silicon Mediate Plant Water Uptake and Loss Under Water Deficiency? **Frontiers in Plant Science**, v. 5, art. 281, 2018. doi: 10.3389/fpls.2018.00281.

CIAT – Centro Internacional de Agricultura Tropical. **Phenotyping common beans for adaptation to drought: protocol for field evaluation.** Disponível em: https://cgspace.cgiar.org/bitstream/handle/10568/41626/field_evaluation_final.pdf?sequence=1&isAllowed=y Acessado em: 25 set. 2018.

CLAASSEN, N.; BARBER, S.A. A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. **Plant Physiology**, v. 54, p. 564-568, 1974.

CRAIG, H. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. **Geochimica et Cosmochimica Acta**, v. 12, p. 133-149, 1957.

CRIPPA, M.; SOLAZZO, E.; GUIZZARDI, D.; MONFORTI-FERRARIO, F.; TUBIELLO, F.N.; LEIP, A. Food systems are responsible for a third of global anthropogenic GHG emissions. **Nat Food.,** v. 2, p. 198–209, 2021. https://doi.org/10.1038/s43016-021-00225-9

CURRIE, H.A.; PERRY, C. Silica in plants: Biological, biochemical and chemical studies. **Ann. Bot**., v. 100, p. 1383-1389, 2007.

DEBONA, D.; Rodrigues, F.A.; DATNOFF, L.E. Silicon's role in abiotic and biotic plant stresses. **Annu. Rev. Phytopathol.,** v. 55, p. 4.1-4.23, 2017.

DOOREMBOS, J.; KASSAM, A. H. **Yield response to water**. Rome: FAO, 1979. p. 193, art. 33.

ELLIOT, C.L.; SNYDER, G.H. Autoclave – Induced digestion for the colorimetric determination of silicon in rice straw. **J. Agric. Food Chem.**, v.39, n. 6, p. 1118-1119, 1991.

EPSTEIN, E. Silicon. **Annu. Rev. Plant Physiol. Plant Mol.,** v. 50, p. 641-644, 1999.

EPSTEIN E. Silicon: its manifold roles in plants. **Ann. Appl. Biol.,** v. 155, p. 155–160, 2009.

EPSTEIN, E.; BLOOM, A. J. **Mineral Nutrition of Plants**: principles and perspectives. 2. ed. Massachusetts: Sinauer Associates, 2006. 380 p.

EPSTEIN, E.; HAGEN, C.E. A kinetic study of the absorption of alkali cations by barley roots. **Plant Physiol.**, v. 27, p. 457-474, 1952.

FAO. **The future of food and agriculture**. Trends and challenges. Rome, 2017. https://www.fao.org/3/i6583e/i6583e.pdf

FAO. **The future of food and agriculture -** Alternative pathways to 2050. Rome, 2018. 60 p. License: CC BY-NC-SA 3.0 IGO. https://www.fao.org/3/CA1553EN/ca1553en.pdf. 2018.

FARQUHAR, G.D.; EHLERINGER, J.R., HUBICK, K. T. Carbon isotope discrimination and photosynthesis. **Annual Review of Plant Physiology and Plant Molecular Biology**, v . 40, p. 503-537, 1989.

FARQUHAR, G.D.; O'LEARY, M.H.; BERRY, J.A. On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. **Australian Journal of Plant Physiology**, v. 9, p. 121-137, 1982.

FERREIRA, D., F. Sisvar: um sistema computacional de análise estatística. **Ciênc. Agrotec**. v. 5, 2011 https://doi.org/10.1590/S1413-70542011000600001

FERNÁNDEZ, F.; GEPTS, P.; LOPEZ, M. **Etapas de desarrollo de la planta de frijol común (*Phaseolus vulgaris* L.**). Cali: CIAT, 1986. 34 p.

FRANÇA, M.G.C.; THI, A.T.P.; PIMENTEL, C.; ROSSIELLO, R.O.P.; ZUILY-FODIL, Y.; LAFFRAY, D. Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. **Environmental and Experimental Botany**. V. 43, p. 227–237, 2000. https://doi.org/10.1016/S0098-8472(99)00060-X

FREW, A.; POWELL, J.R.; SALLAM, N.; ALLSOPP, P.G.; JOHNSON, S.N. Trade-offs between silicon and phenolic defenses may explain enhanced performance of root herbivores on phenolic-rich plants. **J. Chem. Ecol.** v. 42, p.768-771, 2016.

FREW, A.; WESTON, L.A.; REYNOLDS, O.L.; GURR, G.M. The role of silicon in plant biology: a paradigm shift in research approach. **Ann. Bot.**, v. 121, p. 1265–1273, 2018. doi: 10.1093/aob/mcy009

GAO, X.; ZOU, C.; WANG, L.; ZHANG, F. Silicon decreases transpiration rate and conductance from stomata of maize plants. **J. Plant Nutr**., v. 29, p. 1637-1647, 2006.

GIANNOPOLITIS, C.N; RIES, S.K. Superoxidedismutases: I. Occurrence in higher plants. **Plant Physiol.**, v. 59, p. 309-314, 1977.

GONG H.; CHEN K. The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in feld drought conditions. **Acta Physiol. Plant.,** v. 34, p. 1589–1594, 2012.

GUIMARÃES, C. M. Relações hídricas. In: ARAUJO, R.S.; RAVA, C.A.; STONE, L.F.; ZIMMERMANN, M.J.O. (Ed.). **Common bean crop in Brazil**. Piracicaba: Potafos**,** 1996. p. 139-168.

HASSAN ETESAMI; BYOUNG RYONG JEONG. Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants, **Ecotoxicology and Environmental Safety**, v. 147, p 881-896, 2018. https://doi.org/10.1016/j.ecoenv.2017.09.063.

HEATH, R.L.; PACKER, L. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. **Arch. Bioc. Bioph.**, v. 125, p. 2141-2145, 1968.

HERNÁNDEZ, I.; ALEGREL, L.; MUNNE´-BOSCH, S. Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions. **Tree Physiol**, v. 24, p. 1303–1311, 2004.

HOAGLAND, D.R.; ARNON, D. I. **The water culture method for growing plants without soils**. Berkeley: California Agricultural Experimental Station, 1950. 347 p.

HOSSEINI, S.A.; MAILLARD, A.; HAJIREZAEI, M.R.; ALI, N.; SCHWARZENBERG, A.; JAMOIS, F.; YVIN, J.C. Induction of barley silicon transporter HvLsi1 and HvLsi2, increased silicon concentration in the shoot and regulated Starch and ABA Homeostasis under osmotic stress and concomitant potassium deficiency. **Frontiers in Plant Science**, v. 8., p. 1359, 2017).

IPCC. A Report of the Intergovernmental Panel on Climate Change. CLIMATE CHANGE 2023 **Synthesis Report**. https://www.ipcc.ch/report/ar6/syr/

JEER, M.; TELUGU, U.M.; VOLETI, S.R.; PADMAKUMARI, A.P. Soil application of silicone reduces yellow stem borer, *Scirpophaga incertulas* (Walker) damage in rice. **J. App. Ent.**, v. 141, p. 189-201, 2017.

KANG, J.; ZHAO, W.; ZHU, X. Silicon improves photosynthesis and strengthens enzyme activities in the C3 succulent xerophyte *Zygophyllum xanthoxylum* under drought stress. **J. Plant Physiol.**, v. 199, p. 76–86, 2016.

KIM, Y. H.; KHAN, A. L.; SHINWARI, Z. K.; KIM, D. H.; WAQAS, M.; KAMRAN, M. Silicon treatment to rice (*Oryza sativa* L. cv.'Gopumbyeo') plants during different growth periods and its effects on growth and grain yield. **Pak. J. Bot.**, v. 44, p. 891–897, 2012.

KIM, Y. H.; KHAN, A. L.; WAQAS, M.; SHAHZAD, R.; LEE, I. J. Silicon-mediated mitigation of wounding stress acts by up-regulating the rice antioxidant system. **Cereal Res. Commun.**, v. 44, p. 111–121, 2016. doi: 10.1556/0806.43.2015.031

KONAPALA, G., MISHRA, A. K., WADA, Y., MANN, M. E. Climate change will affect global water availability through compounding changes in seasonal precipitation and evaporation. **Nat Commun**., v. 11, p. 3044, 2020. https://doi.org/10.1038/s41467-020-16757-w

KRAUS, T.E.; MCKERSIE, B.D.; FLETCHER, R.A. Paclobutrazol-induced to tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. **J. Plant Physiol.**, v. 145, p. 570-576, 1995. doi: 10.1016/S0176-1617(11)81790-6

KRISHAN K. VERMA, PRATIKSHA SINGH, XIU-PENG SONG, MUKESH KUMAR MALVIYA, RAJESH KUMAR SINGH, GAN-LIN CHEN, SUSHIL SOLOMON, AND YANG-RUI LI. "Mitigating Climate Change for Sugarcane Improvement: Role of Silicon in Alleviating Abiotic Stresses" **Sugar Tech** v. 22, p. 741-749, 2020. doi: 10.1007/s12355-020-00831-0

KURDALI, F.; AL-CHAMMAA, M. Growth and nitrogen fixation in silicon and/or potassium fed chickpeas grown under drought and well-watered conditions. **J. Stress Physiol. Biochem.**, v. 9, p. 385–406, 2013.

KUMAR, S., MILSTEIN, Y., BRAMI, Y., ELBAUM, M., ELBAUM, R. Mechanism of silica deposition in sorghum silica cells. **New Phytologist**, v. 213, p. 791–798, 2017. https://doi.org/10.1111/nph.14173

LIANG Y; SUN W; ZHU YG; CHRISTIE P. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. **Environ Pollut**. v. 8, p. 422, 2007. doi: 10.1016/j.envpol.2006.06.008.

MA, D.; SUN, D.; WANG, C. Silicon application alleviates drought stress in wheat through transcriptional regulation of multiple antioxidant defense pathways. **J. Plant Growth Regul.,** v. 35, p. 1–10, 2016. doi:10.1007/s00344-015-9500-2.

MA, J.F.; TAKAHASHI, E**. Soil, Fertilizer, and Plant Silicon Research in Japan**. Amsterdam: Elsevier, 2002.

MA, J.F.; YAMAJI, N.; MITANI-UENO, N. Transport of silicon from roots to panicles in plants. **Proc. Jpn. Acad. Ser. B.**, v. 87, p. 377–85, 2011.

MARSCHNER, H.; OBERLE, H.; CAKMAK, I.; RÖMHELD, V. Growth enhancement by silicon in cucumber (Cucumis sativus) plants depends on imbalance in phosphorus and zinc supply. **Plant and Soil,** v. 124, p. 211–219, 1990. https://doi.org/10.1007/BF00009262

MATEOS-NARANJO, E.; GALLE, A.; FLOREZ-SARASA, I.; PERDOMO, J. A.; GALMÉS, J.; IBAS-CARBÓ, M. Assessment of the role of silicon in the Cutolerance of the C 4 grass Spartina densiflora. **J. Plant Physiol.**, v. 178, p. 74–83, 2015. doi: 10.1016/j.jplph.2015.03.001

MENEGAT, S.; LEDO, A.; TIRADO, R. Greenhouse gas emissions from global production and use of nitrogen synthetic fertilisers in agriculture. **Sci Rep** v. 12, p. 14490, 2022. https://doi.org/10.1038/s41598-022-18773-w

MEENA, V.D., DOTANIYA, M.L., COUMAR, V.; RAJENDIRAN S.; AJAY; KUNDU S. RAO A. S. A Case for Silicon Fertilization to Improve Crop Yields in Tropical Soils. **Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci**. v. 84, p. 505–518, 2014. https://doi.org/10.1007/s40011-013-0270-y

MONAKHOVA, O.F.; CHERNYAD'EV, II. Protective role of kartolin-4 in wheat plants exposed to soil draught. **App. Biochem. Microbiol.**, v. 38, p. 373–380, 2002.

MOREIRA, J.A.A.; SILVEIRA, P.M.; STONE, L.F. Irrigação. In: ARAÚJO, R.S.; RAVA, C.A.; STONE, L.F.; ZIMMERMANN, M.J.O. (Ed.). **Common bean crop in Brazil**. Piracicaba: **Potafos**, p. 465-522, 1996.

MURATA, S.; TAKAHASHI, E.; MATOH, T. Effect of silicate application on photosynthesis of rice [Oryza sativa] plants Japanese **Journal of Soil Science and Plant Nutrition**, v. 62. p. 248-251, 1991.

NWUGO, C.C; HUERTA, A.J. Effects of silicon nutrition on cadmium uptake, growth and photosynthesis of rice plants exposed to low-level cadmium. **Plant Soil**, v. 311, p. 73-86, 2008.

OLIVEIRA, M.G. de C.; OLIVEIRA, L.F.C. de; WENDLAND, A.; GUIMARÃES, C.M.; QUINTELA, E.D.; BARBOSA, F.R.; CARVALHO, M. da C.S.; LOBO JUNIOR, M.; SILVEIRA, P.M. da. **Conhecendo a fenologia do feijoeiro e seus aspectos fitotécnicos**. Etapa de desenvolvimento da planta. Santo Antônio de Goiás: Embrapa Arroz e Feijão, 2018. 59 p. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/173690/1/CNPAF-2018-lvfeijoeiro.pdf

PILON-SMITS E.A.; QUINN C.F.; TAPKEN W.; MALAGOLI M.; SCHIAVON M. Physiological functions of beneficial elements. **Curr Opin Plant Biol.**, v. 3, p. 267-274, 2009. doi: 10.1016/j.pbi.2009.04.009.

POLANCO LR, RODRIGUES FA, NASCIMENTO KJT, CRUZ MFA, CURVELO CRS. Photosynthetic gas exchange and antioxidative system in common bean plants infected by *Colletotrichum lindemuthianum* and supplied with silicon. **Trop. Plant Pathol.,** v. 39, p.35–42, 2014.

RABÊLO, F. H. S., JORDÃO, L. T., LAVRES, J. A glimpse into the symplastic and apoplastic Cd uptake by Massai grass modulated by sulfur nutrition: Plants well-nourished with S as a strategy for phytoextraction. **Plant Physiology and Biochemistry**. v. 121 p. 48–57, 2017.

RAHMAN, A.; WALLIS, C.M.; UDDIN, W. Silicon-induced systemic defense responses in perennial ryegrass against infection by *Magnaporthe oryzae*. **J. Phytopathol.,** v. 105, p. 748–57, 2015.

REIS, A. R., LAVRES, J. **Genotypic Influence on the Absorption, Use and Toxicity of Manganese by Soybean**. Rijeka, Croacia: InTech, 2011. doi: 10.5772/14596.

RUIZ, H.A.; FERNANDES FILHO, E.I. Cinética: Software para estimar as constantes Vmax e Km da equação de Michaelis-Menten. In: Reunião Brasileira de Fertilidade do Solo e Nutrição de Plantas, 1992, Piracicaba. **Anais.** Piracicaba, Sociedade Brasileira de Ciência do Solo, 1992. p. 124-125.

SCHMUTZ, J.; MCCLEAN, P.E.; MAMIDI, S.; WU, G.A.; CANNON, S.B.; GRIMWOOD, J.; JENKINS, J.; SHU, S.; SONG, Q.; CHAVARRO, C.; TORRES-TORRES, M.; GEFFROY, V.; MOGHADDAM, S.M.; GAO, D.; ABERNATHY, B.; BARRY, K.; BLAIR, M.; BRICK, M.A.; CHOVATIA, M.; GEPTS, P.; GOODSTEIN, D.M.; GONZALES, M.; HELLSTEN, U.; HYTEN, D. L.; JIA, G.; KELLY, J.D.; KUDRNA, D.; LEE, R.; RICHARD, M.M.S.; MIKLAS, P.N.; OSORNO, J.M.; RODRIGUES, J.; THAREAU, V.; URREA, C.A.; WANG, M.; YU, Y.; ZHANG, M.; WING, R.A.; CREGAN, P.B.; ROKHSAR, D.S.; JACKSON, S. A. A reference genome for common bean and genome-wide analysis of dual domestications.**Nat. Genet.**, v. 46, n. 7, p. 707–13, 2014.

SHEN, X.; ZHOU, Y.; DUAN, L.; LI Z.; ENEJI, A.E.; LI, J. Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation **J. Plant Physiol**., v. 167, p. 1248-1252, 2010.

SOURESHJANI, H. K.; NEZAMI, A.; KAFI, M.; TADAYON. M. Responses of two common bean (Phaseolus vulgaris L.) genotypes to deficit irrigation. **Agricultural Water Management**, v. 213, p. 270–279, 2019. https://doi.org/10.1016/j.agwat.2018.09.038

STETTER, C.; SAUER, J. Greenhouse Gas Emissions and Eco-Performance at Farm Level: A Parametric Approach. **Environ Resource Econ**, v. 81, p. 617–647, 2022. https://doi.org/10.1007/s10640-021-00642-1

STOUT, P.R.; ARNON, D.I. Experimental methods for the study of the role of cooper, manganese, and zinc in the nutrition of higher plants. **American J. Bot**., v. 26, p. 144-149, 1939.

VAUGHAN, M.M.; BLOCK, A.; CHRISTENSEN, S.A.; ALLEN L.H; SCHMELZ E.A. The effects of climate change associated abiotic stresses on maize phytochemical defenses. **Phytochem. Rev.**, v. 17, p. 37–49, 2018. https://doi.org/10.1007/s11101-017-9508-2

WANG, M.; WANG, R., MUR; L.A.J; RUAN J.; SHEN Q.; GUO S. Functions of silicon in plant drought stress responses. **Hortic. Res.,** v. 8, p. 254, 2021. https://doi.org/10.1038/s41438-021-00681-1 https://www.nature.com/articles/s41438-021-00681-1

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of Plant Physiology**, v. 144, p. 307-313, 1994.

ZAMBROSI, F.C.B.; MATTOS JR.; SYVERTSEN, J.P. Plant growth, leaf photosynthesis, and nutrient-use efficiency of citrus rootstocks decrease with phosphite supply. **Journal of Plant Nutrition and Soil Science**, v. 174, p. 487-495, 2011.

ZARGAR, S.M.; MAHAJAN, R.; BHAT, J.A.; NAZIR, M.; DESHMUKH, R. Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. **3 Biotech**., v. 9, art. 73, 2019. doi: 10.1007/s13205-019-1613-z.

ZHANG, Y.; SHI, Y.; GONG, H.; ZHAO H.; LI H.; HU Y.; WANG, Y. Beneficial effects of silicon on photosynthesis of tomato seedlings under water stress. **Journal of Integrative Agriculture**, v. 17, p. 2151–2159, 2018. doi: 10.1016/S2095-3119(18)62038-6.