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SÂMIA PAIVA DE OLIVEIRA

SILÍCIO: ALÍVIO A ESTRESSES ABIÓTICOS EM AMBIENTES NATURAIS

FORTALEZA

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Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, da Universidade Federal do Ceará, como parte dos requisitos para obtenção do título de Doutora em Ecologia e Recursos Naturais. Área de concentração: Conservação e Manejo dos Recursos Naturais.

Orientador: Prof. Dr. Teogenes Senna de Oliveira

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RESUMO

O Silício (Si) foi, durante muito tempo, ignorado pelos ecologistas de planta. No entanto, é inegável sua participação nos ecossistemas terrestres. A hipótese desse trabalho é que as plantas, em ambientes naturais, são submetidas a estresses abióticos, isolados ou combinados, que podem estimular a absorção de Si. Assim, essas plantas são igualmente beneficiadas pela absorção e acúmulo do elemento. Deste modo, foram levantadas as seguintes previsões: I) As concentrações de silício disponível na solução do solo ampliariam a distribuição espacial e consequente produção de biomassa de plantas, II) populações de plantas localizadas em ambientes com condições edafoclimáticas adversas terão a absorção e acúmulo de silício maiores, a fim de desenvolver mecanismos de resistência ao estresse fisiológico estabelecido. Para testar essas previsões, foi selecionada a espécie *Eugenia punicifolia*, de ampla distribuição geográfica, e que ocupa três diferentes fitofisionomias com condições abióticas limitantes no Estado do Ceará: Savana Costeira, Arbustaria Densa Decídua e Floresta Estacional Decídua. Inicialmente, foi estudado se a espécie apresentava características germinativas que indicasse uma aclimação da mesma aos seus locais de origem. Em seguida, foi realizada uma metanálise com os artigos publicados que tratam dos efeitos amenizadores de estresse do Si, no intuito de identificar se seus efeitos são comprovados, se existem variação na resposta ao elemento entre as espécies, se os resultados positivos são corroborados ao longo do tempo e se existem avanços no conhecimento sobre os mecanismos de alívio a estresses pelo elemento. Foram conduzidos experimentos em laboratório e casa de vegetação que simulassem condições climáticas de estresses comuns às áreas selecionadas e avaliados a absorção do Si, concentração nos tecidos e produção de biomassa de plantas. Por fim, esses mesmos parâmetros foram analisados em campo, nas três fitofisionomias, no intuito de identificar alterações estruturais e ou fisiológicas ocasionadas pelo Si em plantas sob estresse abióticos em condições naturais, e se essas facilitarão a compreensão dos mecanismos de alívio a estresses abióticos em estudos ecológicos. Observou-se que a espécie apresenta adaptação ao clima de seu habitat natural, o que ajuda a compreender sua ampla colonização em ambientes climaticamente distintos e que o cumprimento dos requisitos de temperatura, garantem maior sucesso em germinação. Confirmou-se nos estudos com Si, que não existe diferenças nas respostas em relação aos tipos de estresse a que as plantas são submetidas, o que confere um efeito mais geral do elemento. Além disso, a principal novidade é que o efeito amenizador do elemento vem diminuindo ao longo dos anos de estudo sobre o mesmo. Em laboratório, observou-se que a absorção de Si pela espécie estudada é aumentada pelo estresse hídrico e variações na temperatura quando as

plantas são originárias de fitofisionomias com maior disponibilidade de Si no solo. Por fim, as plantas de *E. punicifolia* absorvem mais Si do solo quando estimuladas pela presença de fatores limitantes, refletindo mais uma vez, adaptações aos seus locais de origem. Assim, esse estudo permite inferir sobre funções de alívio do Si a estresses em sistemas naturais, confirmando os benefícios do elemento já discutidos para culturas agrícolas.

Palavras-chave: biomassa; distribuição geográfica; absorção; ecofisiologia; aclimação.

ABSTRACT

Silicon (Si) has long been ignored by plant ecologists. However, their participation in terrestrial ecosystems is undeniable. The hypothesis of this work is that plants in natural environments are subjected to isolated or combined abiotic stresses that can stimulate the uptake of Si. Thus, these plants are also benefited by the absorption and accumulation of the element. In this way, the following predictions were made: I) The concentrations of silicon available in the soil solution would increase the spatial distribution and consequent biomass production of plants, favoring their occupation in areas with limiting conditions, II) plant populations located in environments with adverse edaphoclimatic conditions that result in physiological stresses in the plant will have the greater absorption and accumulation of silicon in order to develop mechanisms of resistance to established physiological stress. To test these predictions, the *Eugenia punicifolia* species, with a wide geographic distribution, was selected and occupies three different phytophysionomies with limiting abiotic conditions in the State of Ceará: Coastal Savanna, Seasonal Deciduous Forest, and Dense Deciduous Shrub. Initially, it was studied whether the species presented dormancy or germination characteristics that indicated an acclimatization of the same to their places of origin. Subsequently, a meta-analysis was carried out with the published articles dealing with the stress-damaging effects of Si, in order to identify if their effects are proven, if there are variations in response to the element between species, if the positive results are corroborated over of the time and if there are advances in the knowledge about the mechanisms of stress relief by the element. Experiments were conducted in a laboratory and greenhouse to simulate climatic conditions of stresses common to the areas selected for this study and evaluated the Si absorption, tissue concentration and biomass production of plants. Finally, these same parameters were analyzed in field, on the three phytophysionomies, in order to identify structural and/or physiological changes caused by the Si in plants under stress under natural conditions, and if these would facilitate the understanding of the mechanisms of relief to abiotic stresses in ecological studies. It was observed that the species presents adaptation to the climate of its natural habitat, which helps to understand its wide colonization in climatically distinct environments. It was confirmed in studies with Si, that there are no differences in the responses to the types of stress (water and temperature) at which the plants are submitted, which gives a more general effect of the element. In addition, the main novelty is that the smoothing effect of the element has been decreasing over the years of study on it. In the laboratory, it was observed that the absorption of Si by the studied species is increased by water stress and temperature variations when the plants originate from

phytophysiognomies with higher availability of Si in the soil. Finally, under natural conditions, the plants of *E. punicifolia* absorb more Si from the soil when stimulated by the presence of limiting factors, reflecting once again, adaptations to their places of origin. Thus, this study allows inferring Si stress relief functions in natural systems, confirming the benefits of the element already discussed for agricultural crops.

Keywords: biomass; geographic distribution; absorption; ecophysiology; acclimatization.

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1 INTRODUÇÃO GERAL

O Silício (Si) foi, durante muito tempo, ignorado pelos ecologistas de planta. No entanto, é inegável sua participação nos ecossistemas terrestres, pois o mesmo é o oitavo elemento mais comum na natureza e o segundo mais comum encontrado no solo após o oxigênio (SAHEBI et al., 2015). Apesar de ser possível encontrar Si em todas as plantas, a maior parte das pesquisas sobre Si vem de áreas afins como agronomia, paleontologia e biogeoquímica (COOKE; LEISHMAN, 2011).

As funções mais bem estudadas do Si são provenientes de estudos realizados em plantas domesticadas, associando o elemento a uma gama de estresses abióticos, que incluem: toxicidade por metais pesados (LU et al., 2014; DRESLER et al., 2015), desequilíbrios funcionais (MA; TAKAHASHI, 2002), salinidade (LIU et al., 2014; SIDDIQUI et al., 2014), temperaturas elevadas (LIANG et al., 2008) e estresse hídrico (HABIBI, 2014; SAYED; GADALLAH, 2014). Conseqüentemente, são aplicados, rotineiramente, em muitos sistemas agrícolas fertilizantes contendo Si para melhorar o crescimento da planta e aliviar estresses (DATNOFF et al., 2001).

Embora quase todos os estudos tenham sido conduzidos com espécies agrícolas, esses estudos têm incorporado uma série de famílias incluindo Asphodelaceae (XU et al., 2015), Asteraceae (SAVVAS et al., 2002), Cucurbitaceae (SHI et al., 2005), Fabaceae (IWASAKI et al., 2002), Pinaceae (RYDER et al., 2003), Poaceae (LIANG et al., 2003), Rosaceae (TREDER; CIESLINSKI, 2005), Solanaceae (ROMERO-ARANDA et al., 2006) e Mirtaceae (CARVALHO et al., 2003; RAMOS et al., 2009).

Se o Si contribui para resistência celular e permite que as plantas respondam de forma adaptativa aos estresses ambientais, o elemento pode aumentar a aptidão da planta em muitos aspectos fundamentais da ecologia, incluindo interações com o ambiente, aclimações e alívio a estresses abióticos em comunidades naturais de plantas (COOKE; LEISHMAN, 2011). Elementos comuns de estresses abióticos, como temperatura, umidade e toxidez por metais pesados, são importantes fatores ecológicos que servem de estímulos para desenvolvimento de mecanismos de resistência em plantas superiores (ECKARDT et al., 2001; KENNEDY; WILSON, 2004). E, de acordo com Fauteux et al. (2005), os efeitos benéficos do Si serão mais evidentes quando as plantas estiverem submetidas a algum tipo de estresse.

Se as vantagens oferecidas pelo Si estão diretamente ligadas ao estresse a que as plantas estão submetidas, a ocorrência de vegetação em áreas com condições climáticas adversas poderia estar condicionada à maior absorção de Si pelas plantas, ou ainda à existência

de algum mecanismo de resistência a estresse, associado ao elemento. Identificar alterações estruturais e ou fisiológicas ocasionadas pelo Si em plantas sob estresse em condições naturais poderia facilitar a compreensão dos mecanismos de alívio a estresses em estudos ecológicos. Espécies amplamente distribuídas, que ocorrem em diferentes tipos de vegetação, como *Eugenia punicifolia* (Kunth) DC, representam um bom estudo de caso para analisar o efeito de mudanças nas condições ambientais e a absorção do Si pelas plantas e identificar mecanismos de resistência desencadeados pelo elemento. Nesse estudo, é possível se fornecer uma perspectiva ecológica para pesquisas em diversas áreas, confirmando que o Si é um elemento importante na ecologia vegetal e que merece maior atenção dos cientistas dessa área.

O presente trabalho foi desenvolvido com o objetivo de ampliar o conhecimento acerca do Si para comunidades de plantas naturais, bem como sobre aclimações de plantas da mesma espécie que ocupa diferentes fitofisionomias a estresses abióticos, favorecidas pelo elemento. Os aspectos levantados acima foram abordados em quatro manuscritos, onde cada um busca responder questões específicas a respeito do papel do Si em aliviar estresses em ambientes naturais. Os manuscritos foram delimitados pelas seguintes perguntas: **Manuscrito 1**- A espécie estudada exibe dormência fisiológica a germinação? E existem variações nas taxas de germinação entre as populações de diferentes fitofisionomias com condições climáticas distintas, quando submetidas à mesma temperatura? Baseia-se na hipótese de que os requisitos para a germinação das sementes e a presença de mecanismos de dormência, são frequentemente uma consequência da adaptação da espécie a ambientes imprevisíveis e / ou sazonais e podem ajudar a entender a ampla colonização da espécie objeto desse estudo. **Manuscrito 2** – Existe um efeito positivo do silício em plantas sob estresses abióticos? E as plantas submetidas a diferentes tipos de estresse abiótico são igualmente beneficiadas pela presença de Si no solo? Esse manuscrito provê uma perspectiva numérica usando dados de pesquisas publicadas anteriormente com Si e em várias áreas de estudo, em diferentes espécies de plantas sujeitas a estresse abiótico e discute avanços relacionados com o estudo de silício, juntamente com os efeitos no alívio de estresses ao longo do tempo. Esse manuscrito trata-se da primeira meta-análise que avalia os efeitos de silício em plantas sob condições de estresse, se tratando de uma informação nova e relevante para os cientistas de plantas. Além disso, discute se o efeito do elemento permanece constante mesmo após anos de estudos relacionados à sua ação. **Manuscrito 3** – Estresses abióticos aumentam a absorção de Si pelas plantas? A presença de Si estimula um melhor desenvolvimento das plantas em ambientes naturais? E As plantas de diferentes ambientes diferem também na absorção de Si quando em condição de estresse? O objetivo deste trabalho foi avaliar a absorção de Si e produção de biomassa em plantas da

mesma espécie, originárias de diferentes fisionomias e submetidas a estresses causados por altas e baixas temperaturas e estresse hídrico, visando identificar diferenças nas respostas e se essas respostas são diferentes nas plantas oriundas de fitofisionomias distintas. Por fim, o **Manuscrito 4** – Os efeitos benéficos do Si também são observados nas plantas em seu ambiente natural? Existe diferença no efeito do Si quando as plantas são submetidas a estresses simultâneos no campo? Esse manuscrito considera os efeitos benéficos do elemento, amplamente documentados para as plantas cultivadas submetidas a condições de estresse, e os relaciona a alívios a estresses ambientais em condições naturais a que *E. punicifolia* é submetida. O estudo surgiu da necessidade de compreender e documentar possíveis relações de alívio a estresses abióticos pelo Si em ambientes naturais e objetiva relacionar estresses abióticos simultâneos com a presença do elemento no solo e a distribuição de uma espécie vegetal.

2 MANUSCRITOS

2.1 Manuscrito 1

Germination requirements for the seeds of a widely distributed species: a case study

Abstract

Requirements for seed germination, and the presence of dormancy mechanisms, are often a consequence of species adaptation to unpredictable and/or seasonal environments. Temperature is one of the abiotic variables that affect germination, but responses to that variable can be different in populations of species that occur along climatic gradients. Seeds of *Eugenia puniceifolia* were obtained from three plant physiognomies: Coastal Savanna, Seasonal Deciduous Forest, and Dense Deciduous Shrub; and two experiments were conducted: 1) a test for breaking seed dormancy, and 2) the seeds submitted to three temperatures: 15, 25 and 35°C after mechanical scarification. The species displays a degree of dormancy, possibly physical and confirmed by the imbibition test, which justifies the use of tests of breaking dormancy in this study. The use of treatments to break dormancy, when complying with temperature requirements, ensures greater success in germination. Variations in the rate of germination at different temperatures are the result of the climate conditions to which the plants are subjected in their places of origin, and reflect acclimatisation of the species. This condition may help in understanding the broad colonisation of the species in climatically different environments.

Keywords: acclimatisation; mechanical scarification; phenotypic plasticity; rate of germination. risk spreading.

2.1.1 Introduction

Germination is a critical stage in the life cycle of plants, particularly in areas with seasonally dry tropical climates (KRICHEN et al., 2014). In such environments, the requirements for seed germination are often the consequence of species adaptation (MEYER et al., 1990; DÜRR et al., 2015). Some of the factors that control seed germination are genetic in nature, as for example, seed dormancy and integument thickness (GUTTERMAN, 1993), while others depend on the environment, such as water, light and temperature (DÜRR et al., 2015).

Temperature is one of the abiotic factors that most affect seed germination (BEWLEY; BLACK, 1994; BASKIN; BASKIN, 1998; VERMA et al., 2010; KUMAR et al., 2011), and has been linked to the inhibition or stimulation of seed germination in several forest species (TEKETAY, 1994; AMRI, 2010; CHANYENGA et al., 2012). However, germination response to temperature can vary in populations of species which occur along a climatic gradient due to selection pressure, which results in a suitable phenotypic variation for each climatic condition (RIBEIRO; COSTA, 2015). Phenotypic variation in populations can increase the number of regeneration niches for the species by increasing the range of conditions at which germination takes place, being a strategy for spreading risk, important for survival in environments that are climatically unpredictable (PHILIPPI, 1993).

The environment may have a proven effect on seed dormancy and germination (JAYASURIYA et al., 2007). Seeds which ripen under different environmental conditions may also display different levels of dormancy (i.e. ROACH; WULFF, 1987; BENECH-ARNOLD, 2004). Dormancy is an intrinsic factor of seeds (Footitt et al. 2011), and is fundamental in mediating the interaction between the ecological environment and seedling, one of the most vulnerable stages of plant life (WILLIS et al., 2014). This interaction involves different physiological, morphological, anatomical and mechanical mechanisms (LINKIES et al., 2010; Willis et al., 2014) and indicates a complex evolutionary history (NIKOLAEVA, 1999; BASKIN; BASKIN, 2004) that may explain the distribution patterns of some species.

Physiological dormancy is the most frequent type of dormancy (BASKIN; BASKIN, 2004; 2014). In such a case, the integument can be an obstacle to germination, as it causes mechanical retention, or contains chemical inhibitors capable of producing physiological seed dormancy (PEREZ, 2004). The impermeability to water of the seed, common in integument dormancy, develops during seed maturation (JAYASURIYA et al., 2007; VAN STADEN et al., 1989; BASKIN; BASKIN, 1998). In this type of dormancy, the palisade layer is responsible for the impermeability (JAYASURIYA et al., 2007). The seed becomes permeable to water when an opening is formed in the palisade layer, allowing water into the seed (BASKIN; BASKIN, 2000). This kind of dormancy results in seasonal cueing, and ensures that germination occurs only under specific abiotic conditions of moisture, light or temperature (FINCH-SAVAGE; LEUBNER-METZGER, 2006; BASKIN; BASKIN, 2014). Integument dormancy is therefore an important strategy in seasonally dry environments, as it allows species to be resistant to adverse abiotic conditions such as long periods of heat and drought (NOODÉN et al., 1985; DI SALVATORE et al., 2016).

Information about the types of dormancy and average germination time (LONG et al., 2012) is important in understanding the life history strategies of a species, since it can help to explain species abundance and distribution (REES, 1997; HANDLEY; DAVY, 2005). Given the lack of knowledge of the conditions suitable for ensuring maximum germination (ALVES et al., 2008), awareness of the requirements for germination is important for the conservation and/or proper management of a species (CHANYENGA et al., 2012). It also helps to increase efficiency in the production of seedlings, and meet the growing demand for seedlings of native forest plants for the recuperation of degraded areas and restoration of the landscape (RIBEIRO et al., 2012).

Species that are widely distributed, such as *Eugenia punicifolia* (Kunth) DC, which occurs in various types of Brazilian vegetation, represent a good case study for analysing the effect of changes in abiotic variables on the germination of populations occurring in different habitats. As yet there are no reports in the literature about dormancy or germination requirements in *E. punicifolia*, but studies of species of the genus indicate physiological dormancy, caused mainly by impermeability of the integument (MARTINOTTO et al., 2007; MASETTO et al., 2009; MENDES; MENDONÇA, 2012).

With an view to understanding the temperature requirements that maximise germination, identifying possible differences in germination behaviour in populations of *E. punicifolia*, and finally establishing a germination protocol, as yet unpublished, for this species, the aim was to study whether the species displays physiological dormancy at germination, and whether there are variations in the rates of germination between populations from different climatic conditions when subjected to the same temperature.

2.1.2 Materials and Methods

Focal species

The Myrtaceae family is widely represented in many phytophysiognomies in Brazil, from more humid climates such as in the Tropical Atlantic Rainforest (MORI et al., 1983) and the *Restinga* (LEMOS et al., 2001; ASSIS et al., 2004) to seasonally dry environments, such as the Cerrado (BATALHA; MARTINS, 2004; BATALHA; MARTINS, 2007; COSTA et al., 2007; CARVALHO; MARTINS, 2009; MORO et al., 2011) and the deciduous vegetation on the acid soils of northeastern Brazil (ARAÚJO et al., 1998; LIMA et al., 2009; ARAUJO et al., 2011). The genus *Eugenia* L. is among those displaying the greatest wealth in the Myrtaceae

family, with around 1,000 species (OLIVEIRA et al., 2005; PRISCILA et al., 2010). The species selected for this study, *Eugenia punicifolia* (Kunth) DC, occurs almost throughout Brazil (SOBRAL, 1987), and has been frequently reported in floristic and phytosociological studies carried out in several forest formations (PEIXOTO; GENTRY, 1990; FABRIS; CESAR, 1996) and in non-forest formations such as the *Cerrado* and *Carrasco* (KAWASAKI, 1989; PROENÇA, 1994; NIC LUGHADHA, 1996; ARAUJO et al., 2011).

Seed collection and germination trials

To check whether climate has an influence on germination response in *E. punicifolia*, seeds were collected from populations occurring in three distinct types of vegetation: 1) Coastal Savanna (CS), 2) Dense Deciduous Shrubland (DDS) and 3) Seasonal Deciduous Forest (SDF). The fragment of CS is located on the coast of the State of Ceará, Brazil (3°43'02"S and 38°32'35"W), within the urban area of Fortaleza, at 16 m a.s.l., has a hot sub-humid tropical climate, an average annual rainfall of 1,338 mm and an average temperature of 30°C (MORO et al., 2011, INMET, 2014).

The other two types of vegetation, DDS and SDF, are located on the Ibiapaba Plateau, in an area of the Serra das Almas Private Natural Heritage Reserve (PNHR) in Crateús, Ceará. The area of DDS is located on the dryer slope of the basin (5°8'45"S, 40°55'43"W), 700 m above sea level, and has an average annual rainfall of 636. 61 mm. The mean annual minimum and maximum temperatures were $19.14 \pm 1.78^{\circ}\text{C}$ and $33.6 \pm 3.85^{\circ}\text{C}$ respectively (VASCONCELOS et al., 2010). The other area (SDF), is a forest physiognomy, divided into two well-defined strata – a canopy at 18 m and a lower stratum at not more than 8 m – located on the most humid slope of the basin at 650 m above sea level, with an average annual rainfall of 1044 mm and a mean annual temperature of 24°C (LIMA et al., 2011).

Fruits were randomly picked from 10 plants in each type of physiognomy, avoiding diseased plants or those with signs of damage. Immediately after collection, the fruits were taken to the laboratory to begin the treatments, placed in a sieve and depulped under running water. The seeds were dried at room temperature for 24 hours on absorbent paper, disinfested with 2% active-chlorine sodium hypochlorite for 15 minutes and rinsed with distilled and deionized water (BRASIL, 1992). They were then placed into paper bags and kept for up to three days until the experiments had been prepared.

Two experiments were conducted in this study. The first aimed to carry out the test for breaking dormancy using mechanical and chemical scarification, in order to understand a

little more about dormancy in the species. For this test, only seeds from the CS were used, since as Finch-Savage and Leubner-Metzger (2006), in general, responses to methods for breaking dormancy do not vary between plants of the same species. The second experiment consisted of an analysis of the effects of temperature, carried out on scarified seeds from each of the three phytophysiognomies, with each being subjected to three different temperatures: 15, 25 and 35°C.

Initially the imbibition curve was obtained so as to verify impermeability of the integument, thereby justifying the use of mechanisms for breaking dormancy in the seeds. For this, seeds from the three areas were submitted to tested mechanisms of breaking dormancy and weighed to obtain their initial weight (W_i). They were then immersed in distilled water at 25°C at a ratio of 25 seeds to 500 mL of distilled water. Then, every 12h, the seeds were dried on absorbent paper and reweighed (weight over time, W_t). The test was carried out over a total period of 72 hours, and imbibition (E) for each period calculated according to the equation $E = (W_t - W_i) / W_i \times 100$, with the results expressed as a percentage.

In the tests for breaking dormancy, the seeds were divided into three treatments: 1) manual scarification, using P60 sandpaper on the abaxial end of the seed surface; 2) chemical scarification with sulphuric acid; and 3) a control, to obtain germination data for the species without a procedure for the breaking of dormancy. For the chemical breaking of dormancy, the seeds were immersed in concentrated sulphuric acid (98%) for 15 minutes and then washed in running water for 1 hour (DAVIDE et al., 1995). All the seeds from each physiognomy were kept at a constant temperature of 25°C during the germination period. To run the tests for temperature, the seeds from each of the three phytophysiognomies were scarified manually, since that method results in a greater percentage of seed germination according to preliminary tests carried out on the species. The seeds were then submitted to three different temperatures: 15, 25 and 35°C. The temperatures used in the experiment were based on the minimum and maximum temperatures recorded in the environments selected for study that did not entail a lethal stress for the seeds. Tests of alternating temperature were not carried out, since, according to Lamarca et al. (2011), the seeds of other species of *Eugenia* are indifferent to variations in temperature.

For each experiment (breaking dormancy and germination at different temperatures), four replications of 50 seeds were used per treatment. The seeds were placed on petri dishes with germitest paper (previously autoclaved) (BRASIL, 1992) and kept in BOD (Biochemical Oxygen Demand) germination chambers. All the treatments were illuminated

with 20-watt fluorescent lamps (GE), giving a radiant flux of around $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Rosa and Ferreira 1998), for 12 hours daily.

Variables analysed and statistical analysis

A completely randomised design was used with four replications. Water content and seed dry weight were measured gravimetrically after drying at $105 \pm 3^\circ\text{C}$ for 24 hours, using 10 seeds with four replications for each treatment (BRASIL, 1992). The thousand-seed weight of *E. punicifolia* was 420 g, displaying 68% humidity. The germination variables being evaluated were: 1) Percentage germination (PG), 2) Mean Germination Time (MGT) and 3) Germination Speed Index (GSI).

The PG was obtained at the end of the germination test (60 days after sowing), calculated with the formula $\text{PG} = (n / N) \times 100$ where: n = number of germinated seeds at the end of the test, and N = total number of seeds in each replication. Seeds with a radicle protrusion of 0.5 cm were considered as having germinated, a cut-off commonly used in other studies of germination (MAGUIRE, 1962; PAULSEN; HÖGSTEDT, 2002; SOUZA et al., 2007). The MGT was obtained by a daily count of germinated seeds at 30 days after sowing, calculated with a formula proposed by Labouriau (1983), $\text{TMG} = (\sum n_i t_i) / \sum n_i$ where: n_i = number of germinated seeds per day, t_i = length of time of the experiment, $i = 0$ to 60 days, with the results expressed in days. The GSI was calculated by a daily count of germinated seeds 60 days after sowing, following an equation proposed by Maguire (1962): $\text{GSI} = \sum (n_i / t_i)$ where: n_i = number of germinated seeds for time ' i ', t_i = time after carrying out the test, $i = 0$ -60 days.

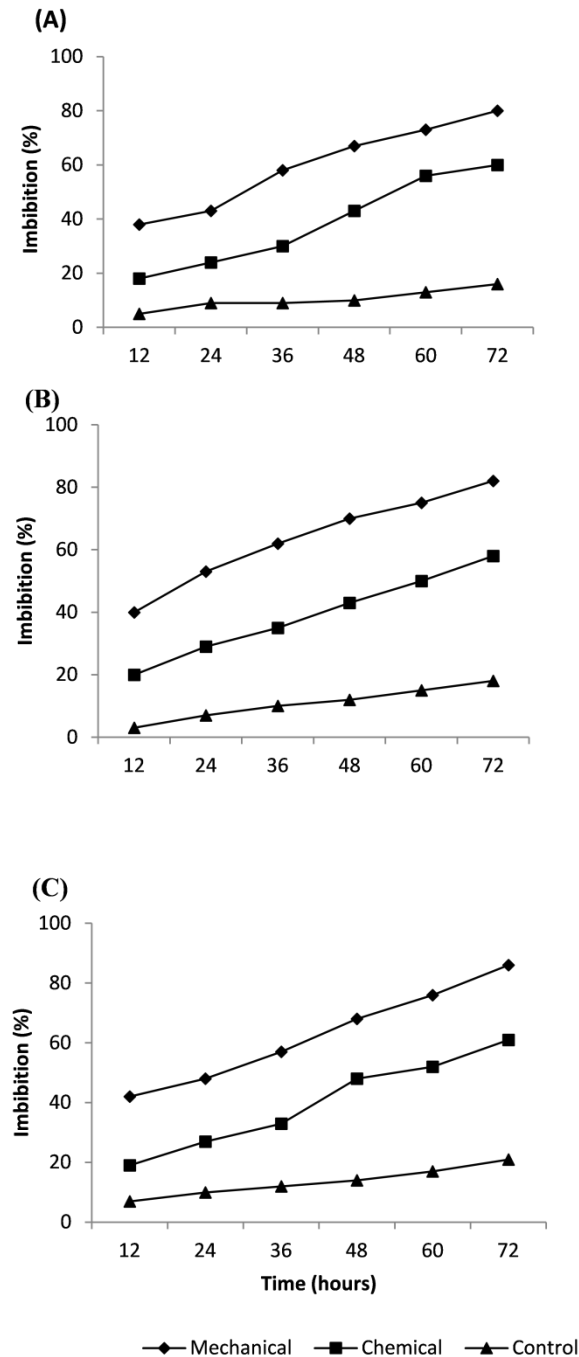
Normality of the data and homogeneity of the residuals was tested by the Kruskal-Wallis test. Analysis of variance (ANOVA) was then performed for the variables PG, GSI and MGT using Tukey's test at 1% probability to compare mean values for the treatments. These tests were carried out using the Assistat v. 7.4 Beta statistical assistance software (SILVA; AZEVEDO, 2006). The manner of accumulated germination (accumulated proportion of germinated seeds each day) varying between treatments was also evaluated by means of generalised linear models (GLM), using the binomial error family and logit link function, considering that the values for accumulated germination range between 0 and 1 (GUILLÉN et al., 2009; BENITEZ-MALVIDO et al., 2014). Accumulated germination was the response variable, and time (number of days after sowing), time^2 , treatment, $\text{time} \times \text{treatment}$, $\text{time}^2 \times \text{treatment}$, were the explanatory variables. This linear model allows testing of the variation between treatments for three parameters: 1) initial germination (intercept of the regression), 2)

initial rate of germination, and 3) variation in rate of germination, or inflection point. Analysis of deviance (ANODEV) with chi-square tests was used to evaluate whether these parameters varied between treatments (GUILLÉN et al., 2009; BENÍTEZ-MALVIDO et al., 2014). Z-tests at a significance level of 5% ($P < 0.05$) were used to evaluate the differences between treatments. The GLMs were carried out with the R software v. 3.1.2 (R CORE TEAM, 2014).

2.1.3 Results

Regardless of period, the unscarified seeds absorbed smaller amounts of water and generally displayed constant imbibition throughout the 72-hour period, with averages of around 20% (Figure 1A-C). In turn, the scarified seeds, especially those scarified mechanically, show a mean value for imbibition of about 80% for seeds from the three phytophysiognomies. In this case, it can be inferred that the seeds have some degree of dormancy, possibly physical, thereby justifying the use of mechanisms for breaking dormancy in this study.

Figure 1 - Imbibition curves of seeds of *Eugenia puniceifolia* originating in phytophysiognomies of Coastal Savanna (A), Dense Deciduous Shrubland (B) and Seasonal Deciduous Forest (C) after mechanical (sandpaper) and chemical (H_2SO_4) scarification. Unscarified seeds were used as the control.



In addition to facilitating imbibition of the seeds, breaking dormancy helped to increase the percentage and initial rate of germination, but mean germination time was not influenced by mechanical or chemical scarification (Table 1). This implies that there are seeds

that germinate quickly under favourable abiotic conditions, and that there are others that take longer to soak and trigger germination.

Germination response varied with the treatments for breaking seed dormancy (Table 1), and demonstrated that mechanical scarification resulted in a greater percentage germination compared to the other treatments ($P < 0.001$), with a mean value which was twice that of the control treatment. The same pattern of results was seen for the variable GSI. Mean values for MGT however, were not significantly different between treatments (Table 1).

Table 1 - Mean values for percentage germination (PG), germination speed index (GSI) and mean germination time (MGT) in seeds of *Eugenia punicifolia* subjected to treatments for breaking dormancy. Standard errors are shown in parentheses ($n = 4$). CV: coefficient of variation

| Germination response | | | |
|-----------------------------|--------------------|----------------------|-----------------------|
| Scarification | PG (%) | GSI | MGT (days) |
| Control | 38c (± 4.76) | 0.29c (± 0.05) | 35.46a (± 1.18) |
| Mechanical | 83a (± 2.51) | 0.85a (± 0.03) | 32.12a (± 1.46) |
| Chemical | 60b (± 1.63) | 0.60b (± 0.04) | 32.60a (± 0.84) |
| CV (%) | 10.77 | 14.80 | 7.14 |

Mean values followed by the same letter in a column do not differ by Tukey's test at a level of 1%.

Eugenia punicifolia displayed variations in germination response with the temperature treatments, depending on the original environment of the seed populations. In general, with the treatments for temperature, the seeds originating in the DDS displayed higher values for GSI at the three temperatures being tested, resulting in faster germination over time, regardless of the temperature. As for MGT, it can be seen that temperature had no influence on the results (Table 2).

Table 2 - Mean values for percentage germination (PG), germination speed index (GSI) and mean germination time (MGT) in seeds of *Eugenia punicifolia* subjected to treatments for breaking dormancy. Standard errors are shown in parentheses (n = 4). CV: coefficient of variation

| Area | Temperature (°C) | | |
|--------------------|------------------|----------------|----------------|
| | 15 | 25 | 35 |
| PG | | | |
| Coastal Savanna | 47cB(±1.91) | 87aA(±2.51) | 65bA(±3.00) |
| Seasonal Deciduous | 61bAB(±5.29) | 79aA(±3.78) | 51bB(±7.72) |
| Dense Deciduous | 75aA(±6.11) | 82aA(±1.91) | 68aA(±5.77) |
| CV (%) | 7.59 | 15.20 | 17.24 |
| GSI | | | |
| Coastal Savanna | 0.49cB(±0.05) | 1.18aA(±0.06) | 0.79bAB(±0.06) |
| Seasonal Deciduous | 0.62bAB(±0.05) | 1.14aA(±0.12) | 0.61bB(±0.13) |
| Dense Deciduous | 0.78bA(±0.03) | 1.12aA(±0.11) | 0.81bA(±0.08) |
| CV (%) | 15.20 | 11.15 | 10.47 |
| MGT | | | |
| Coastal Savanna | 32.82aA(±4.00) | 28.03aA(±0.67) | 29.03aA(±1.86) |
| Seasonal Deciduous | 33.04aA(±3.66) | 26.48aA(±1.22) | 28.91aA(±1.73) |
| Dense Deciduous | 32.34aA(±2.77) | 27.77aA(±1.34) | 30.13aA(±1.74) |
| CV (%) | 17.24 | 14.36 | 10.94 |

The interaction time² x treatment indicating a difference in variation of the rate of germination for treatment (Table 3). There is a reduction in the initial speed of germination near the end of the experiment, and a plateau is reached for accumulated germination before the other treatments (Figure 2A).

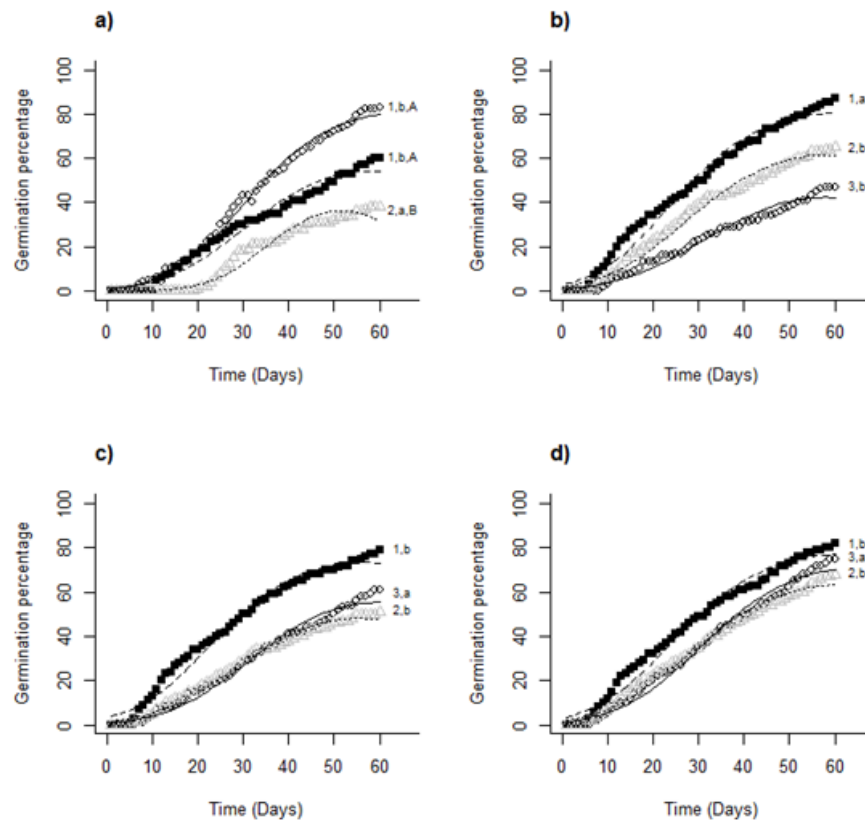
Table 3 - Analysis of deviance carried out to evaluate the significant predictors in the model using chi-square tests. The response variable was the percentage of accumulated germination in the seeds of *Eugenia puniceifolia*, and the explanatory variables were treatment, time and time²

| Explanatory variable | DF | Deviance | P |
|----------------------------------|-----------|-----------------|----------|
| Breaking of dormancy | | | |
| Time | 1 | 4056.7 | <0.001 |
| Time ² | 1 | 320.5 | <0.001 |
| Treatment | 2 | 1076.8 | <0.001 |
| Time:Treatment | 2 | 44.0 | <0.001 |
| Time ² :Treatment | 2 | 32.5 | <0.001 |
| Residual | 171 | 174.6 | |
| Temperature | | | |
| <u>Coastal Savanna</u> | | | |
| Time | 1 | 3718.4 | <0.001 |
| Time ² | 1 | 286.3 | <0.001 |
| Treatment | 2 | 1104.6 | <0.001 |
| Time:Treatment | 2 | 14.7 | <0.001 |
| Time ² :Treatment | 2 | 3.7 | 0.16 |
| Residual | 171 | 196.5 | |
| <u>Seasonal Deciduous Forest</u> | | | |
| Time | 1 | 3517.6 | <0.001 |
| Time ² | 1 | 295.2 | <0.001 |
| Treatment | 2 | 698.8 | <0.001 |
| Time: Treatment | 2 | 15.2 | <0.001 |
| Time ² : Treatment | 2 | 0.9 | 0.65 |
| Residual | 171 | 160.0 | |
| <u>Dense Deciduous Shrubland</u> | | | |
| Time | 1 | 4629.0 | <0.001 |
| Time ² | 1 | 230.3 | <0.001 |
| Treatment | 2 | 239.8 | <0.001 |
| Time:Treatment | 2 | 16.5 | <0.001 |
| Time ² :Treatment | 2 | 0.2 | 0.92 |
| Residual | 171 | 188.9 | |

At a temperature of 25°C greater percentage germination, speed of germination and initial germination can be seen in seeds from the three phytophysiognomies being analysed (Table 2, Figure 2B) when compared to the other temperatures under test, showing that this is the most suitable mean temperature for germination in the species. However, when subjected to a temperature of 15°C, differences between the areas became clearer when it was seen that seeds from the SDF and DDS displayed higher percentage germination (Table 2) and higher initial rate of germination than those from the CS (Figure 2C - D). The temperature of 35°C resulted in a higher average percentage germination for seeds from the CS and DDS, when compared to seeds from the area of SDF, the first two physiognomies being located in

environments with higher daily temperatures. The inflection point of the curve was similar between the different treatments for the three populations, i.e. accumulated germination reaches a plateau at similar time intervals.

Figure 2 - Percentage of accumulated germination in seeds of *Eugenia punicifolia* during the experimental period. Fig. 2A) ○ = Treatment 1: mechanical scarification; ■ = Treatment 2: chemical scarification and △ = Control treatment: no breaking of dormancy. Fig. 2B), C) and D) ○ = Treatment 1: 15°C; ■ = Treatment 2: 25°C and △ = Treatment 3: 35°C, for the areas of Coastal Savanna, Seasonal Deciduous Forest and Dense Deciduous Shrubland. Different numbers indicate differences in intercept (initial germination) between treatments; lowercase letters indicate differences in initial rates of germination between treatments; uppercase letters indicate differences in variation of the initial rates of germination.



2.1.4 Discussion

The use of sandpaper for mechanical scarification caused fragmentation of the integument, making it more permeable to the inflow of water during imbibition; for this reason, the imbibition curve for the seeds submitted to this treatment indicated a rapid absorption of water by the tissue. According to Orozco-Segovia et al. (2007). Under natural conditions, these

seeds would be classified as dormant, as they do not absorb water without a breaking mechanism. The integument of species of *Eugenia* is normally pachychalazal, consisting of a complex structure built up by multiplication of the cells of the two fused integuments (CORNER, 1976; VAN WYK; BOTHA, 1984). The presence of this characteristic is usually associated with other characteristics that are considered primitive, such large embryo, woody habit and tropical habitat (VON TEICHMAN; VAN WYK, 1991), as are those of above species.

The increase in percentage germination after the application of mechanical scarification shows this to be the most efficient method for breaking dormancy in this species. This result was also confirmed by Lameira et al. (2008). In the field, it was seen that these seeds are eaten by primates; Bravo (2009) noted that passage of the seeds through the digestive tract of monkeys increased the speed of germination in *E. puniceifolia*. This condition confirms the dormancy seen in the species, and may be considered as a natural mechanism for breaking dormancy. Despite exposure to acid being a common method for breaking dormancy widely reported in the literature (ZAIDAN; CARREIRA, 2008), chemical scarification with sulphuric acid displayed less efficiency in germination compared to mechanical scarification, showing this not to be the most suitable method for breaking dormancy in this species.

Based on evidence that dormancy is an adaptive response to local environmental conditions (NOODÉN et al., 1985), the resistance of the integument seen in *E. puniceifolia* indicates an adaptive response by the species to seasonal drought and the high temperatures of the Brazilian semi-arid area; since, according to Argel and Humphreys (1983), dormancy is a way of delaying germination until water availability is sufficient for seedling establishment.

Since the object species of this study has wide geographical distribution (SOBRAL et al., 2015) and occurs under different conditions of soil and climate (CONCEIÇÃO; ARAGÃO, 2010), a rapid germination, but with different percentage germination for mechanical and chemical treatments of scarification and with no difference in mean germination time, indicates differences in germination requirements between the three populations. When germination takes place over time, according to Venable (1997) and Silveira et al. (2013), it is a strategy for spreading risk, which guarantees persistence and reproductive success to the population in areas with an unpredictable supply of resources (SILVEIRA et al., 2013).

As regards the germination requirements of such seeds; a mean temperature of 25°C has been reported in germination protocols for seeds from other species of Myrtaceae (ALVES et al., 2015; DRESCH et al., 2012) and *Eugenia* (LAMARCA et al., 2011) originating in

different kinds of Brazilian vegetation. However, different responses for rates of germination as a result of the temperature and type of vegetation at the origin of the seeds, according to Besnier (1989), reflect the temperature of the geographic origin of each population. Differences, as expressed for germination, are expected in populations from areas with heterogeneous environmental conditions (BISCHOFF et al., 2006; LOPEZ et al., 2003; PICO et al., 2003). In this study, populations of *E. puniceifolia* collected in the phytophysionomies seen on the Ibiapaba plateau (DDS and SDF) are subjected to daily variations in temperature, with the occurrence of minimum temperatures of 10-15°C at night (data from the portable weather station located at the headquarters of the Serra das Almas PNHR), which explains the greater germination of seeds from these areas at the lowest temperature tested. Furthermore, the areas of CS and DDS have higher temperatures in the warmer months with less daily variation, which may have contributed to the greater germination success of these seeds at the temperature of 35°C (INMET, 2014).

The variation in germination response of seeds of *E. puniceifolia* originating in regions of different climatic conditions indicates that in addition to the strong influence of the environment on this characteristic, there is great phenotypic variation in the germination processes of the plant. According to Lamarca et al. (2011), the difference in germination behaviour seen among populations of the same species, indicates that selection pressure in the environment of origin acts to select individuals adapted to the local conditions of temperature. Many species exhibit different physiological responses to the adversities imposed by their habitat, which consist of a set of adaptive characteristics, resulting from the interaction between genetic makeup and environmental conditions (DAWS et al., 2005; GEBLER et al., 2005; RENAUT et al., 2005).

The mean germination time, similar in seeds from the three areas, is a result of phylogenetic determination. However, variability in germination behaviour reinforces the influence of the environment on the remaining characteristics. If the seeds of any one species are to display similar germination, it does not mean they should display the same germination behaviour, since the start of germination and its distribution may be different, displaying different speeds and rates of initial germination (BORGHETTI; FERREIRA, 2004). Such a condition was expressed in this study by the results obtained for initial germination and rates of germination from the accumulated germination curves.

Knowledge of the biology and germination behaviour of seeds from phylogenetically related species, but which occupy different habitats, is important for understanding the processes that shape communities throughout a succession, seedling

establishment and natural regeneration (RANIERI et al., 2003). According to Botezelli et al., (2000), it is important to work with plants from different locations, finding different phenotypic expressions for variations in temperature, photoperiod and precipitation, among other factors that highlight certain aspects of genetic makeup, and that would not be seen in other environments. In this way, the expression of each genotype made possible by the environmental conditions can be noted.

The wide geographic distribution of *E. puniceifolia* denotes a character which is adaptive to environments of different temperatures. This is an aspect to be considered in the management of the species for purposes of reforestation, or in studies aimed at understanding its distribution.

2.1.5 Conclusions

The germination protocol for *E. puniceifolia* indicates that the use of pre-germination treatments to break dormancy, together with compliance with temperature requirements, guarantee greater success in germination. The species displays integument dormancy, which is best broken by the method of mechanical scarification. Variation in the rates of germination as a result of the temperature between populations coming from environments with different climatic conditions, reflects adaptation to the climate of the native habitat, and helps in understanding the wide colonisation over climatically distinct environments.

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2.2 Manuscrito 2

Beneficial effects of silicon in plants under abiotic stress conditions: A new approach

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Abstract

Considering the importance of Silicon (Si) for plants, many studies have focused to evaluate the ability of the element to alleviate abiotic stress in different species, mainly of agricultural interest. For all species, Si offers a range of benefits in reducing the effects of biotic and abiotic stress, being especially associated with increases in plant biomass. However, after performed a systematic quantitative review of studies linking Si to the relief of abiotic stress in plants, it was demonstrated that there is a reduction in the effects of Si over time and that the effects of Si were smaller in recent studies. Furthermore, was found that plants under different types of stress had similar responses to Si, suggesting that abiotic stress relief mechanisms in plants may be common among conditions of water stress, salinity, and heavy metals — the main environmental stresses studied in the literature. This study can guide future research, which should not be limited to simply evaluating the existence of any Si stress-relieving effects, but also to understand the variation that exists in these mechanisms and considered that they depend on the type of stress and the factors involved in reducing these effects.

Keywords: relief mechanisms; systematic review; reduced effect; biomass.

2.2.1 Introduction

All plants grown in soil contain silicon (Si) in their tissue (COOKE; LEISHMAN, 2011; EPSTEIN, 1994). Si is the second most abundant element in the Earth's crust, accounts for approximately 32% of its weight (NEERU et al., 2019) and its ubiquity in the biosphere makes it difficult to prove its essentiality for plants. Unfortunately, the benefits of Si were mostly neglected until the early 20th century, in part due to the element's abundance in nature, but also due to the lack of visible symptoms of deficiency or toxicity (ZELLNER et al., 2021).

However, several dysfunctions in plant growth and development can be caused by Si deficiency (CHAKMA et al., 2021), which together with numerous evidences proving

beneficial effects on plants, especially under abiotic stress conditions (ARAÚJO et al., 2022; DHIMAN et al., 2021), makes it “almost essential”.

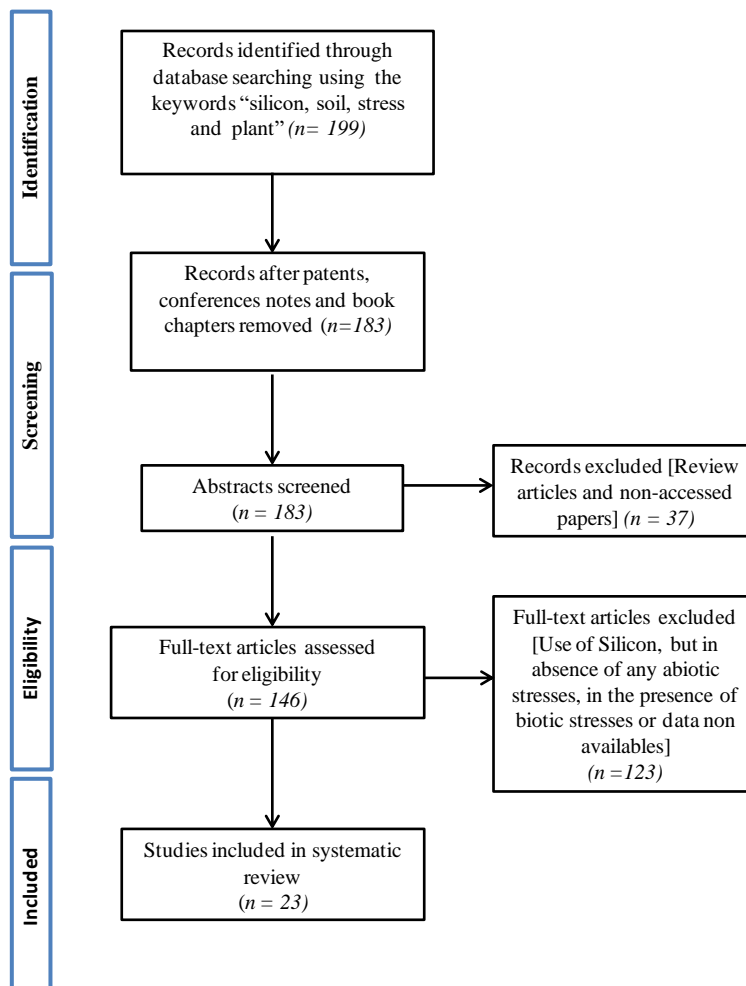
Most of these abiotic stresses are due to climate change and threaten agricultural productivity, with significant losses in crop yields, for example (KHAN et al., 2020; KHAN et al., 2021; Z'ORB et al., 2019). Plant exposure to multiple abiotic stresses results in changes in physiological responses ranging from seed germination to maturity and causing severe losses in growth and productivity (KHAN et al., 2021; MAHALINGAM, 2015). Numerous studies in the literature, recent or not, establish the ability of Si to neutralize the effects of various stresses such as water deficit (HABIBI, 2014; OTHMANI et al., 2020), salinity (ABDELAAL et al., 2020; LIU et al., 2014) heat (KHAN et al., 2020), toxicity of heavy metals (DE JESUS et al., 2017; DRESLER et al., 2015), among others. Therefore, the element has been widely used over decades as an important ally of crops of agricultural interest, even though there is still a gap in understanding the action of Si in various metabolic processes of different plant species. Functions of Si associated with agricultural studies carried out in domestic plants are the most discussed (COOKE; LEISHMAN, 2016). In these cases, the main benefits from Si in terms of a reduction in the effects of biotic and abiotic stress in agricultural systems are well-documented under conditions of Si fertilisation; with most studies focusing mainly on surveying data from almost 30 years of the use of the element in agriculture (i.e., ADREES et al., 2015; COOKE; LEISHMAN, 2016; LIANG et al., 2007; SAVANT et al., 1999). Besides that, hundreds of studies have assessed the capacity of Si to alleviate abiotic stresses in single species by single-stress experiments (COOKE; LEISHMAN, 2016). Therefore, it is important to have access to a systematic review that objectively synthesises the effects of Si on different types of stress over time and confirms whether studies have been conducted to obtain advances in scientific knowledge about Si.

We assumed that 1) There is a difference in the effect of Si depending on the type of stress to which the plant is subjected, and 2) The effect of relief to abiotic stress is confirmed by the studies carried out with the element over time. For this, we search for publications in the literature about the influence of Si on plant response to the different types of abiotic stress: salinity, water, and heavy metal, thereby providing a meta-analysis evaluating silicon effects in plants under stress conditions, a relevant information to plant and soil scientists. Based on that, we aimed to perform a systematic quantitative review linking Si to the relief of abiotic stress in plants and contribute to the advancement of Si studies in the soil and serve as a basis for future research with the element.

2.2.2 Materials and Methods

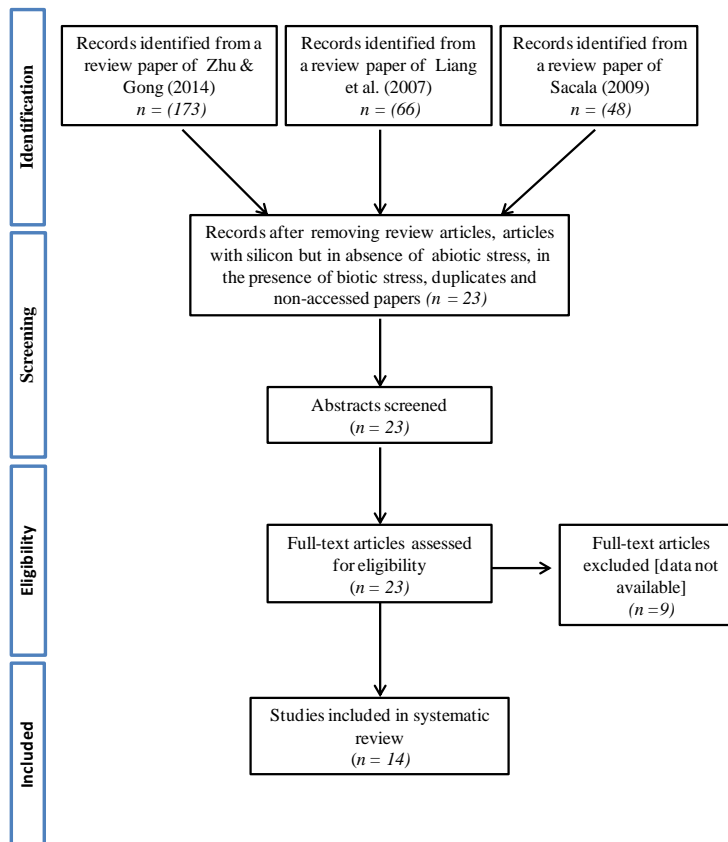
The literature in the ISI Web of Science database was searched with reference to the influence of Si on the response of plants to stress (“silicon” and “soil” and “stress” and “plant”). See Figure 1 for a detailed explanation of the inclusion criteria used for selecting the papers and Supporting Information for the references of the studies included in the meta-analysis. Within the resulting data, the references of two consistence review articles (SACALA, 2009; ZHU; GONG, 2014) were also checked for further papers.

Figure 1 - PRISMA flow diagram detailing how the literature search was performed. Adapted from Koricheva and Gurevitch (2014)



The same approach described in Figure 1 was followed to select studies from these reviews (See Fig. 2).

Figure 2 - PRISMA flow diagram detailing how the literature search was performed. Adapted from Koricheva and Gurevitch (2014).



Hedges' g was used as a measure of effect size, with a correction factor that reduces problems related to low sample sizes (BORENSTEIN et al., 2009). To calculate this index for each study, one of the following approaches was employed depending whether the response variable of each study was mass or silicon concentration: 1) when the response variable of the study was any measure of plant weight, the experimental group under stress conditions with Si was compared with the group under stress conditions with no silicon (Mass of the Treatment_{stress+Si}–Mass of the Treatment_{stress}); 2) when the response variable of the study was Si concentration, the experimental group under stress conditions with Si was compared to the group under non-stress conditions with Si (Concentration of the Treatment_{stress+Si}–Concentration of the Treatment_{Si}). Positive values for Hedges' g represent the positive effects of Si in plants under stress conditions. For studies with multiple treatments, the mean and variance were determined for the effect group following Borenstein et al. (2009), by calculating a weighted mean for the mean values and the pooled variance respectively. When the studies had multiple outcomes, causing problems of lack of independence among the data, the formula proposed by Borenstein et al. (2009) was used, where the non-independent effect sizes from

each outcome were combined in a single value for each study. However, using this approach demands to specify the correlation between the different related results of a study, information which is generally absent from the papers. This combination was carried out therefore, using three different correlation values (0.25, 0.5 and 0.75) in order to cover a range of possible relationships between the variables, as suggested by Borenstein et al. (2009).

As there was no evidence suggesting that all the different studies should converge in a single effect size, a meta-analysis with random effects was performed. Furthermore, since the study units were species, a mixed-effect model was used to incorporate the relationship between the species in the analyses. The taxonomic distances between species were calculated following an approach proposed by Clarke and Warwick (1998), since there was no phylogeny available which covered all the studied species together with their varieties. These distances were used as a correlation matrix between species to correct the phylogenetic dependence among the variety of species after performing simple standardisation $[(100 - \text{distance}) / 100]$, where 100 is the maximum distance provided by the formula of Clarke and Warwick], making the main diagonal equal to 1, following the correlation matrix commonly used in meta-analyses (NAKAGAWA; SANTOS, 2012).

The significance of the overall influence of Si in plants under stress conditions was evaluated by checking whether the 95% interval confidence (CI) for the overall effect size (a weighted mean of the effect sizes for each study) crossed zero. The Q statistic was employed, evaluated using a Chi-square distribution, to measure heterogeneity in models from the present work (BORENSTEIN et al., 2009). In order to investigate the causes of variation in plant response to stress conditions, two moderators (or explanatory variables) were included in the meta-analysis: 1) the type of stress evaluated in the study (salt, hydric or heavy metal) and 2) the year the study was carried out. Since the mechanism of the effect of Si in plants under stress conditions changes according to the type of stress, it is expected that different stress situations should produce different effect sizes. In the current work, salt, hydric and heavy metal stress were used because they were the abiotic stress that most appeared in the literature. The year of publication was also included to account for temporal changes in effect size (KORICHEVA; GUREVITCH, 2014). The Akaike Information Criterion, corrected for small sample sizes (AICc), was used to select which model containing moderators best described the selected data. Differences in AICc greater than 2 were considered as evidence of difference between models. Since some studies included in our search evaluated different varieties of a plant and the present work considered each variety as an independent study, previous analyses were rerun using study reference (ID) as a random effect, to account for possible problems of non-independence

existing in the studies carried out by the same research group (KORICHEVA; GUREVITCH, 2014).

Publication bias was verified using a funnel plot of effect sizes; this analysis was also carried out using the residuals of the meta-regressions. The residual approach appears to be better than using effect size, as there may be a large amount of heterogeneity with the latter, causing funnel plot asymmetry that is not due to publication bias (NAKAGAWA; SANTOS, 2012). In order to assess asymmetry in the funnel plot, an adaptation of Egger's regression test was used, which uses residuals instead of effect size and has been used in other studies (NAKAGAWA; SANTOS, 2012). The presence of publication bias in an analysis is supported when the intercept of the regression line is significantly different from zero (NAKAGAWA; SANTOS, 2012).

All analyses were performed with the R metafor package (VIECHTBAUER, 2010). Taxonomic distances were calculated in Vegan (OKSANEN et al., 2013).

2.2.3 Results and Discussion

After applying all the inclusion criteria, 37 studies were included in the meta-analysis which provided standardised mean differences and variances for 81 different varieties of plants. Sensitivity analysis, using three different correlation values (0.25, 0.50 and 0.75) gave very similar results; only the results of those analyses for a correlation value of 0.50 are presented here, this being an intermediate value among those used in the study (see Supporting Information for results using the remaining correlation values).

In general, the plants produced more biomass or accumulated more Si under stress conditions. The overall influence of Si in plants under stress conditions was not significant when study identification (ID) was not used as a random effect ($g = 1.36$, $CI = -0.45-3.17$), but changed with the inclusion of this random effect ($g = 1.73$, $CI = 0.31-3.17$; see Supporting Information, Figures S1-S3 and Table S1). The result highlights the positive effect of Si in plants under stress conditions. Heterogeneity was both very high and significant in the two models ($Q = 491.55$, $P < 0.001$), reinforcing the importance of using moderators to understand the causes of variation between studies and between the varieties of plants.

The increase in biomass production with the addition of Si, confirmed in this study covering many species of plants of different taxonomic groups, is well-documented in studies focusing only in one species, whether with experiments are conducted in soil or under nutrient solution (e.g. KHAN et al., 2017; MUSHTAQ et al., 2020; OTHMANI et al., 2020). According

to a study conducted by Chen et al. (2019), Si increased the biomass of rice plants by approximately 50%. The reason for such beneficial effects of Si in increasing plant biomass, in addition to other gains, is still not well understood, but some research has pointed out that Si absorbed by the plant would deposit in the epidermis, forming a double-layer "cuticle structure-silicon". This could greatly increase the cell wall resistance, making the plant more erect and straight, with elongated leaves (FAROUK et al., 2020). In addition, Si can increase the chlorophyll content and optimize the light condition, reducing the angle between the stem and the leaf, which is beneficial for photosynthesis (FAN et al., 2016; HUSSAIN et al., 2019). It is important to highlight that Si considerably improves photosynthesis and ion homeostasis, as well as the activation of the antioxidant capacity, regulation of genes needed in several physiological processes and in the production of secondary metabolites (FAROUK et al., 2020). Furthermore, Si can promote the absorption of nutrients such as N, P and K, improving the oxidation efficiency and root absorption rate (KELLER et al., 2015; MA et al., 2021). More recently, Mir et al. (2022) extensively discussed the intervention of Si in triggering the synthesis of phytohormones, regulating gene expression in alleviating abiotic stress conditions, in addition to reinforcing the element's role in increasing plant growth and metabolism.

The meta-regression, whether including the study ID or not as a random effect, provided similar general results, although the models with ID had a lower AICc (Table 1 and Table S2).

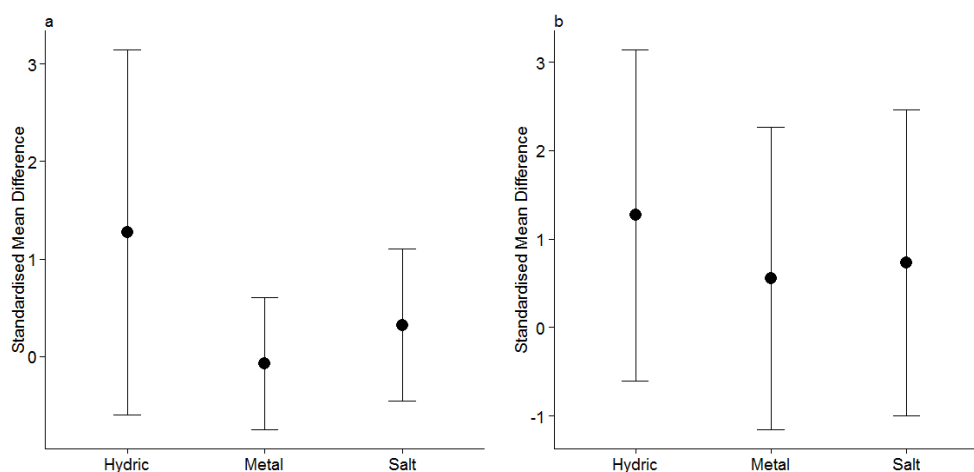
Table 1 - Model selection based on the Akaike Information Criterion corrected for small differences in sample size (AICc). Moderator effect = amount of heterogeneity in effect size explained by the moderator (Q statistic); P = p-value evaluation of the moderator. The models presented in the table were obtained using effect sizes estimated for a correlation value of 0.50. (See supplementary material for results for other correlation values)

| Model | Moderator effect | P | AICc | Δ AICc |
|-------------|------------------|---------|--------|---------------|
| Year + ID | Q = 5.55 | 0.02 | 338.02 | 0.00 |
| Year | Q = 22.86 | < 0.001 | 397.64 | 59.62 |
| Stress + ID | Q = 0.71 | 0.70 | 340.11 | 2.09 |
| Stress | Q = 1.37 | 0.50 | 418.26 | 80.24 |

The best model was that using the year of publication as a moderator and including study ID as a random effect. The type of stress did not influence the effect of Si in plants under stress conditions (Figure 3 and Figure S4), but the year of publication had a negative effect

(when using ID as a random effect: b (angular coefficient) = - 0.11, $P = 0.02$; when not using ID: $b = -0.10$, $P < 0.001$; Figure 4).

Figure 3 - Standardised mean differences (Hedges' g) for the three groups: hydric, heavy metal and salt, a: without study identification (ID) as random variable, b: with study ID as random variable. The results are from data for a correlation value of 0.50



Most data used in this analysis comes from studies that provided raw data relating the role of Si under isolated and controlled stress conditions. However, it is known that plants grown in field are constantly subjected to a number of different types of combined stresses. Despite the existence of an overall positive effect of Si under stress conditions, the heterogeneity observed in our general model suggests that the plant response to stress in the presence of Si can be explained by specific factors. It is necessary to better understand the function of Si in a larger number of species with more complex environmental interactions. Field studies would be needed to understand the functions of Si under multiple types of stress, as they would allow comparisons between Si-supplied plants and other stress coping mechanisms, such as wax accumulation in leaves, succulence, or other adaptations under stressful conditions, for example (COOKE; LEISHMAN, 2011).

The effect of Si associated with plants under adverse stresses, expressed in terms of increases in element concentrations in plant tissues, was also confirmed in our analysis. The discussion about the wide variation in Si concentrations in plant tissue may be related to differences in the absorption and transport characteristics of the element within groups of plants before exposure to stress (EPSTEIN, 1994; LIANG et al., 2007). For example, grasses absorb much more Si than other species, while most dicots absorb it passively and others, such as legumes, exclude the element from absorption (LIANG et al., 2007). Such phylogenetic

influences were considered in the present study with the inclusion of the variance-covariance distance matrix between the studied species, avoiding possible bias in the results due to the use of different proportions of monocotyledons and dicotyledons (indeed it was found that the AIC values for those models incorporating the distances between species were far lower than for models that did not incorporate this relationship – data not shown, reinforcing the phylogenetic effect on plant response). Several authors associate the different action mechanisms of Si with the type of stress the plants are subjected to. In general, under conditions of water or salt stress, the effects of Si for both conditions are associated with regulating the rates of transpiration (ZHU; GONG, 2014), and for salt stress, inhibiting the transport of Na and Cl to the leaves, and/or their accumulation in the roots (TUNA et al., 2008; ZHU; GONG, 2014). For stress caused by heavy metals, the most common mechanisms are a reduction in the absorption of these elements by the plant, and an improvement in gas exchange and photosynthetic pigments (ADREES et al., 2015). However, even with specific mechanisms for each type of stress, in the present study the Si displays a similar relieving effect regardless of the type of abiotic stress being tested. The data used in our meta-analysis and much of the Si research are from studies conducted in one or at most two types of stress at a time. It is understood that plants under natural conditions in field can be affected by more than one stress-producing factor, such as salt and water stress, for example, which has a strong association of occurrence, reinforcing the need for future case studies that cover different combinations of stress conditions.

It is necessary to highlight that there is still a great variability in the benefits of adding Si when the environmental levels of the element are low (THORNE et al., 2022), in which some plants are even negatively impacted by Si, others do not respond, and others show positive results. The data suggest that the effectiveness of Si is greater in varieties that are more tolerant to one or more types of stress. Thus, it is important that cultivar-specific data be collected in broader studies, with the aim of evaluating the benefits of Si supplementation. In terms of practical applications and future studies, it would be very useful for such studies to include assessments of the economic viability of Si supplementation, especially with reference to different cultivation and production systems.

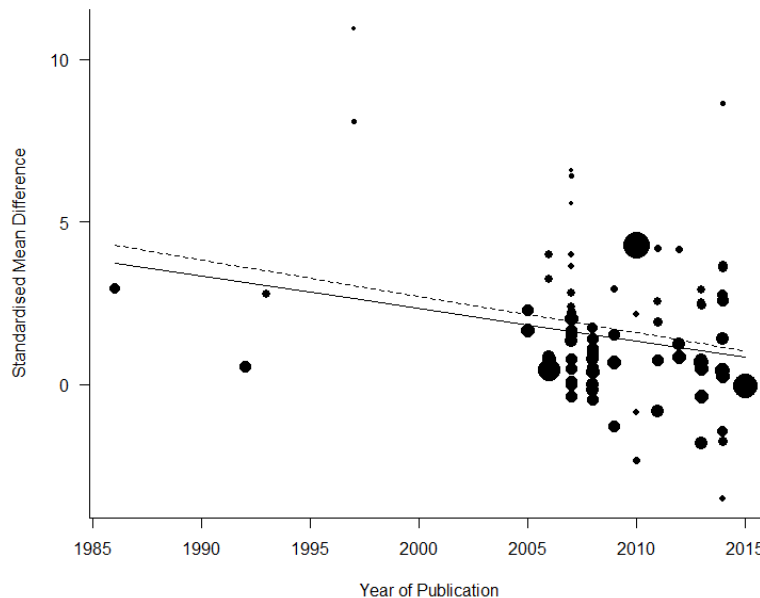
The occurrence of a defense mechanism stimulated by Si, common to the different types of stress used in these studies, would explain the absence of any difference found in this meta-analysis.

Si seems to act in general mechanisms common to most plant species, such as those that lead to the expression of stress-related genes. Increases in biomass and productivity, in addition to Si concentration, were common variables in the selected publications for this meta-

analysis, for a wide variety of plant species. In some cases, these effects were associated with amorphous silica deposition while in others, a consequence of monosilicic acid bioactivity. Recently, Hall et al. (2019) reported that Si improves several defense responses through signal transduction, especially the regulatory pathway of jasmonic acid (JA). The application of Si can also increase the tolerance to abiotic stresses by modifying the homeostasis of phytohormones and regulating endogenous hormone levels (KHAN et al., 2021; MORADTALAB et al., 2018).

In the most recent studies used in our analysis, the beneficial functions of Si are associated with an increase in enzymatic and non-enzymatic antioxidants, demonstrated for adverse stress conditions. This is increasingly reported in the literature, such as in the studies by Rahman et al. (2017) and Liu et al. (2019). It is possible, then, that a common action of Si under these conditions explains this more general action of the element, regardless of the type of stress the plants are subjected to or the studied species.

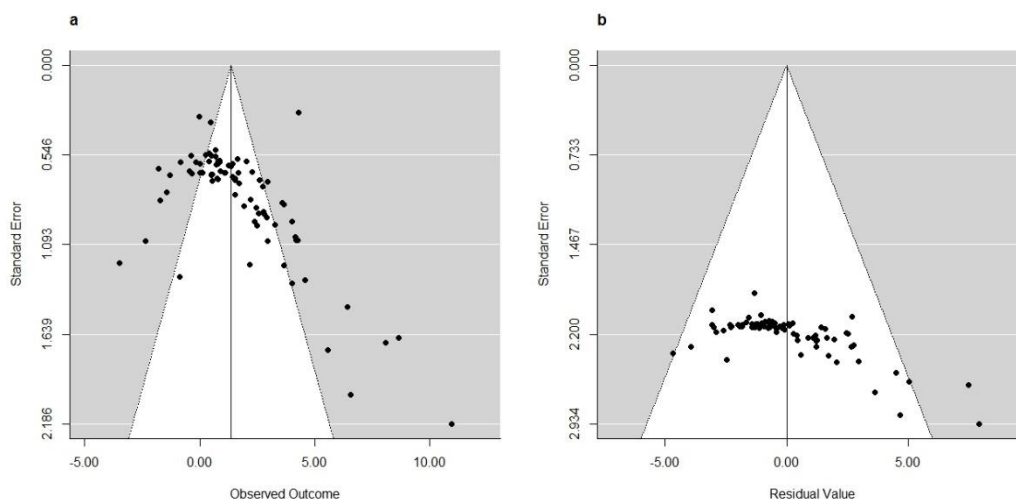
Figure 4 - Scatterplot of the meta-regression, representing the influence of year of publication on the standardised mean difference (Hedges' g). Full line: model without study identification (ID) as a random effect; dashed line: model with study ID as random effect. Dot size is proportional to the weights (inverse of the square root of the variance) for each study



Funnel plots for the best model showed a low signal for publication bias, as most of the effect sizes and residuals are almost equally distributed around zero (Figure 5; see Figure S5 for graphs of publication bias in the dataset for correlation values of 0.25 and 0.75). Egger's

regression test, using the residuals of the regression, suggests a low signal for publication bias (intercept = 1.55, $t = 1.99$, $P = 0.05$; see also Appendix S1).

Figure 5 - Funnel plots of effect size (a) and residuals of the meta-regression (b)



The result that most caught attention in our research is that the effect of Si in conditions of abiotic stress has become less evident over time. There are many aspects that can be considered for this statement. One could be the lack of a universally applied method for the determination of Si available to plants in the soil, or even in plants.

According to Zellner et al. (2021), there is a lack of agreement on Si test procedures in soil and plant. The two main components of soil Si testing are the extraction procedure and the method of its quantification in the soil extracts. According to the authors, the establishment of different procedures for extracting Si from soil began in the 1960s. Since then, several procedures have been established, and almost all of them had several modifications made mainly to reduce the extraction time requirement (TUBAÑA; HECKMAN, 2015). The amount of Si extracted from the soil differs depending on the extraction procedure used (PAYE et al., 2018). In addition, the method by which the concentration of Si in soil extracts is quantified, also affects the soil Si test values, and current standard procedures for digestion of plant tissues can interfere with the determination of the contents, as they affect the solubility of the element. If we consider the number of crops cultivated, the soil classes in the landscapes and the amount of research that is multiplying rapidly, it is worrying that, after more than five decades, there is no agreement on the use of procedures, a database with critical Si levels and soil test interpretations for economically important crops that could more accurately guide the use of the element in agriculture.

In the studies used as database for this work, the use of different methods to determine Si concentrations in leaves was observed, using different extractors such as $\text{H}_2\text{SO}_4 + \text{HF}$ (LIU et al., 2014), CaCl_2 (PULZ et al., 2008), $\text{NaOH} + \text{H}_2\text{O}_2$ (BALAKHNINA et al., 2012), as well as the use of modern equipments, such as inductively coupled plasma atomic emission spectroscopy (ICP-AES) (HABIBI, 2014) and electron microscopy transmission (SIDDIQUI et al., 2014). According to our analysis, current studies on the action of Si in plants under stress conditions do not confirm the results obtained in previous studies. These conditions may have been identified because of technological advances in the field of soil and plant research, thanks to the use of more modern equipment that ensure greater accuracy in data collection and, especially, less possibility of contamination (VERCHOT et al., 2007). Furthermore, it is important to consider that the most recent studies used in this review include the use of genetically modified species or species resistant to abiotic stress, such as the studies by Ahmed, Hassen and Khurshid (2011), Liu et al. (2014) and Habibi (2014). Although there has been a gradual increase in the production of major crops since the 1960s due to the development of agronomic practices such as the use of genetically modified plants, susceptibility to climate change has increased, resulting in the loss of harvests (MICKELBART et al., 2015). As a result, the adoption of resistant plants has guaranteed global food security, allowing plants to successfully occupy differing environments under limiting conditions. In this scenario, the reliever function of Si will possibly be eliminated or become "unnecessary". Gene transcription analysis has shown that the addition of silicic acid has no impact on gene expression for Si absorption in the absence of stress (FAUTEUX et al., 2006), so any advantages provided by the Si may not be evident unless the plants are forced to (CHERIF et al., 1994; COOKE; LEISHMAN, 2011). If genetically modified plants are already seen as being resistant to stress, absorption of the element by the plants would be reduced.

2.2.4 Conclusions

1. No differences for Si responses in relation to the types of stress selected for this study were found, confirming a more general effect of the element. The identification of a common and general effect of a greater number of stresses, or under conditions where the species are subjected to more than one stress simultaneously, may direct further studies with the element and help to clarify the mechanisms of stress relief.

2. The main finding refers to the reduction in the effect of Si as a stress reliever over time; this should be better investigated aiming to identify any possible interference from

advances in science and technology in studies that address resistance to various stresses. Future studies therefore should not be restricted to simply evaluating the existence of positive effects, but also to understanding the variations between them and whether they depend on the type of stress, and the factors involved in the reduction of these effects over time.

3. Considering the ability of Si to create a tolerance in plants to abiotic stress, future research should include aspects of plant adaptation to climate change and/or strategies for environmental remediation, making the element relevant to current research. In this way, plant scientists from other areas than agronomy could be involved, bringing new insights and ideas that would contribute to advances in the area.

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2.2.5 Supplementary information

Appendix S1 - Egger's regression analysis of data sets using correlation coefficients of 0.25 and 0.75 to combine multiple dependent outcomes from the same study.

Egger's regression test shows no signal of publication bias when using a correlation coefficient of 0.25 (intercept = 1.37, $t = 1.67$, $P = 0.10$) to combine multiple dependent outcomes from any one study, but detected publication bias with a correlation coefficient of 0.75 (intercept = 1.68, $t = 2.25$, $P = 0.03$).

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Table S1 - Overall effect sizes of the different data sets used in the present study both with and without study identification (ID) as a random effect. Cor = Correlation value used to combine multiple dependent effect sizes from the same study into a single effect size following *Borenstein et al. (2009)*

| Model | Hedges' g | SE | Z | P | Lower CI | Upper CI | Q | P |
|-------------------------------------|------------------|-----------|----------|----------|---------------------|---------------------|----------|----------|
| Without the ID random effect | | | | | | | | |
| Cor = 0.25 | 1.375 | 0.9628 | 1.428 | 0.153 | -0.512 | 3.26 | 559.58 | <0.001 |
| Cor = 0.5 | 1.358 | 0.923 | 1.472 | 0.141 | -0.450 | 3.167 | 491.55 | <0.001 |
| Cor = 0.75 | 1.345 | 0.897 | 1.499 | 0.139 | -0.413 | 3.103 | 445.23 | <0.001 |
| With the ID random effect | | | | | | | | |
| Cor = 0.25 | 1.761 | 0.806 | 2.184 | 0.029 | 0.181 | 3.342 | 559.58 | <0.001 |
| Cor = 0.5 | 1.739 | 0.729 | 2.387 | 0.017 | 0.311 | 3.168 | 491.55 | <0.001 |
| Cor = 0.75 | 1.716 | 0.682 | 2.516 | 0.012 | 0.379 | 3.053 | 445.23 | <0.001 |

Table S2 - Model selection based on the Akaike Information Criterion corrected for small differences in sample size (AICc). Moderator effect = amount of heterogeneity in effect size explained by the moderator (Q statistic); P = p-value evaluation of the moderator

| Model | Moderator effect | P | AICc | ΔAICc |
|--------------|-------------------------|----------|-------------|--------------------------------|
| Cor = 0.25 | | | | |
| Year + ID | Q = 5.74 | 0.02 | 342.41 | 0 |
| Year | Q = 28.17 | <0.001 | 414.05 | 71.64 |
| Stress + ID | Q = 0.73 | 0.69 | 344.51 | 2.1 |
| Stress | Q = 1.20 | 0.55 | 440.26 | 97.85 |
| Cor = 0.75 | | | | |
| Year + ID | Q = 5.35 | 0.02 | 335.24 | 0 |
| Year | Q = 18.87 | <0.001 | 387.18 | 51.94 |
| Stress + ID | Q = 0.70 | 0.70 | 337.27 | 2.03 |
| Stress | Q = 1.50 | 0.47 | 403.62 | 68.38 |

Figure S1 - Forest plot containing the effect sizes and confidence interval of the effect of silicon (Si) on 81 variety of plants under abiotic stress. Effect size and variance for each variety of plant were obtained using a formula for multiple non-independent outcomes found in Borenstein et al. (2009) using a correlation value of 0.25. The polygon in the bottom of the forest plot represents the overall effect size. Study reference and the relationship among the variety of plants were used as random variables.

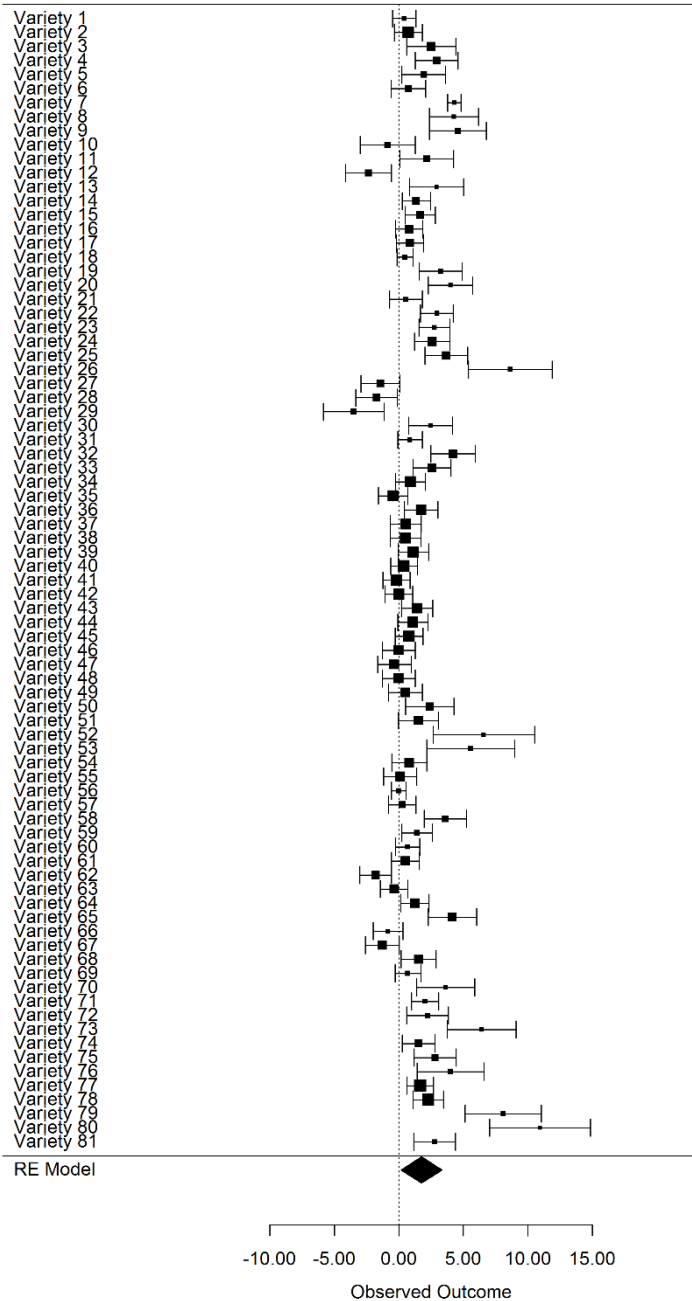


Figure S2 - Forest plot containing the effect sizes and confidence interval of the effect of silicon (Si) on 81 variety of plants under abiotic stress. Effect size and variance for each variety of plant were obtained using a formula for multiple non-independent outcomes found in Borenstein *et al.* (2009) using a correlation value of 0.50. The polygon in the bottom of the forest plot represents the overall effect size. Study reference and the relationship among the variety of plants were used as random variables.

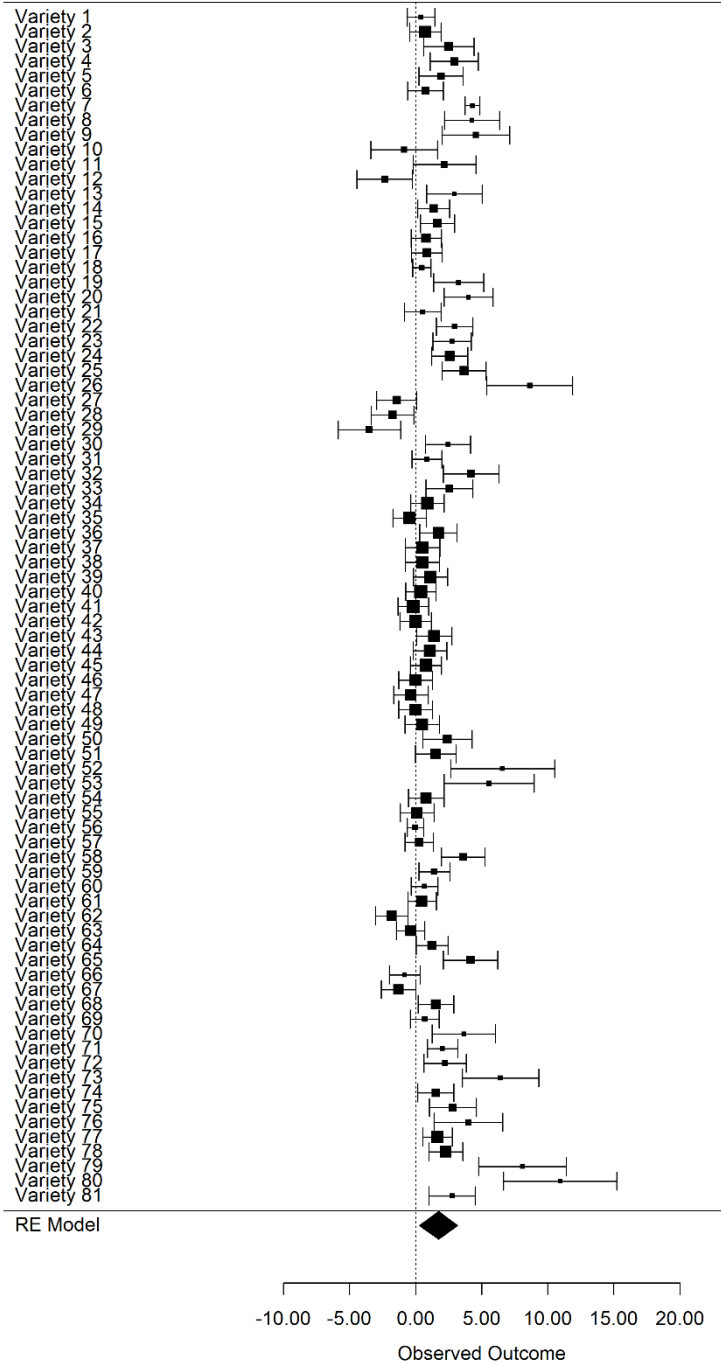


Figure S3 - Forest plot containing the effect sizes and confidence interval of the effect of silicon (Si) on 81 variety of plants under abiotic stress. Effect size and variance for each variety of plant were obtained using a formula for multiple non-independent outcomes found in Borenstein et al. (2009) using a correlation value of 0.75. The polygon in the bottom of the forest plot represents the overall effect size. Study reference and the relationship among the variety of plants were used as random variables.

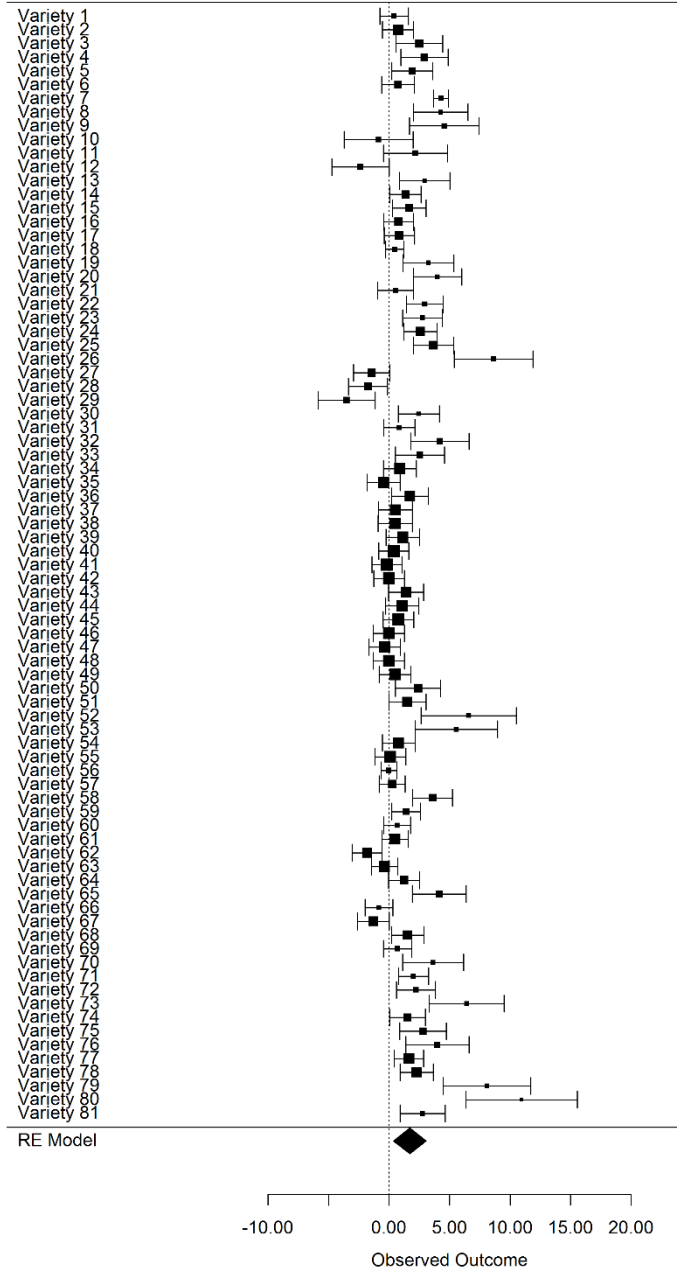


Figure S4 - Standardised mean differences (Hedges' g) for the three groups: hydric, metal and salt, using correlation coefficients of 0.25 (a, b) and 0.75 (c, d) to combine multiple dependent outcomes from the same study: a and c) without using study identification (ID) as a random variable; b and d: using study ID as a random variable.

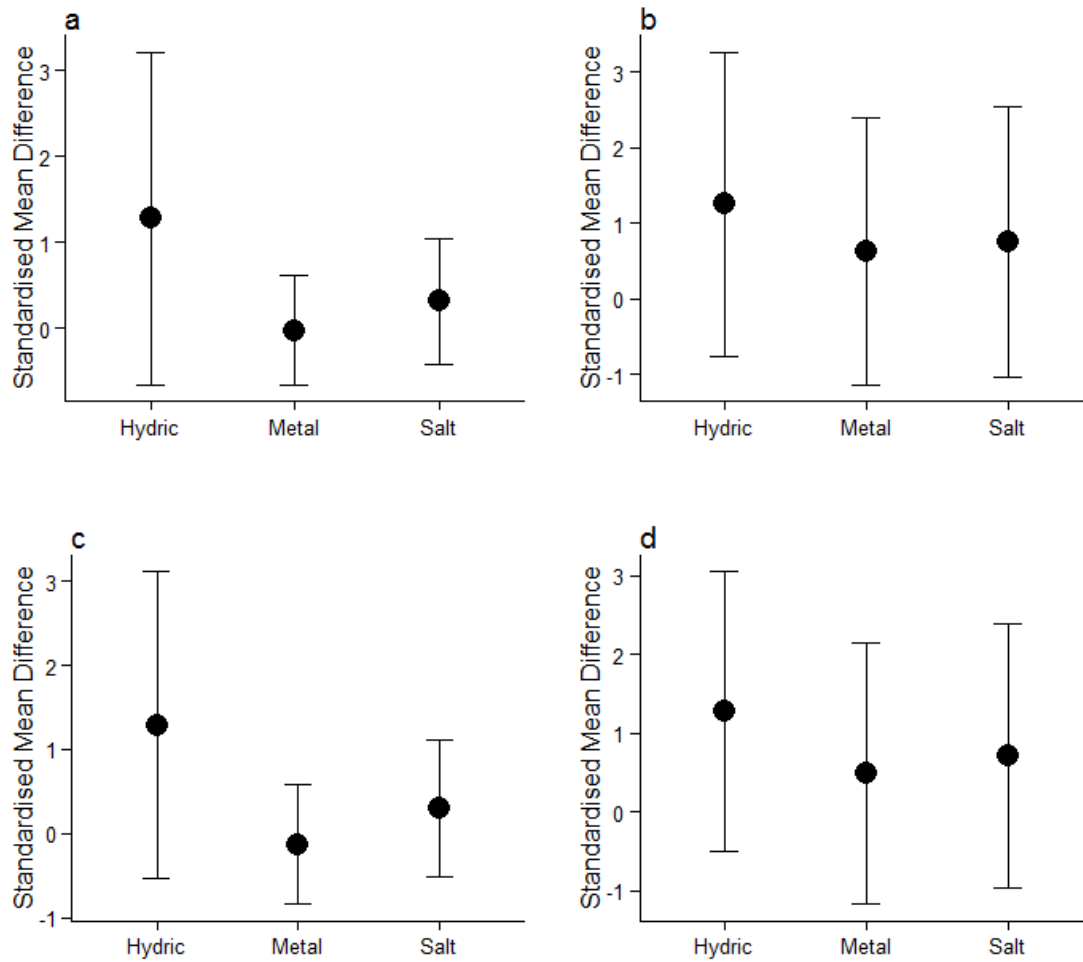
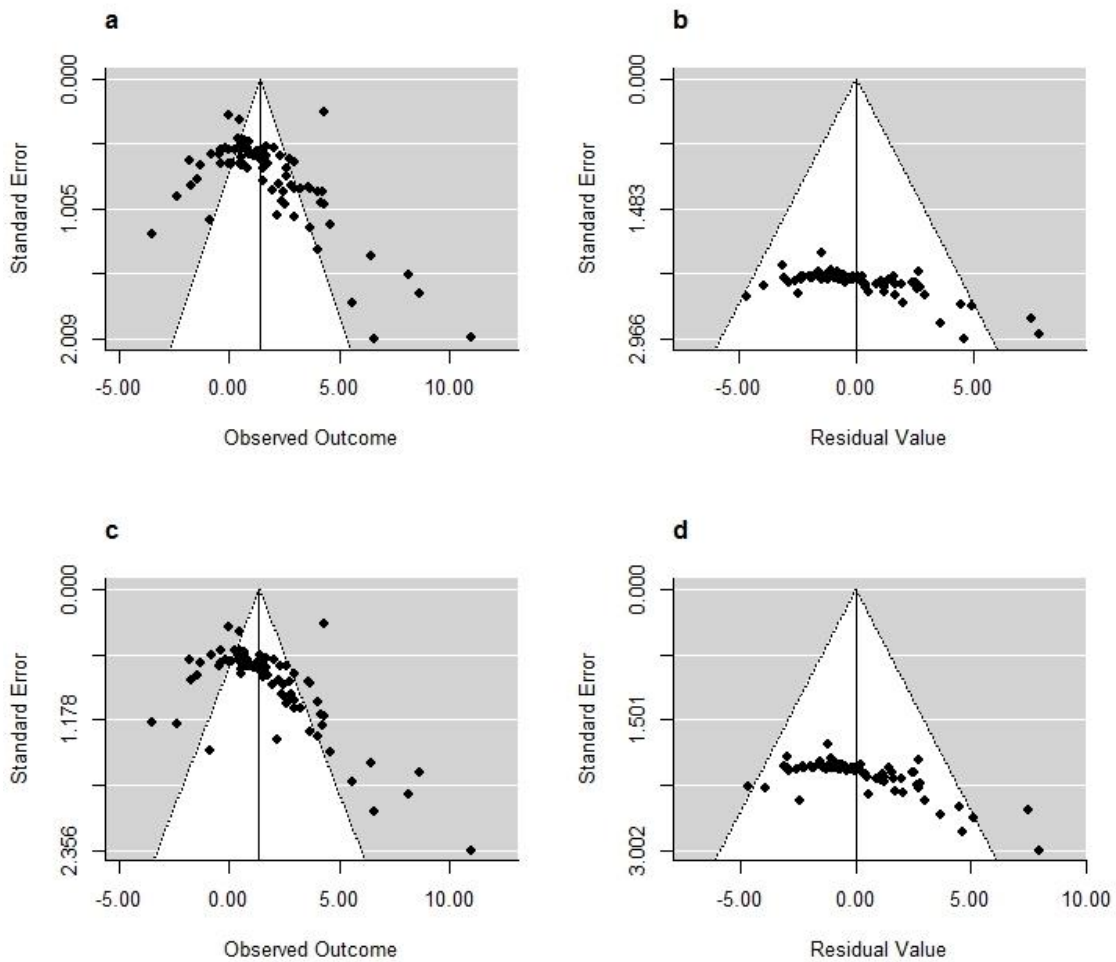


Figure S5 - Funnel plots of the effect sizes (a, c) and residuals of the meta-regression (b, d) using correlation coefficients of 0.25 (a, b) and 0.75 (b, d) to combine multiple dependent outcomes from the same study.



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2.3 Manuscript 3

Silicon absorption by plants in response to the environment

Abstract

Despite the importance given to Silicon in the relief of stress in cultivated plants, there are no studies that address this function of Si in plants under natural environments, aiming to identify responses that would indicate acclimatisation to the conditions at their place of origin. The goal of this study was to answer the following questions: 1) Does abiotic stress increase Si absorption? 2) Does the presence of Si stimulate biomass production in natural environments? and 3) Do plants from different environments display differences in Si absorption? To do so, the species *Eugenia punicifolia* was selected considering its wide distribution and occurrence in three different types of physiognomy, Coastal Savanna, Dense Deciduous Shrubland and Seasonal Deciduous Forest. Increases in Si absorption were directly related to increases in dry matter production in plants, suggesting that this may be a relief mechanism for temperature and hydric stresses. Differences in the response to stress conditions may be a result of the phenotypic plasticity which occurs in *E. punicifolia* and could be important information that brings a new perspective of Si to cropped plant scientists.

Keywords: water stress; temperature; phenotypic plasticity; acclimatisation; relief .

2.3.1 Introduction

Silicon is an element of brittle crystalline structure with enormous application in the field of plant science (GAUR et al., 2020). It is the second most abundant element, constituting about 32% (by weight) of the earth's crust (NEERU et al., 2019), and its common presence in the biosphere entangle to prove its essentiality as a nutrient for higher plants (CHAKMA et al., 2021). Several dysfunctions in plant growth and development can be caused by Si deficiency. However, the element is categorized as “non-essential” since cultivated plants accumulate less Si during normal environmental conditions (MIR et al., 2022).

Sustainable agricultural production is highly conditioned by the irregularity of favorable environmental conditions and the reduction in productivity, mainly influenced by abiotic stress factors such as drought, heat, cold, salinity and nutrient imbalance is 51 to 82%

(ZAHRA et al., 2021; RAZA et al., 2022). Current climate change induces drought and heat stress, two of the main abiotic stress factors that result in crop and productivity loss (ZANDALINAS et al., 2018). In the last four decades, studies have identified the role of silicon in increasing resistance and tolerance to this abiotic stress (HORIGUCHI; MORITA, 1987; EPSTEIN, 1999; LIANG et al., 2007; MIR et al., 2022).

The main benefits associated with Si to alleviate stress in agroecosystems have already been documented in terms of biomass and grain yield increase (WANG et al., 2021). Other goods related to Si are stimulus to root system development (ETESAMI; JEONG, 2018); absorption and nutrients assimilation gains (KIM et al., 2017) and maintenance of the water balance in plants (COSKUN et al., 2016). Among all benefits attributed to Si, Cooke and Leishman (2011) suggested that plants can use Si to reduce the effects of stress, allowing the permanence and occupation of areas with adverse conditions, or even to obtain an advantage in reproductive capacity over other plants. Research regarding the role of Si in the alleviation of environmental stresses in natural environments would reinforce the condition of its “essentiality” for plant science, in addition to clear the unknowing distribution and occupation of species that determine patterns of biodiversity (ALSTAD et al., 2016).

The Brazilian semi-arid tropical zone presents great environmental heterogeneity due to mosaic of associations between climate, soil, and relief, which directly influence the degree of aridity of the areas. As the altitude increases, for example, there is a reduction in temperature, an increase in precipitation, a greater availability of water in the soil and, consequently, a greater availability of some elements (TISDALE et al., 1985). In addition, the position of relief slope in relation to winds direction (windward and leeward) influences environmental humidity and consequently the composition of plant species.

Despite the importance given to the alleviation of stress in cultivated plants, there are no studies that address this function of Si in plants under natural environments and that identify their adaptation to these conditions. Considering this knowledge gap, our study explored the following questions: 1) Does abiotic stress increase Si absorption in plants? 2) Does the presence of Si stimulate biomass production in natural environments? and 3) Do plants from different environments display differences in Si absorption? To answer the above questions, we evaluated Si absorption and biomass production in plants of the same species, from different physiognomies and subjected to stress caused by high and low temperatures and water deficit. In addition, we used the responses to demonstrate the possible mechanisms of acclimatisation of these plants to their places of origin.

2.3.2 Materials and Methods

Focal species and study sites

The object of study was *Eugenia puniceifolia* (Kunth) DC, a species of wide geographical distribution, being frequently reported in various types of vegetation in tropical South America: Seasonal Forest (RODRIGUES et al., 1989), Coastal Forest (FABRIS; CESAR, 1996), Cerrado (PROENÇA, 1994), and occurs under different conditions of soil and climate (CONCEIÇÃO; ARAGÃO, 2010). Besides this wide distribution, the species was selected in view of the capacity of the Myrtaceae family to alleviate the physiological stress caused by adverse climatic conditions through the accumulation of Si (RAMOS et al., 2009).

Aiming to test how abiotic stresses (temperature and hydric stress) influence silicon absorption in *E. puniceifolia*, were collected seeds from three sites with different types of physiognomy. The sites were selected considering the different soil properties and climatic conditions, which may influence the concentration of available Si in the soil and the mechanisms of Si absorption by plants in response to the environment. The first site was a fragment of savanna vegetation, classified by Moro et al. (2011) as Coastal Savanna (CS), located in an urban coastal zone of Fortaleza (3°43'02"S, 38°32'35"W), in the State of Ceará, Brazil, at 16 m above sea level. The climate is tropical with dry summer (Koppen's classification, Alvares, et al., 2014), with average annual rainfall of 1338 mm, concentrated from January to March, and mean temperature of 28 to 30°C, with few or absence daily and/or monthly variation (MORO et al., 2011). The region includes areas of coastal plain (dunes and paleo dunes), pre-coastal tableland (*Barreiras* Formation) and fluvial plains, where different physiognomies can be found (CASTRO et al., 2012; IPECE, 2008). According to Jacomine et al., (1975), these soils originate from sandy-clay sediments (information confirmed by soil granulometry, Table 1), that related to the particular conditions of climate, such as rainfall and high temperatures, contribute to greater desilication, i.e. the removal of Si due to intense weathering (KORNDÖRFER et al., 2006). The soil of the area was classified as Ultisol (Soil Taxonomy).

Table 1 - Physicochemical characteristics at different layers of the soil in Coastal Savanna, Dense Deciduous Shrubland and Seasonal Deciduous Forest

| Property | Sites/Layers | | | | | | | | |
|---|-----------------|-------|-------|-----------------|-------|-------|--------------------|-------|-------|
| | Coastal Savanna | | | Dense Deciduous | | | Seasonal Deciduous | | |
| | 0-10 | 10-20 | 20-40 | 0-10 | 10-20 | 20-40 | 0-10 | 10-20 | 20-40 |
| Exchangeable Ca ⁺⁺ | 1.02 | 1.00 | 1.23 | 0.60 | 0.58 | 0.67 | 0.62 | 0.50 | 0.50 |
| Exchangeable Mg ⁺⁺ | 0.80 | 0.50 | 0.54 | 0.51 | 0.52 | 0.60 | 1.02 | 0.51 | 0.50 |
| Exchangeable Na ⁺ | 0.09 | 0.07 | 0.05 | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 |
| Exchangeable K ⁺ | 0.08 | 0.09 | 0.06 | 0.08 | 0.04 | 0.03 | 0.07 | 0.05 | 0.05 |
| Available P (mg kg ⁻¹) ^a | 6 | 5 | 8 | 12 | 13 | 11 | 16 | 17 | 12 |
| Al ³⁺ (cmol _c kg ⁻¹) ^b | 0.00 | 0.00 | 0.00 | 1.29 | 1.00 | 1.10 | 1.30 | 1.28 | 1.74 |
| Sum of bases | 1.99 | 1.66 | 1.88 | 1.21 | 1.17 | 1.32 | 1.73 | 1.08 | 1.07 |
| Potential Acidity | 0.90 | 0.83 | 0.83 | 7.12 | 4.70 | 4.38 | 5.50 | 5.16 | 6.62 |
| CEC (cmol _c kg ⁻¹) | 2.89 | 2.49 | 2.71 | 8.33 | 5.87 | 5.70 | 7.23 | 6.24 | 7.69 |
| Base Saturation (%) | 77 | 80 | 82 | 15 | 20 | 25 | 27 | 19 | 16 |
| pH in water (1:2.5) | 6.0 | 6.0 | 6.0 | 4.3 | 4.5 | 4.4 | 4.5 | 4.3 | 4.6 |
| Coarse Sand (g kg ⁻¹) | 693 | 661 | 622 | 353 | 332 | 300 | 120 | 80 | 100 |
| Fine Sand (g kg ⁻¹) | 230 | 252 | 261 | 530 | 534 | 523 | 730 | 712 | 641 |
| Silt (g kg ⁻¹) | 70 | 69 | 80 | 39 | 89 | 88 | 82 | 130 | 132 |
| Clay (g kg ⁻¹) | 23 | 44 | 51 | 74 | 58 | 59 | 79 | 160 | 168 |

Extracted using: ^aMehlich-1, ^bKCl 1M, ^cAmmonium acetate (EMBRAPA, 1997).

The remaining sites occur in the eastern margin of the Mid-North Sedimentary Basin, which forms an asymmetric cuesta, known as the *Ibiapaba* Plateau. One side with high-density small size vegetation (3 to 4 m), classified as Dense Deciduous Shrubland — DDS (locally known as *carrasco*), located on the dryer slope of the Basin (5°08'45"S, 40°55'43"W) at 700 m above sea level. The climate is semi-arid, with average annual rainfall of 636.61 mm, concentrated from January to May, and mean annual minimum and maximum temperatures of 19.14 ± 1.78°C and 33.6 ± 3.85°C respectively (VASCONCELOS et al., 2010), which shows a large variation in temperature for this area. The other side was a Seasonal Deciduous Forest (SDF), located on the most humid slope of the basin (5°08'29"S, 40°54'05"W), at 650 m above sea level. The climate is also tropical with dry summer, with an average annual rainfall of 1044 mm, concentrated from January to April (corresponding to more than 80% of the annual precipitation) and mean annual temperature of 24.8°C (LIMA et al., 2011). The soils were

classified as Typic Quartzipsamment and Lithic Udipsamment, respectively (Soil Taxonomy). The sites from the Ibiapaba Plateau differ from the site from the coastal zone in terms of soil physicochemical properties (pH, soil texture and concentration of available Si) (Table 1), and especially regarding the soil depth (being shallower, which allowed sampling only up to 40 cm depth). According to Pulz et al., (2008), low pH values (as seen in DDS and SDF; mean = 4.5) contribute to higher solubility of the Si, reducing Si adsorption at these sites. The morphological description of the profiles showed that desilication was less intense in DDS and SDF compared to CS, possibly due to the lower rainfall and temperatures. Herpin et al. (2004) established the same relationship between desilication, temperature and precipitation under conditions similar to this study. According to Tisdale et al. (1985), younger soils display greater levels of Si. It can therefore be inferred that these soils are younger than the CS, with a higher content of available Si, as shown in Table 2.

Sampling and laboratory experiment

Ten plots of 100 m² (10 x 10 m) on each site were selected, and four samples in each plot randomly distributed were collected, homogenized, air-dried, sieved (2 mm grid) and stored for further physicochemical analysis. Fruits from different individuals (5 plants from each plot) were collected and taken to the laboratory for pulping, disinfection, dormancy breaking by mechanical scarification, and germination, according to protocol established by Brazilian Ministry of Agriculture, Livestock and Food Supply (BRASIL, 1992). The seeds were kept in B.O.D (Biochemical Oxygen Demand) germination chambers at constant temperature of 25°C and lightened for 12 hours with GE 20-watt fluorescent light bulbs, which allowed a radiant flux of about 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (ROSA; FERREIRA 1998). Only seeds with 0.5 cm of emerged roots were considered germinated. After emergence, the seedlings were kept in humidity chambers in washed-autoclaved sand for about 30 days, until the emergence of three or more definitive leaves.

To test the influence of temperature on Si absorption, were conducted experiments simulating temperature stress at 15 °C, 25 °C, and 45 °C, adopting an approximate average temperature at the three sites of study (25 °C), and values above and below found in the field (45 °C and 15 °C). Three seedlings were transferred to a plastic container constantly aerated, and added a nutrient solution as Johnson et al. (1957). Sodium metasilicate was added (0.9 mmol L⁻¹ ~ 25 mg L⁻¹) to the nutrient solution (as source of Si, referred as +Si treatment) to five containers, each with three seedlings, for each temperature treatment. Treatments without

Si (-Si treatment) were also kept. The Si concentration was adopted based on reports involving stress (EPSTEIN, 1999). The nutrient solution was added every seven days for treatments with and without Si.

Initially, the seedlings were kept in the solution at 20% of the total concentration, then the nutrient concentration was raised to 50% (second week), and finally to 100% (third week). The pH of the nutrient solution was monitored every two days and, if necessary, corrected to 6.5 with NaOH or HCl; the initial electrical conductivity was also corrected to 2.5 mS cm⁻¹.

To test the influence of hydric stress on Si absorption, plastic pots containing approximately 2 kg of washed autoclaved sand was used in which three seedlings were placed per pot and kept in a greenhouse at 35 °C under sunlight. For the acclimatisation of the seedlings, the vessels were maintained at 90% soil field capacity (FC) by adding water daily for two weeks. The field capacity was determined on a gravimetric basis according to Nachabe (1998). The pots received a chemical fertiliser containing 0.25g N + 0.125g P₂O₅ and 0.125 g K₂O pot⁻¹. The treatments were then applied, consisting of two moisture levels, 60 and 40% FC, either in the presence of Si at 100 mg kg⁻¹ soil or without Si, with 5 replications. The moisture levels were established considering the precipitation in each area, one level of no stress, approaching to field capacity during the rainy season (60%), and the other associated to stress (40%), as reported in the literature (AMIN et al., 2014). Silicon was applied to the soil in the form of Na₂Si₃O₇. The soil moisture level was checked daily by weighing the pots and adding water to reach the weight corresponding to the moisture level at 60 and 40% FC.

Determination of Si in soil and plant and relative water content

The quantification of Si in soil was carried out according to Snyder (1991) and readings for Si in the extracts were taken with a spectrophotometer at a wavelength of 660 nm.

For the quantification of Si in plants, 0.1 g of ground samples were mixed with 2.0 mL of H₂O₂ at 30% (v/v) and 3.0 mL of NaOH (0.25 mol L⁻¹) and autoclaved for 1 hour at 123°C and 0.15 MPa for digestion. Then the volume was adjusted to 50 mL with ultrapure water, and an aliquot of 1.0 mL was taken and further diluted in 20 mL of ultrapure water. Si concentration was determined spectrophotometrically (CARNEIRO, 2007), at 410 nm of the yellow colour of the molybdosilicic acid formed after reaction between the Si and the ammonium molybdate in an acid environment with HCl (14 mol L⁻¹) (KORNDORFER et al., 2004). The accumulated Si was obtained considering the dry matter production of plants.

To measure Si absorption, one plant was taken at 30, 45 and 60 days, from each replication in each experiment, shoots and roots were separated, washed with deionized water, dried in an oven with force air circulation at 65°C until constant weight to determine the dry matter, and then ground for Si quantification. Plants with -Si treatment were sampled to evaluate the natural occurrence of Si.

The relative water content (%) was determined in the water stress experiment. For this, the youngest fully developed leaf from one seedling in each pot was collected, cut at the base of the blade, and quickly transferred to the laboratory in a sealed plastic bag, to get the leaves fresh weight (LFW) within one and a half hours of collection. The leaves were then soaked for 16-18 hours at room temperature ($25 \pm 2^\circ\text{C}$), dried on paper towels and weighed again to determine the turgid weight (LTW). The dry weight (LDW) was also determined after drying the material in an oven at 65°C for 72 hours. The relative water content (RWC) was calculated from the formula proposed by Turner (1986): $\text{RWC (\%)} = (\text{LFW} - \text{LDW}) / (\text{LTW} - \text{LDW}) \times 100$.

Statistical Analysis

Normality of the data and homogeneity of the residue data were tested with the Kruskal-Wallis Test. Analysis of variance (ANOVA) was then carried out, using Tukey's test to compare treatment means. The experiment was set up in a split-split lot design, considering the three areas as the main lots and temperature, humidity level and sampling period as the sub-lots, with five replications, using R software v. 3.1.2 (R CORE TEAM, 2014).

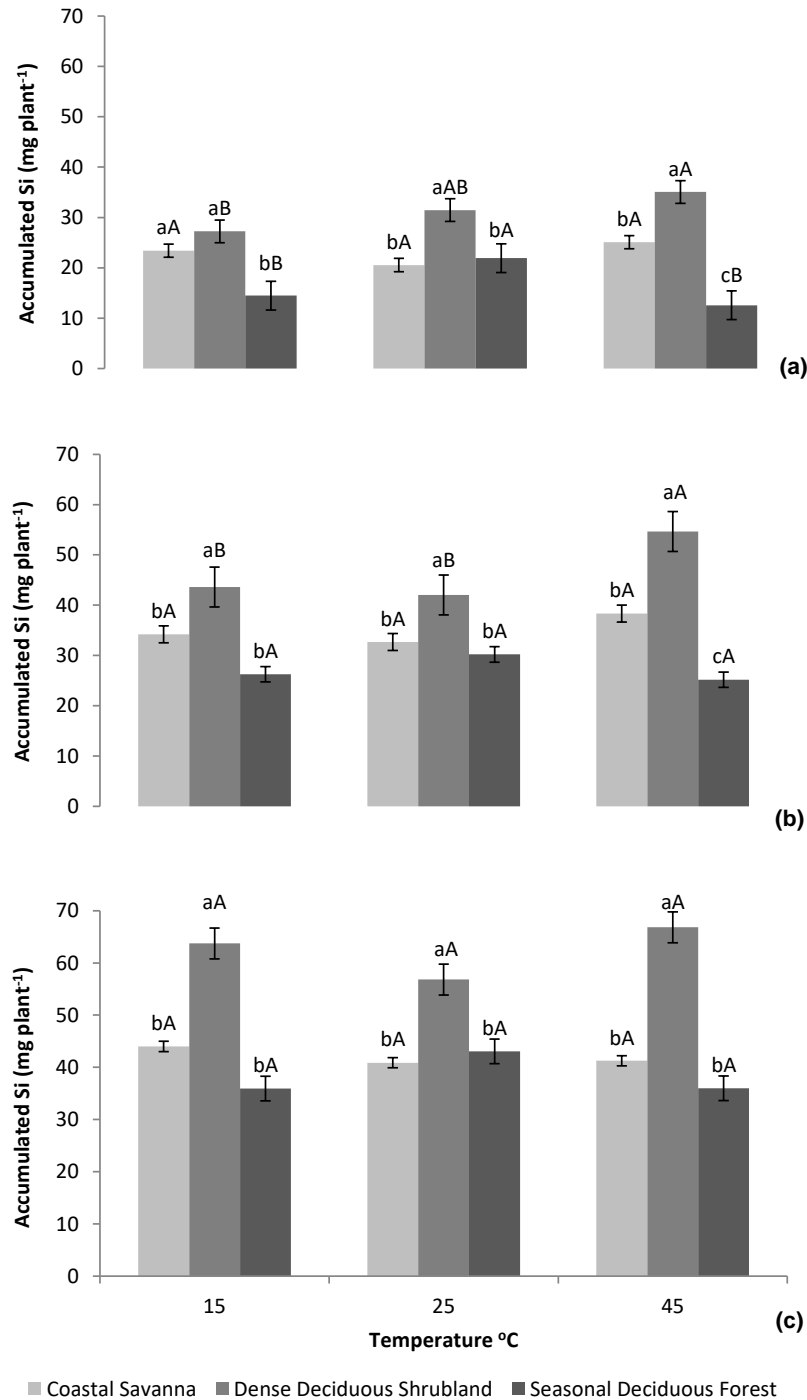
2.3.3 Results

Does abiotic stress increase Si absorption?

The Si absorption varied depending on the temperature (Figure 1). In general, the higher absorption of silicon occurred at the highest temperature (45°C). However, this pattern was more evident in DDS and in the first two-time intervals (30 and 45 days). At 60 days of experiments, there were not differences among sites or among temperatures, indicating that there must be saturation in the silicon absorption capacity over time. At the beginning, the seedlings incorporate the silicon to mitigate the effects of abiotic stress, but this effect

diminishes with time and the plants began to incorporate the Si regardless the greater abiotic stress.

Figure 1 - Accumulated silicon (Si) in plants of *Eugenia punicifolia* from three different areas (Coastal Savanna, Dense Deciduous Shrubland and Seasonal Deciduous Forest), grown in Si-rich nutrient solution under different temperature regimes, at 30 (a), 45 (b) and 60 (c) days of the experiment. Mean values followed by the same uppercase letter do not differ when comparing the effects of temperature for the same area, and mean values followed by the same lowercase letter do not differ when comparing areas with the same temperature, by Tukey's test at 5% probability



Evaluation of accumulated Si in plants grown with nutrient solution without the addition of Si (Figure 2) suggests that plants from DDS and SDF areas - with more available Si in the soil, (Table 2), also have higher concentrations of Si in their tissues.

Figure 2 - Accumulated silicon (Si) in plants of *E. puniceifolia* from three different areas, grown in nutrient solution with no addition of Si, under different temperature regimes, at 60 days of the experiment (mean values followed by the same uppercase letter do not differ when comparing the effects of temperature for the same area, and mean values followed by the same lowercase letter do not differ when comparing areas with the same temperature, by Tukey's test at 5% probability).

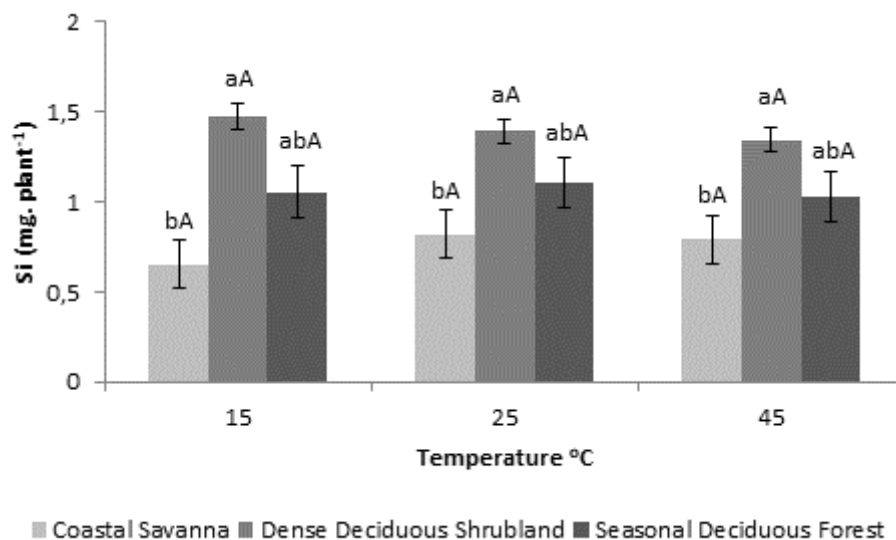


Table 2 - Average available Silicon (Si) content (mg kg⁻¹) at different layers of the soil in sites of Coastal Savanna, Dense Deciduous Shrubland and Seasonal Deciduous Forest

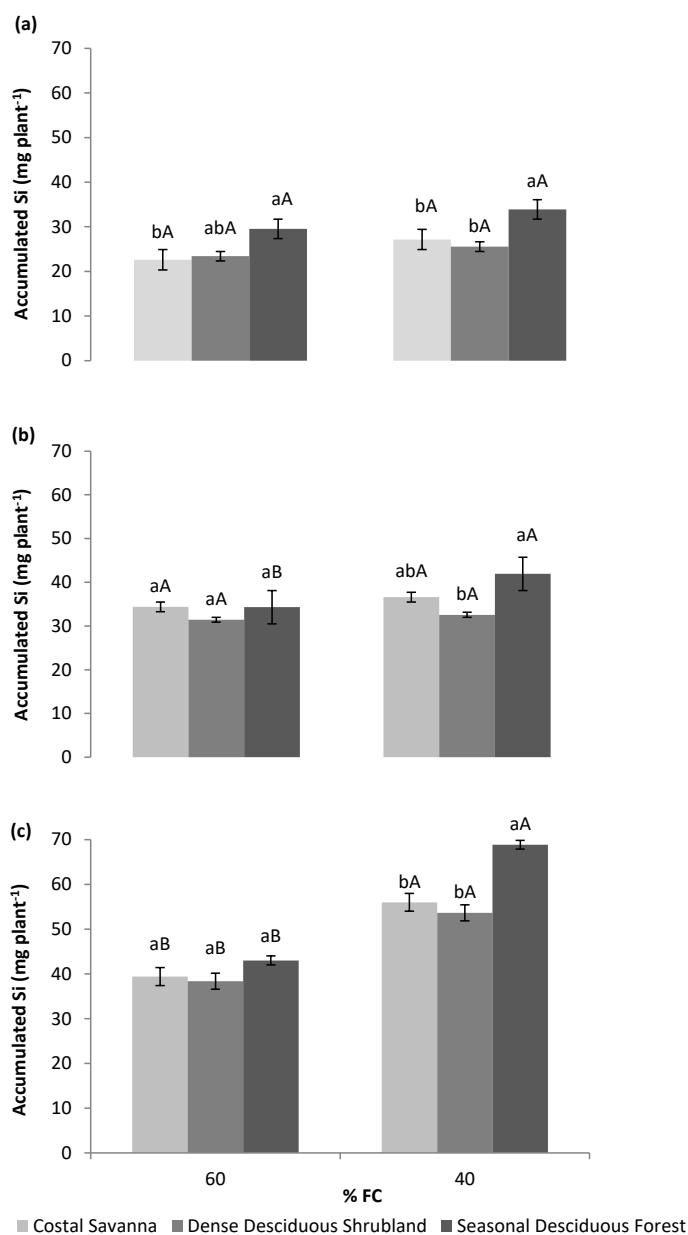
| Sites | Layer (cm) | | |
|-----------------|------------|---------|---------|
| | 0-10 | 10-20 | 20-40 |
| Coastal Savanna | 0.133Ab | 0.147Ac | 0.125Ac |
| Dense | 0.767Aa | 0.970Aa | 0.970Aa |
| Seasonal | 0.705Aa | 0.605Ab | 0.506Ab |

*Mean values followed by the same letter, lowercase in a column and uppercase in a row, do not differ by Tukey test at 5% probability.

Under conditions of water restriction, Si accumulation also was influenced by stress. Seedlings at 40% FC accumulated more Si than seedlings at 60% FC (Figure 3). However, unlike the temperature stress experiments, the effect of stress was more evident at 60

days, indicating that seedlings should take longer to be influenced by the negative effect of water stress than temperature stress.

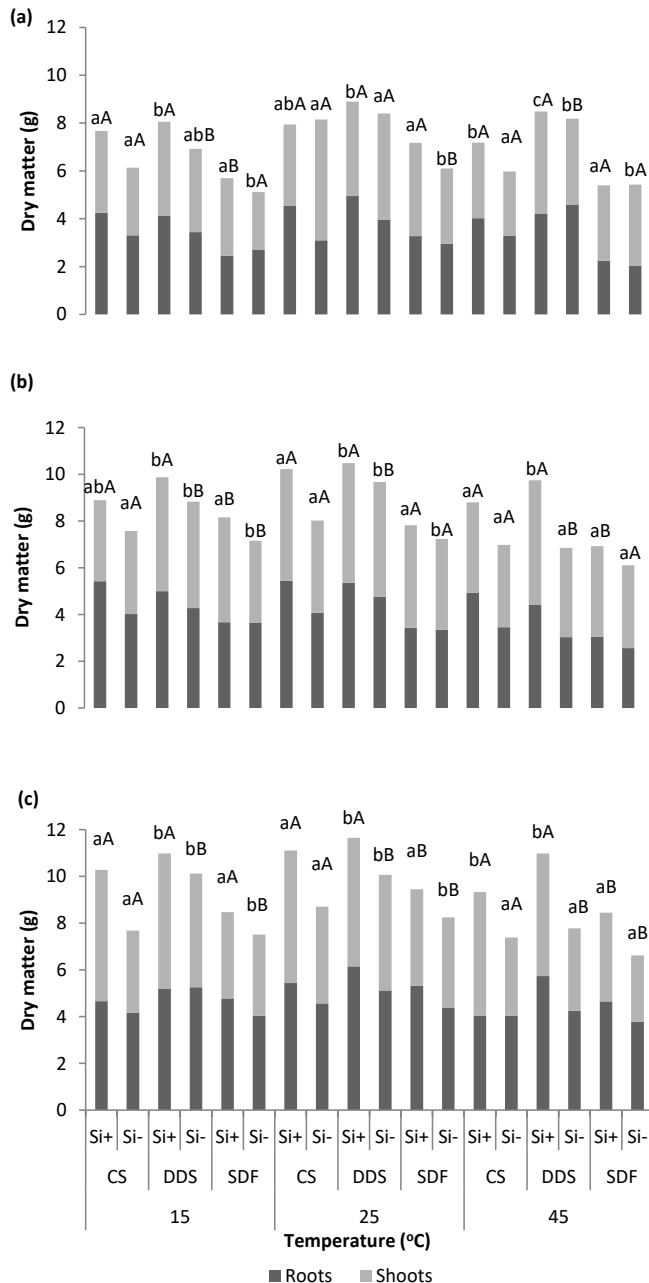
Figure 3 - Accumulated silicon (Si) in plants of *Eugenia punicifolia* from three different areas, grown in nutrient solution with no addition of Si, under different water regimes, at 60 days of the experiment (mean values followed by the same uppercase letter do not differ when comparing the effects of water regime for the same area, and mean values followed by the same lowercase letter do not differ when comparing areas with the same water regime, by Tukey's test at 5% probability).



Does the presence of Si stimulate biomass production in natural environments?

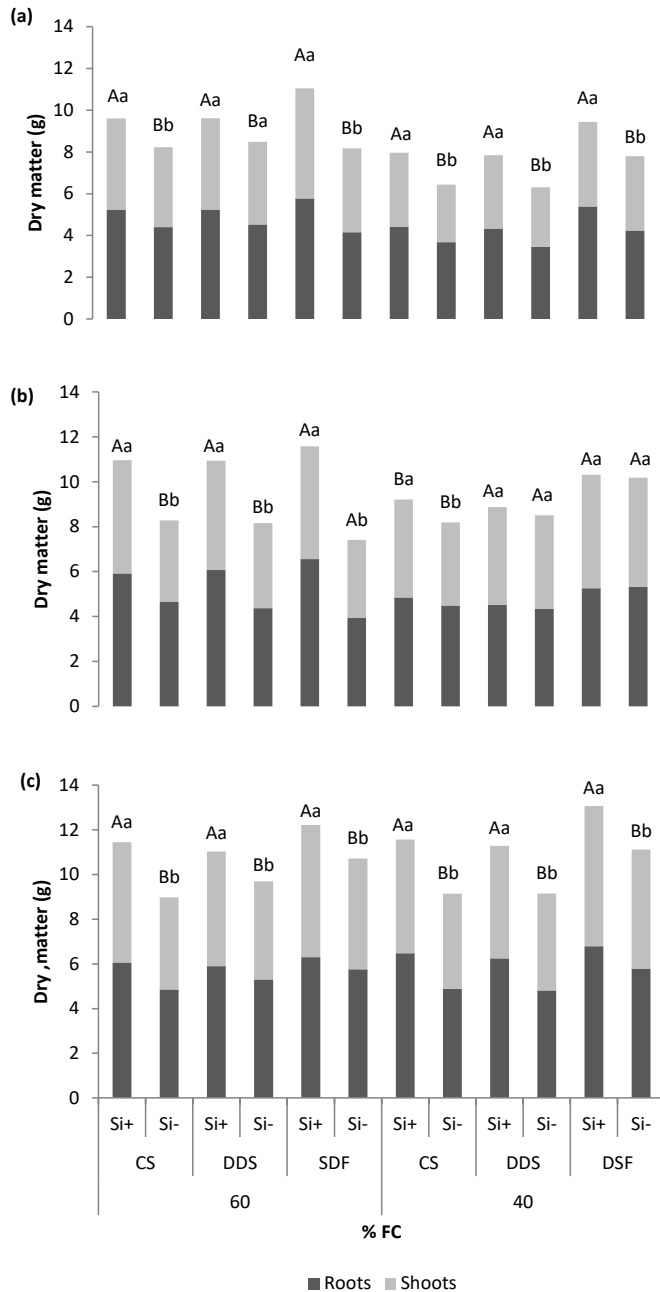
The addition of Si to the nutrient solution increased dry matter production of shoots and roots of *E. puniceifolia* from the three areas during the evaluated periods (Figure 4). Within the areas, the DDS showed higher biomass production compared to the others, being higher at 60 days. Analysing the first sampling period, gains in roots seems higher than shoot except for SDF where the shoots were higher under the three tested temperatures. At 45 days the roots gains were higher only at CS area while in the other areas the presence of Si influences root gains and in its absence was for shoot. At the SDF area shoot was higher than root at 25 and 45°C, while at 15°C the presence of Si promotes shoots gains. Root gains was higher at SDF for the three tested temperatures at 60 days while at DDS at 25 and 45°C. The shoot gain was benefited by the addition of Si at the CS area.

Figure 4 - Dry matter production (roots and shoots) in plants of *Eugenia punicifolia* from three different areas: CS (Coastal Savanna); DDS (Dense Deciduous Shrubland); SDF (Seasonal Deciduous Forest), grown in a nutrient solution with (Si+) and without (Si-) silicon, under different temperature regimes and sampling periods: (a) 30, (b) 45 and (c) 60 days. Mean values followed by the same uppercase letter do not differ when comparing the presence or absence of Si in the nutritive solution for the same area, and mean values followed by the same lowercase letter do not differ when comparing areas with the same tested temperature, by Tukey's test at 5% probability



The presence of Si stimulated the production of biomass in the experiment for water stress, when compared to seedlings from the same area, being root gains higher than shoots with higher values found after 60 days of the experiment (Figure 5). However, there were not differences among the mean values for biomass production in the three sites. The absorption of silicon benefited the growth of seedlings independent of sites.

Figure 5 - Dry matter production (roots and shoots) in plants of *Eugenia punicifolia* from three different areas: CS (Coastal Savanna); DDS (Dense Deciduous Shrubland); SDF (Seasonal Deciduous Forest), grown with (Si+) and without (Si-) silicon, under different moisture regimes and sampling periods: (a) 30, (b) 45 and (c) 60 days (mean values followed by the same uppercase letter do not differ when comparing the presence or absence of Si in the nutritive solution for the same area, and mean values followed by the same lowercase letter do not differ when comparing areas with the same tested temperature, by Tukey's test at 5% probability).



The presence of Si also increased the relative water content (RWC) of the leaves at the two tested moisture levels (Table 3). However, the water deficit affected this variable, reducing the RWC even in the presence of Si, compared to the condition of no water restriction.

Table 3 - Relative leaf water content (RWC) in Coastal Savanna, Dense Deciduous Shrubland and Seasonal Deciduous Forest grown under different moisture regimes (Si+: Si)

| Site | Moisture (%) FC | Si | RLWC (%) |
|---------------------------|-----------------|----|----------|
| Coastal Savanna | 60 | + | 74.43Aa |
| | | - | 49.57Ab |
| | 40 | + | 26.04Ba |
| | | - | 22.07Aa |
| Dense Deciduous Shrubland | 60 | + | 54.59Ba |
| | | - | 42.46Bb |
| | 40 | + | 32.28Aa |
| | | - | 22.38Ab |
| Seasonal Deciduous Forest | 60 | + | 72.99Aa |
| | | - | 53.69Ab |
| | 40 | + | 39.67Aa |
| | | - | 28.85Ab |

Mean values followed by the same letter, lowercase in a column and uppercase in a row, do not differ by Tukey test at 5% probability.

Do plants from different environments display differences in Si absorption?

It should be noted that responses for Si absorption as a function of temperature, was more evident during the first 30 days of the experiment (Figure 1a). During that period, plants from CS and SDF areas did not absorb greater amounts of Si as a function of temperature variations. On the other hand, plants from the DDS displayed greater Si absorption at all tested temperatures, when compared to the other areas.

The same behaviour was seen at 45 days. In the first two periods, plants from the DDS absorbed more Si when subjected to the higher temperature (45°C), whereas for plants from the SDF and CS, the variations in temperature do not appear to affect Si absorption, since no significantly differences were observed.

Finally, after 60 days of the experiment, an increase in Si absorption (30 <45 <60 days) was confirmed for all areas under study (Figure 1c), with no influence of temperature.

On the other hand, when evaluated the absorption of Si under water stress conditions (Figure 3), was noticed that SDF area showed greater sensitivity to stress.

2.3.4 Discussion

Was confirmed the first prediction that abiotic stress causes changes to Si absorption, especially for plants from the DDS at 45°C. According to Lamarca et al. (2011), the optimum temperature for germination and growth in *Eugenia* is 25°C. Additionally, it is known that higher plants, when exposed to excessive heat, characterised by at least 5°C above the optimum growth temperature, display a particular set of cellular and metabolic responses necessary for plants to survive under high temperature (GUY, 1999). Thus, was not expected for *E. puniceifolia* to exhibit high growth at high temperatures (above 30° C). However, it is possible that the presence of Si allowed the growth at high temperatures, due to mechanisms of stress relief associated with use of this element (COOKE; LEISHMAN, 2011). For these plants, the stress conditions stimulated Si absorption, corresponding to a possible mechanism of stress alleviation.

It was expected that plants from the CS display similar behaviour to those from the DDS, since they are subjected to higher temperature ranges under natural conditions than those from DDS. However, low Si availability in the soil from CS induce to low Si absorption, even under extreme conditions of temperature, demonstrating that the environment to which the plants are subjected, influences the adaptation of the species to similar conditions of their place of origin.

To minimise damages due to water shortage, plants develop various strategies to resist or avoid water stress (MIR et al., 2022; YIN et al., 2014), such as reduction in the growth rate of leaves and stem, the synthesis of osmotic solutes that are involved in maintaining cell turgidity, and the synthesis of antioxidant proteins to prevent chlorophyll breakdown (WILKINSON; DAVIES, 2010). Since the plants of the study areas are normally subjected to seasonal rains, these mechanisms may have been activated before Si absorption stimulation under stress condition, thereby causing a delay in the response of the element. In that case, the relative leaf water content in plants under water stress should be considered a more efficient variable to demonstrate changes in the functions of plants under water stress over a short period of time.

The effect of Si preventing water loss from leaves, regardless the physiological mechanism involved in this benefit is not well understood, but literature suggest that may be

related to the formation of a double layer of silica cuticle and silica cellulose (CHANG et al., 2020). This would induce a reduction in the amount of water lost by evapotranspiration throughout the vegetative cycle, requiring less water and becoming more resistant to a possible drought. However, in this study, plants from the three areas did not reflect this beneficial effect from Si when subjected to water stress. Besides the natural adaptation of plants to their original environments, it should be considered that the principal mechanism for absorbing Si is mass flow (Epstein, 1999), that varies depending on plant species (MOTOMURA et al., 2002; GAUR et al., 2020). Considering that the areas of study have no anthropic intervention (e.g. irrigation), and that plants absorb more Si under regular water supply, it can be stated that water was a limiting factor that determined stress in the development of these plants under the studied conditions.

Extreme weather, including high temperatures and water stress, have a negative effect on plant growth and development, leading to a catastrophic loss of biomass production (BITA; GERATS, 2013; WANG et al., 2021). In the current study, plants under high-temperature or water deficit, showed higher biomass production in the presence of Si solution than under its absence. It can therefore be assumed that Si absorption alleviated the temperature and water stress.

Moreover, it was demonstrated that Si absorption positively affects plant biomass. These results agree with other studies using both soil and nutrient solutions (VACULI'K et al., 2009; SHEN et al., 2010). However, seems that the beneficial effect of Si on the production of plant biomass may not be evident unless the plants are subjected to some type of stress (FAUTEUX et al., 2005). In the present study, the affirmation that abiotic variations conditioned the stress in plants and stimulated Si absorption is made possible by the increase in biomass production, especially under extreme temperatures or water stress. It is believed that plants under natural and agricultural systems, subjected to stress temperature suffer by other stress such as water deficit. Considering the increases of temperature and water shortage due to climate change around the world (IPCC, 2007), it is evident the necessity to understand the impact of stress on plant functions, and especially the physiological response mechanisms of plants both during and when recovering from stress (SHEN et al., 2010). The plants in this study were not subjected to simultaneous stresses. However, if the pattern of absorption caused by the temperature increase occurs in the field, the plants will also benefit from Si absorption, which would take place because most of the deposited silica, in particular on the outer walls of the epidermal cells on both sides of the leaves, would form a double layer that would prevent water loss through stomatal transpiration (HATTORI et al., 2005). According to Balakhnina;

Borkowska (2012), the combined effects of Si in plants under stress conditions remain being not understood.

Finally, it was demonstrated that the environment affected Si absorption. Plants of the same species occurring under different environmental conditions reacted differently to temperature and water variations with respect to Si absorption. The absorption of Si by plants seems to be a response of the original environment, e.g. plants from areas with higher temperatures respond to variations in temperature, increasing Si absorption. Such behaviour seems to be related to the natural Si content of these areas (see Table 2), since plants occurring in areas with higher Si availability in the soil absorbed more Si in response to temperature variations. Among the areas under study, plants from the DDS were the most influenced by variations in temperature. This may be related to the location and altitude, where temperature variations up to 10°C during the day are more frequent (ROCHA, et al. 2002), leading this area to be considered as the one with most adverse conditions. In the laboratory, plants from this area had the same behaviour, absorbing more Si regardless of the temperature. In addition, the higher concentrations of Si in plants from this area, even without the addition of Si to the nutrient solution, confirms the higher availability of Si found in the soil (see Table 2), and a tendency to its absorption at temperatures which cause some stress.

Moreover, the Si absorption patterns seen in the present study suggest that *E. punicifolia* has high phenotypic plasticity. According to Scheiner (1993), phenotypic plasticity represents the ability of an organism to change its physiology and morphology in response to its interaction with the environment. Therefore, species with the potential for plasticity in characteristics related to survival or occupation, have adaptive advantages in unstable, heterogeneous, or transitional environments (VIA et al., 1995). In the present study, the absorption of Si in plants from the DDS under higher temperatures, and from the SDF under water stress, could be important mechanisms for the maintenance of *E. punicifolia* in hotter environments, demonstrating a greater capacity of the species to resist variations in temperature and humidity, even with a delay in the stress response of those plants that are not used to such variations naturally.

This would then explain the wide distribution of *E. punicifolia* in different environments, as found by Conceição and Aragão (2010) and Arantes and Monteiro (2002) being important information for plant scientists, since aspects related to the species adaptability, life history, origin, and spatial distribution, could direct studies in genetics and selection of attributes that enable a sustainable production, that reduce the crops susceptibility to environmental fluctuations or the increasing dependence of crop inputs.

2.3.5 Conclusions

Si absorption by *E. puniceifolia* is influenced by variations of temperature and drought, since the plants subjected to these conditions originate from locations with high soil Si availability, and temperatures close to those tested in this study under natural conditions. The plants seem to reflect adaptation to the stresses to which they are subjected, especially water stress, showing late responses to Si absorption. Increases in Si absorption, triggered by water and temperature stress, are directly related to increases in dry matter production, suggesting that this may be a relief mechanism for the stresses under test. The findings could be of relevance importance specially for cropped science, bringing new perspectives about Si behavior in agriculture.

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2.4 Manuscrito 4

Silício na ecologia de plantas: uma ferramenta para auxiliar na compreensão dos padrões de distribuição de espécies

Resumo

Ainda não existe nenhum estudo que forneça evidências das funções do Si associadas ao alívio de estresses em sistemas naturais ou outras áreas de investigação. O presente estudo surgiu da necessidade de compreender e documentar possíveis relações de alívio a estresses abióticos pelo Si em ambientes naturais e objetiva relacionar a absorção de Si, a produção de biomassa e a ocorrência de *Eugenia puniceifolia* em três fitofisionomias com características edafoclimáticas distintas (Savana Costeira, Arbustaria Densa Decídua e Floresta Sazonal Decídua) com a função de alívio a estresses abióticos desempenhada pelo elemento. Observamos que as plantas de *E. puniceifolia* absorvem mais Si do solo quando estimuladas pela presença de fatores limitantes, como condições climáticas ou presença de elementos fitotóxicos no solo, refletindo adaptações aos seus locais de origem. A composição química desses solos, além de estar relacionada com o material de origem, também foi influenciada pelas condições climáticas das áreas. Esse estudo permite inferir sobre funções de alívio do Si a estresses em sistemas naturais, confirmando os benefícios do elemento já discutidos para culturas agrícolas, o que nesse caso, possibilitam a ocupação e distribuição das plantas em áreas com limitações abióticas, especialmente quando submetidas à combinação de dois ou mais fatores que condicionem estresse, como demonstrado aqui. As diversas funções sugeridas em experimentos agrícolas forneceram evidências dos mecanismos que ocorrem nos sistemas aqui estudados, auxiliando outras áreas de conhecimento para explicar questões ainda não resolvidas.

Palavras-chave: biomassa; abundância; microscopia eletrônica; resistência; limitações abióticas

2.4.1 Introdução

Na botânica, os efeitos do silício (Si) sobre o crescimento, desenvolvimento e rendimento das plantas foram durante muito tempo, amplamente ignorados (WANG et al., 2021). Uma das principais razões é que a toxicidade ou deficiência do silício não leva a sintomas

significativos nas plantas (MA; YAMAJI, 2006). Além disso, o silício não atendeu aos critérios de elemento essencial propostos por Arnon e Stout (1939). Após o ano de 2010, um aumento dramático no número de publicações indica que este campo está recebendo mais atenção da pesquisa (ZELLNER et al., 2021). Os biólogos/fisiologistas de plantas reconheceram o significado do Si já no século XIX (HODSON; GUPPY, 2022, SONG et al., 2022) e o silício é agora considerado um elemento benéfico, mas ainda não essencial para o crescimento e desenvolvimento das plantas.

A pedosfera de Si da Terra é estimada em 28,2% em peso (REA et al., 2022, TUBANA et al., 2016). Junto com oxigênio e metais, forma dióxido de silício (Si_2O) e silicatos solúveis em água. Os minerais passam por diversas intempéries físicas e químicas e liberam Si em solução sob condições de pH adequadas (REA et al., 2022), sendo a fonte de sílica e silicatos em solos e argilas os minerais de Si intemperizados, como quartzo e feldspato (SHAKOOR et al., 2014). O elemento está presente no solo principalmente em três fases diferentes: sólido, líquido e adsorvido. As fases sólidas podem ser amorfas ou cristalinas e a sílica amorfa contribui significativamente mais para dissolver o Si na solução do solo devido à sua maior solubilidade do que a forma cristalina (TUBANA et al., 2016). No entanto, a planta não absorve nenhum Si como sílica amorfa; em vez disso, é absorvido pelas plantas na forma de ácido monossilícico (H_4SiO_4) (KEEPING, 2017).

Nas plantas, de um modo geral, pode ser encontrada uma quantidade relativamente alta de Si, quando comparado a outros elementos essenciais como o cálcio, magnésio ou fósforo (BAKHAT, et al., 2018). Devido à abundância de Si e à sua atuação no sistema solo-planta, cada planta cultivada no solo contém uma quantidade apreciável de Si (EPSTEIN, 1999). Mas, apesar de ser um elemento abundante no solo, os solos contêm diferentes concentrações de Si. Por exemplo, os horizontes superiores apresentam baixas concentrações de Si, principalmente em solos arenosos (MARSCHNER, 2011), enquanto os solos argilosos contêm quantidades maiores de filossilicatos que liberam Si (BAKHAT et al., 2018).

Os solos tropicais e subtropicais geralmente têm níveis baixos de Si, devido aos processos de lixiviação e intemperismo (EPSTEIN, 1999). Nessas regiões, existe ainda uma enorme variação de condições climáticas, o que representa um potencial motor para desenvolvimento de mecanismos de adaptação ou plasticidade fisiológica das plantas (ROSA-MANZANO et al., 2017). Sabe-se que a gama de condições ambientais em um local determina quais espécies podem sobreviver e reproduzir com sucesso lá. As espécies de plantas que ocorrem nesses ambientes, muitas vezes, exibem múltiplas estratégias para conviver com as condições limitantes que garantem sua persistência e sucesso reprodutivo (SAHEBI et al., 2015;

SILVEIRA et al., 2013). Os fatores que determinam a distribuição e a ocupação das espécies em ambientes estressantes são cruciais para a compreensão dos padrões de biodiversidade, e, portanto, comumente estudados (ALSTAD et al., 2016). No entanto, ainda não foi feita nenhuma referência ao Si como potencial facilitador da ocupação de espécies em ambientes naturais com condições limitantes.

Embora as plantas possam sobreviver com baixa disponibilidade de Si no solo, é comprovado que as plantas privadas de Si são, muitas vezes, estruturalmente menos resistentes do que plantas crescidas em ares ricas em Si (BAKHAT, et al., 2018). Considerando os efeitos benéficos do Si, amplamente documentados para as plantas agrícolas submetidas a condições de estresse, o Si também poderia estar relacionado a alívios a estresses ambientais em condições naturais. Considerando ainda que o estresse estimula a absorção do elemento, a presença de um ou mais estresses simultâneos, comum em ambientes naturais, resultaria em maior acúmulo de Si pelas plantas. Nessas condições, as plantas poderiam se beneficiar do acúmulo do elemento, o que permitiria, por exemplo, maior ocupação e distribuição dos indivíduos.

Assim, esse estudo se propõe investigar como o Si atua em plantas sem interesse agrônomico, mas com potencial de comprovar como o elemento pode determinar a ocorrência de espécies de ampla distribuição.

2.4.2 Material e Métodos

Espécie focal e caracterização das áreas

A espécie vegetal *Eugenia puniceifolia* (Kunth) DC foi selecionada para esse estudo pela sua ampla distribuição geográfica (SOBRAL, 1987) e ocorrência em variadas condições edafoclimáticas (CONCEIÇÃO; ARAGÃO, 2010). Além disso, existem relatos na literatura do alívio a estresses abióticos pelo Si em espécies da mesma família (RAMOS et al., 2009; CARVALHO et al., 2003). A espécie foi escolhida mediante sua ocorrência em estudos fitossociológicos que indicaram sua presença em três fitofisionomias distintas, a saber: Savana Costeira (MORO et al., 2011), Arbustaria Densa Decídua e Floresta Sazonal Decídua (ARAÚJO et al., 2011).

A primeira delas, Savana Costeira (CS), situa-se em fragmento de vegetação savânica, localizada sob as coordenadas 3°43'02"S e 38°32'35"W, com altitude de 16 m a.n.m, clima tropical quente subúmido, pluviosidade média anual de 1.338 mm, com período chuvoso concentrado nos meses de janeiro a maio e temperatura média de 28 a 30°C (MORO et al.,

2011, INMET 2014). A região abrange terrenos da planície litorânea (dunas e paleodunas), dos tabuleiros pré-litorâneos (Formação Barreiras) e das planícies fluviais (IPECE 2008; FIGUEIREDO; FERNANDES, 1987). O solo encontrado nessa área se encaixa na ordem dos Ultisols (SOIL SURVEY STAFF, 2014).

As outras duas áreas, Arbustaria Densa Decídua (DDS) e Floresta Sazonal Decídua (SDF) estão situadas na Reserva Particular do Patrimônio Natural de Serra das Almas (RNSA). A Reserva abrange uma área de 6.146 hectares, entre as coordenadas 5°8'45"S, 40°55'43"W, estando situadas sobre a bacia sedimentar do Meio Norte, em sua borda oriental, e que forma uma cuesta dissimétrica, denominada planalto da Ibiapaba (altitudes entre 650 e 700 a.n.m; SOUZA, et al., 1979). A área DDS está localizada no topo da bacia sedimentar do Meio-Norte, na vertente mais seca, a 700 m a.n.m, pluviosidade média histórica anual de 636,61 mm, chuvas concentradas de janeiro a maio e temperaturas mínimas e máximas variando de $19,14 \pm 1,78^{\circ}\text{C}$ a $33,6 \pm 3,85^{\circ}\text{C}$ respectivamente (VASCONCELOS et al., 2010). O solo da área foi classificado como Typic Quartzipsamment (SOIL SURVEY STAFF, 2014). Por sua vez, a área SDF está localizada na encosta mais úmida da bacia a 650 m a.n.m., com 80% da precipitação de 1.044 mm, concentrada nos meses de janeiro a abril. A temperatura média anual é 24°C (LIMA et al., 2011) e o solo classificado como Lithic Udipsamment (SOIL SURVEY STAFF, 2014).

Coletas de solo, planta e estimativas de biomassa, densidade, frequência e abundância

Em todas as áreas foram delimitadas dez parcelas, cada uma medindo 10 x 10 m. Em cada parcela, quatro amostras de solo deformadas foram coletadas aleatoriamente, nas profundidades de 0-10, 10-20 e 20-40 cm, homogêneas, secas ao ar, peneiradas (<2 mm) e armazenadas para análises posteriores. Amostras compostas de cada área em cada profundidade foram utilizadas para caracterização química e física (Tabela 1).

Tabela 1- Propriedades químicas e físicas em diferentes profundidades do solo em áreas de Savana Costeira, Arbustaria Densa Decídua e Floresta Sazonal Decídua

| Propriedade | ÁREA | | | | | | | | | |
|--|-----------------------|-------|-------|-------|--------------------------|-------|-------|--------------------------|-------|-------|
| | Savana Costeira | | | | Arbustaria Densa Decídua | | | Floresta Sazonal Decídua | | |
| | -----Camada (cm)----- | | | | | | | | | |
| | 0-10 | 10-20 | 20-40 | 40-60 | 0-10 | 10-20 | 20-40 | 0-10 | 10-20 | 20-40 |
| Acidez potencial ($\text{cmol}_e\text{kg}^{-1}$) ^a | 0,90 | 0,83 | 0,83 | 0,86 | 7,12 | 4,70 | 4,38 | 5,50 | 5,16 | 6,62 |
| CEC ($\text{cmol}_e\text{kg}^{-1}$) | 2,89 | 2,49 | 2,71 | 2,54 | 8,33 | 5,87 | 5,70 | 7,23 | 6,24 | 7,69 |

| | | | | | | | | | | |
|------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| pH em água (1:2.5) | 6,0 | 6,0 | 6,0 | 6,3 | 4,3 | 4,5 | 4,4 | 4,5 | 4,3 | 4,6 |
| Areia grossa (g kg ⁻¹) | 693 | 661 | 622 | 599 | 353 | 332 | 300 | 120 | 80 | 100 |
| Areia fina (g kg ⁻¹) | 230 | 252 | 261 | 399 | 530 | 534 | 523 | 730 | 712 | 641 |
| Silte (g kg ⁻¹) | 70 | 69 | 80 | 89 | 39 | 89 | 88 | 82 | 130 | 132 |
| Argila (g kg ⁻¹) | 23 | 44 | 51 | 99 | 74 | 58 | 59 | 79 | 160 | 168 |

*Extraído com acetato de amônio (EMBRAPA, 1997).

Dentro das parcelas, todas as plantas de *E. puniceifolia* foram contadas, marcadas e medidas sua altura e circunferência a altura do peito (1,3 m). As medidas de circunferência foram convertidas em diâmetro à altura do peito (DAP). A quantidade de biomassa aérea seca disponível foi estimada com uso de equações alométricas desenvolvidas por Sampaio e Silva (2005). A biomassa das plantas com DAP entre 3 e 30 cm foi calculada conforme a equação:

$$\text{Biomassa (kg)} = 0,1730 \times \text{DAP}^{2,2950} \quad R^2=0,9184.$$

Foram calculados os parâmetros fitossociológicos: frequência (índice da ocorrência das espécies em cada parcela); densidade (índice da quantidade de indivíduos da mesma espécie em cada quadrado) e abundância (concentração da espécie nos diferentes pontos da área total) (MUELLER-DOMBOIS; ELLENBERG, 1974).

Em cada parcela, foram selecionadas aleatoriamente quatro plantas sadias e coletadas folhas completas (limbo+pecíolo) do terço médio dos ramos mais recentes, nos diferentes lados da planta. Folhas de cada amostra foram separadas, secas em estufa com circulação de ar a 45 °C e armazenadas para posterior análise foliar em microscopia eletrônica de varredura. O restante das folhas foi lavado com água deionizada e seca em estufa a 65 °C até obterem peso constante para determinação de matéria seca. Em seguida, as amostras foram moídas e acondicionadas até a realização das análises.

Análises químicas em solo e plantas

Para caracterização química do solo e determinação dos teores de Si, 100 ml de água deionizada foram adicionados a 10 g de solo e a mistura incubada por duas semanas em frascos de polietileno. Depois de decorrido esse tempo, a solução foi agitada a 120 rpm por 1 hora, deixada em repouso por 30 minutos e em seguida o sobrenadante foi filtrado

qualitativamente (NONAKA; TAKAHASHI, 1988). Os teores dos elementos: Ca, Mg, P, S, K, Al, Fe, Na, Cl, Ni, Mo, Zn, Cu e Cd foram determinados por espectrometria de emissão por plasma (ICP-OES OPTIMA 8300). Esses elementos foram selecionados a partir de relatos na literatura que apontam a associação entre a presença deles no solo e a ocorrência de estresse nas plantas (GU et al., 2011; YOU-QIANG et al., 2012; RIZWAN et al., 2012). O efeito amenizador do Si em estresses causados por Al (LIANG et al., 2007), Cd (LIANG et al., 2005), Zn (SONG et al., 2011), dentre outros, também foram considerados.

Os teores de Si, por sua vez, foram determinados em extratos usando 1 mL da solução sulfo-molíbdica 7,5% (7,5 g de molibdato de amônio + 10 mL de ácido sulfúrico 9 mol L⁻¹ em 100 mL). Após 10 minutos foram acrescentados 2 mL da solução ácido tartárico 20%, utilizado para complexar o fósforo da solução e, após 5 minutos, adicionados 10 mL da solução de ácido ascórbico 0,3%. A quantificação do Si foi realizada pela redução do complexo β-molibdossilicato amarelo a azul-de-molibdênio, em espectrofotômetro, no comprimento de onda de 660 nm (SNYDER, 1991).

Para quantificação do Si nas plantas utilizaram-se 0,1 g das amostras em 2,0 ml de H₂O₂ a 30% (v/v) e 3,0 ml de NaOH (0,25 mol L⁻¹), sendo autoclavados durante 1 hora a 123°C e 0,15 MPa. O volume foi ajustado para 50 ml com água ultrapura e retirada alíquota de 1,0 ml, novamente diluída em 20 ml de água ultrapura. A concentração de Si foi determinada por espectrofotometria (CARNEIRO et al., 2007) com leitura a 410 nm da cor amarela do ácido molibdosilícico formado após a reação entre o Si e o molibdato de amônio em meio ácido com HCl (14 mol L⁻¹) (KORNDORFER et al., 2004). A quantidade de Si acumulada foi obtida considerando a produção de matéria seca das plantas.

Análise foliar em microscopia eletrônica

As folhas, após secagem a 45°C, foram cortadas em suas partes centrais e coladas em “stubs”, expondo as superfícies adaxial e abaxial e foram recobertas com carbono. Para a detecção da presença dos elementos, as amostras foram analisadas por espectroscopia de energia dispersiva (EDS-Oxford), acoplado ao microscópio eletrônico de varredura MEV — Inspect 50 FEI. Essa determinação teve por objetivo observar como Si se deposita nas folhas dessa espécie e inferir sobre possíveis mecanismos de alívio aos estresses ambientais.

Análises estatísticas

Inicialmente, todos os dados foram analisados quanto a estatística descritiva. Para tal utilizou-se a média e o erro padrão. Com a finalidade de verificar diferenças entre as médias dos teores dos elementos químicos, utilizou-se teste Tukey. Para avaliar o efeito do Si e da área na produção de biomassa da espécie, foi realizada análise de regressão linear múltipla. Todas as análises foram conduzidas no programa R CORE TEAM (2014).

2.4.3 Resultados

Os teores de elementos no solo mostram que existem diferenças entre as áreas, mesmo as que estão sobre a mesma formação geológica, como SDF e DDS (Tabela 2). As diferenças para o Si, por exemplo, são mais evidentes na primeira profundidade estudada, e a área DDS apresentou os maiores teores quando comparada as demais. É importante destacar que esta mesma área também apresentou os maiores teores de Al, especialmente na segunda e terceira profundidades, com médias de até três vezes mais o valor das outras áreas. A análise de regressão reforça a relação entre a absorção de Si pelas plantas e o teor de Al no solo (Tabela 3 e Figura 1).

Tabela 2 - Teores médios dos elementos químicos (mg kg^{-1}) em diferentes profundidades do solo em áreas de Savana Costeira (CS), Floresta Estacional Decídua (SDF) e Arbustaria Densa Decídua (DDS)

| Áreas | Profundidades (cm) | | |
|-----------|--------------------|------------|------------|
| | 0-10 | 10-20 | 20-40 |
| Si | | | |
| CS | 0,9253 bA | 1,0048 aA | 1,1200 aA |
| SDF | 0,9538 bB | 1,3027 aAB | 1,4421 aA |
| DDS | 1,7917 aA | 1,2546 aB | 1,3089 aB |
| Ca | | | |
| CS | 25,6027 aA | 21,0354 aA | 16,8655 aA |
| SDF | 23,5573 aA | 16,4249 aA | 16,4792 aA |
| DDS | 13,9813 aA | 8,6561 aA | 21,2915 aA |
| Mg | | | |
| CS | 22,3391 aA | 16,8248 aA | 15,0012 aA |
| SDF | 25,9837 aA | 20,3461 aA | 24,2413 aA |
| DDS | 10,3648 aA | 5,4429 aA | 7,4719 aA |
| P | | | |

| | | | |
|-----------|-------------|-------------|--------------|
| CS | 7,8387 aA | 6,3308 aAB | 4,4846 abB |
| SDF | 3,5667 bA | 3,7499 abA | 6,0071 aA |
| DDS | 3,0399 bA | 2,4427 bA | 2,4440 bA |
| S | | | |
| CS | 58,5845 bAB | 70,2805 bA | 51,4059 bB |
| SDF | 109,6634 aA | 98,7996 aA | 100,8866 aA |
| DDS | 40,2140 bA | 26,9552 cA | 28,9131 bA |
| K | | | |
| CS | 2,1870 aA | 1,3012 bAB | 1,0886 bB |
| SDF | 2,7732 aA | 2,7059 aA | 1,8034 bA |
| DDS | 2,0329 aA | 2,8002 aA | 2,9184 aA |
| Al | | | |
| CS | 44,4415 bA | 49,2479 bA | 48,2892 bA |
| SDF | 58,0795 bA | 71,6388 bA | 74,3508 bA |
| DDS | 142,3404 aB | 210,9931 aA | 167,3675 aAB |
| Fe | | | |
| CS | 15,3760 aA | 15,7112 aA | 13,0426 aA |
| SDF | 18,4156 aA | 21,1997 aA | 21,6349 aA |
| DDS | 36,8334 aA | 33,7313 aA | 24,7840 aA |
| Na | | | |
| CS | 47,0716 aA | 47,6057 aA | 48,3139 aA |
| SDF | 29,2577 aA | 29,9908 aA | 31,1012 aA |
| DDS | 33,8154 aA | 29,8043 aA | 35,7305 aA |
| Cl | | | |
| CS | 0,4962 bA | 0,2915 bA | 0,1955 bA |
| SDF | 2,2219 aA | 1,4581 aB | 1,5923 aB |
| DDS | 0,3658 bA | 0,0506 bA | 0,0767 bA |
| Ni | | | |
| CS | 0,5933 aA | 0,6360 aA | 0,5918 abA |
| SDF | 0,6325 aA | 0,6074 aA | 0,4472 bB |
| DDS | 0,6727 aA | 0,6593 aA | 0,6185 aA |
| Mo | | | |
| CS | 0,2899 aA | 0,1771 aA | 0,2931 aA |
| SDF | 0,2564 abA | 0,2795 aA | 0,2848 aA |
| DDS | 0,1398 bA | 0,2377 aA | 0,2475 aA |
| Zn | | | |
| CS | 0,3351 aA | 0,2285 aA | 0,1895 aA |
| SDF | 0,2569 aA | 1,1402 aA | 1,1398 aA |
| DDS | 0,3921 aA | 0,2063 aA | 0,3266 aA |
| Cu | | | |

| | | | |
|-----------|------------|-----------|------------|
| CS | 0,2784 aA | 0,2773 aA | 0,3302 abA |
| SDF | 0,3001 aAB | 0,2231 aB | 0,5275 aA |
| DDS | 0,1919 aA | 0,1706 aA | 0,2077 bA |
| Cd | | | |
| CS | 0,0591 aA | 0,0453 aA | 0,0599 aA |
| SDF | 0,0385 aA | 0,0385 aA | 0,3792 aA |
| DDS | 0,0894 aA | 0,0613 aA | 0,0746 aA |

*Valores médios seguidos pela mesma letra minúscula na coluna e maiúscula na linha, não diferem entre si pelo teste de Tukey a 5% de probabilidade.

As diferenças entre as áreas também são percebidas para os teores de P na área CS, e para os teores de S e Cl na área SDF, apresentando maiores médias dos teores destes elementos quando comparadas as outras áreas deste estudo. Essas duas áreas, no entanto, apresentam semelhança quanto aos teores de alguns elementos como Mo e Cu, com maiores teores de Mo ocorrendo na primeira profundidade e de Cu na última profundidade, quando comparadas a DDS.

Por sua vez, as diferenças quanto aos teores de Ni foram percebidos apenas na última profundidade estudada, com as maiores médias observadas para as áreas DDS e CS. Comportamento semelhante foi observado para os teores de K, onde os maiores teores do elemento foram encontrados nas áreas DDS e SDF também em profundidade (10-20 e 20-40 cm).

Para os teores de Ca, Mg, Fe, Na, Zn e Cd não foram observadas médias que significativamente diferentes entre áreas e profundidades.

Tabela 3 – Análise de regressão linear do efeito das áreas e do teor de Al no teor de Si em folhas de *E. puniceifolia*.

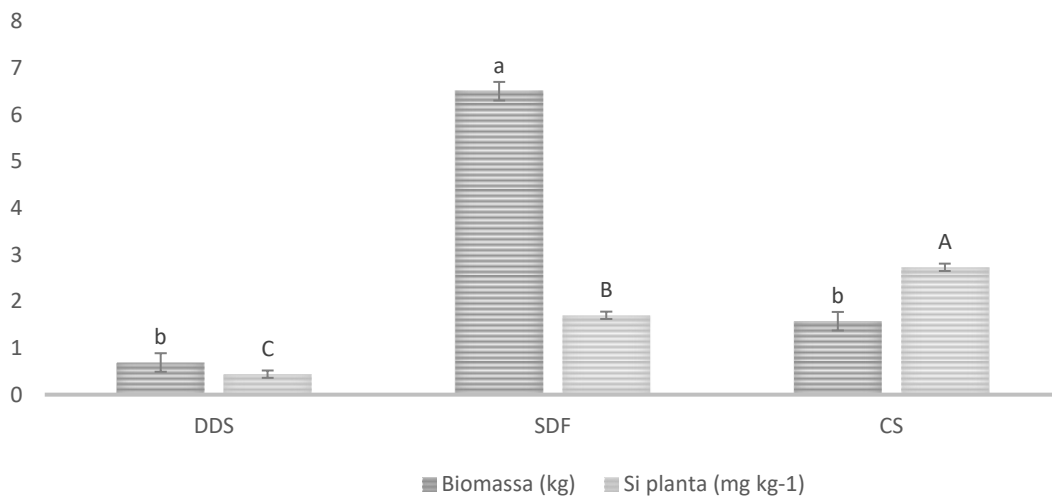
| | Coef. Estimado | Erro padrão | valor - T | valor - P |
|------------|----------------|-------------|-----------|--------------------------|
| Intercepto | 2.8157927 | 0.1341985 | 20.982 | <2e ⁻¹⁶ *** |
| Área DDS | -2.3519142 | 0.1375489 | -17.099 | <2e ⁻¹⁶ *** |
| Área SDF | -1.0801283 | 0.1306472 | -8.268 | 1.52e ⁻¹² *** |
| Al | -0.0004823 | 0.0006212 | -0.776 | 0.44 |

R-quadrado: 0.8277, R-quadrado ajustado: 0.8217. F=137.7, gl: 86, p=<2.2e⁻¹⁶

Os maiores teores de Si no solo para DDS não implicaram no maior acúmulo nas folhas de *E. puniceifolia* desta área (Figura 1), que apresentou teores médios aproximados de 0,44 mg kg⁻¹. Por sua vez, folhas da área CS apresentaram maior acúmulo de Si, com valores de 2,74 mg kg⁻¹. O maior acúmulo de Si em folhas, no entanto, não resultou na maior produção

de biomassa da espécie, pois, de acordo com a mesma figura, as médias da produção de biomassa não diferem entre si nas áreas DDS e CS enquanto a área SDF apresentou a maior biomassa.

Figura 1 - Silício (Si) acumulado em folhas e produção de biomassa de plantas de *E. puniceifolia* oriundas de três diferentes áreas (valores médios seguidos da mesma letra maiúsculas para Si e minúscula para biomassa não diferem entre si pelo teste de Tukey a 5% de probabilidade).



A tabela 4 apresenta os parâmetros fitossociológicos da espécie. A área de Savana Costeira apresenta maior abundância, ou seja: maior concentração de plantas de *E. puniceifolia* nos diferentes pontos da área total amostrada quando comparada as demais áreas; e maior densidade (índice da quantidade de indivíduos de uma mesma espécie em cada quadrado), mesmo com os menores teores de Si em folha observados para esta área.

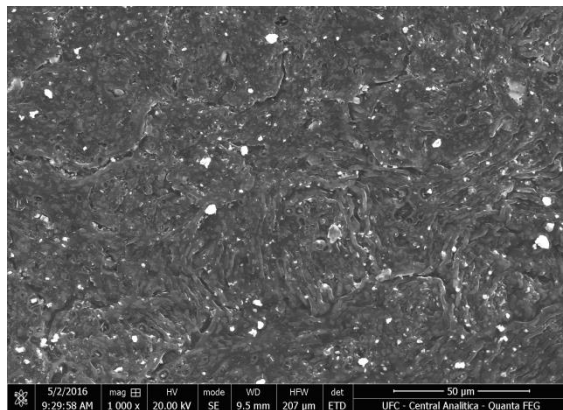
Tabela 4 - Parâmetros fitossociológicos de *E. puniceifolia* em áreas de Floresta Estacional Decídua, Arbustaria Densa Decídua e Savana Costeira

| Área | Parâmetros fitossociológicos | | |
|-----------------------------|------------------------------|-----------|------------|
| | Frequência | Densidade | Abundância |
| Floresta Estacional Decídua | 1 | 0,02 | 1,7 |
| Arbustaria Densa Decídua | 0,2 | 0,004 | 2 |
| Savana Costeira | 1 | 0,04 | 4 |

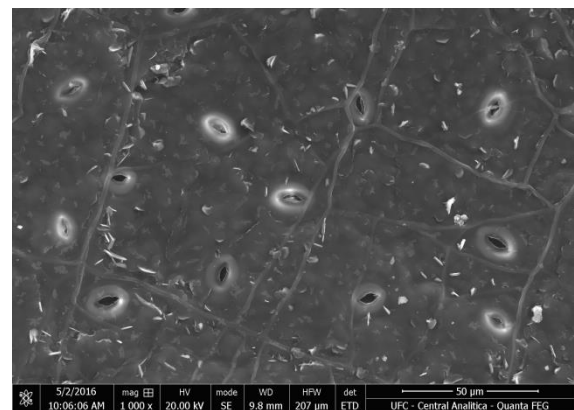
Frequência (índice da ocorrência das espécies em cada quadrado); Densidade = n° de plantas m²; Abundância = concentração da espécie nos diferentes pontos da área total.

Nas imagens das superfícies adaxial e abaxial das folhas, observou-se a presença de cutícula mais espessa na superfície abaxial das folhas (Figura 2B, D e F) recobrendo parcialmente os estômatos, especialmente em DDS (Figura 2F). Nas outras áreas, essas estruturas podem ser visualizadas ainda com mais clareza.

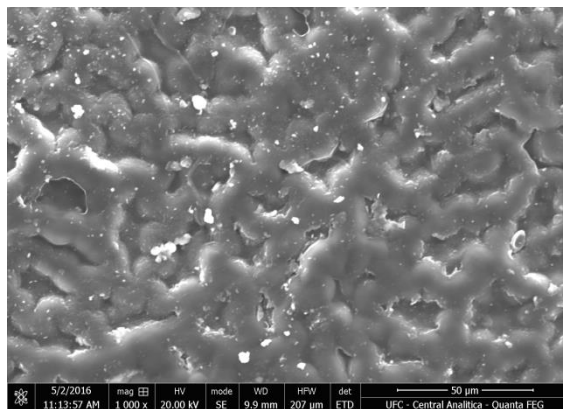
Figura 2 - Superfície inferior e superior de folhas de *E. puniceifolia* oriundas de três diferentes áreas: Savana Costeira (A,B), Floresta Estacional Decídua (C,D) e Arbustaria Densa Decídua (E,F).



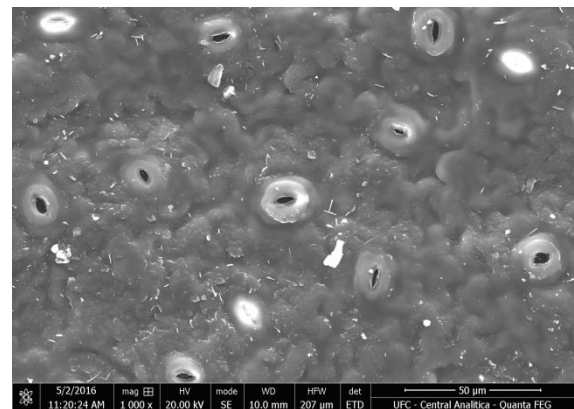
(A)



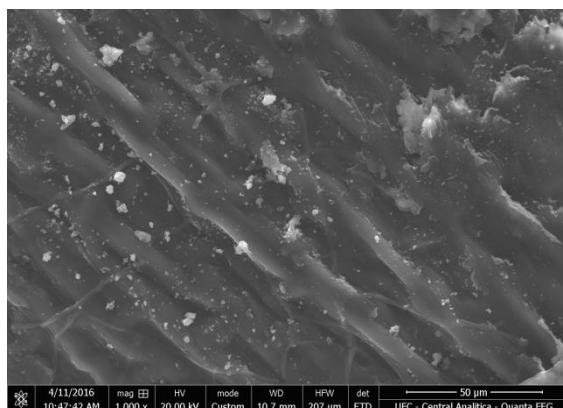
(B)



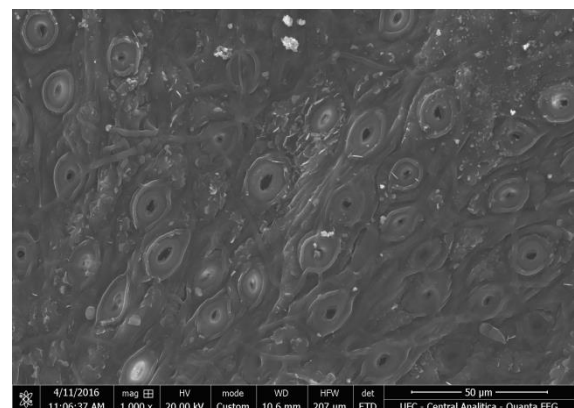
(C)



(D)



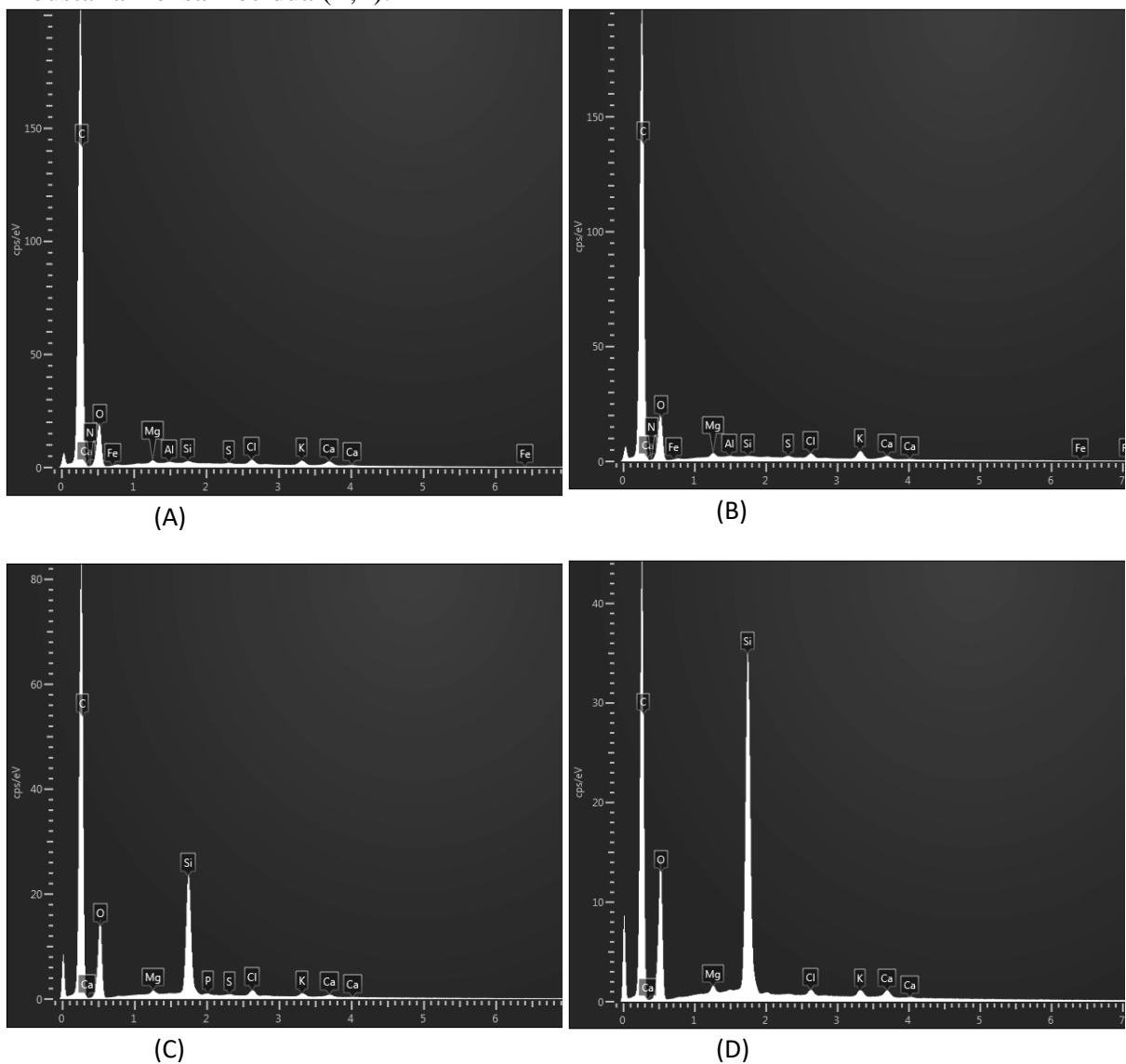
(E)

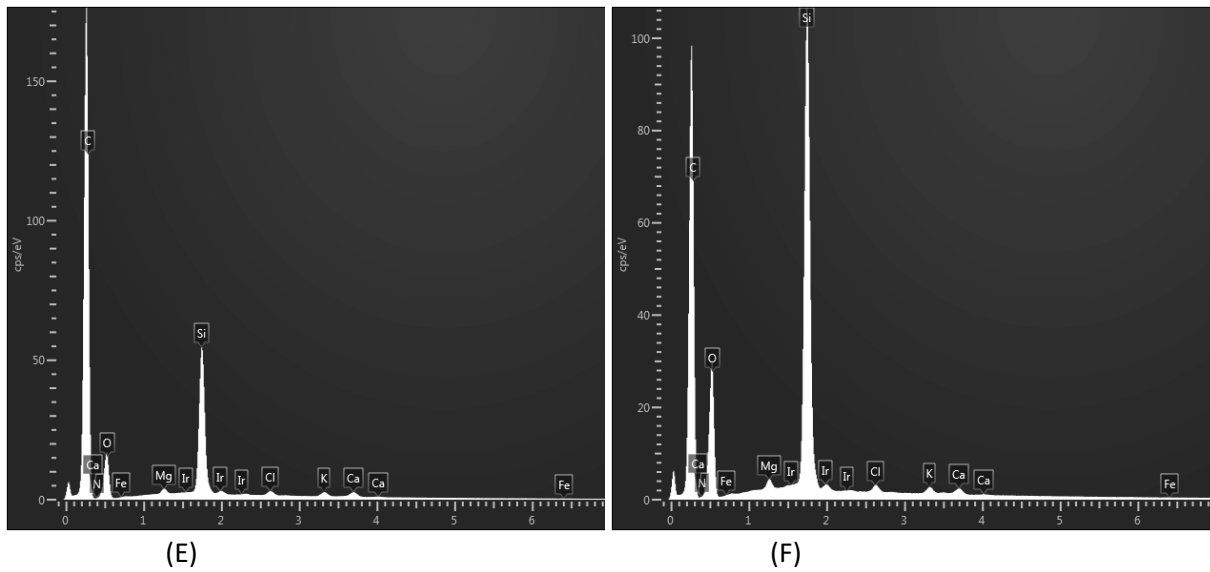


(F)

A Figura 3, por sua vez, confirma a maior concentração de Si e dos demais elementos nessas amostras. As áreas SDF e DDS mostram picos de Si que não ocorrem na área CS, confirmando os maiores teores de Si em folha, que foram observados na Figura 1. No entanto, não são encontrados maiores teores de Al em folhas da área DDS como foi observado na Tabela 2. Na Figura 3 é observado ainda a presença de Cl nas folhas das três áreas e de Irídio (Ir) nas áreas SDF e DDS.

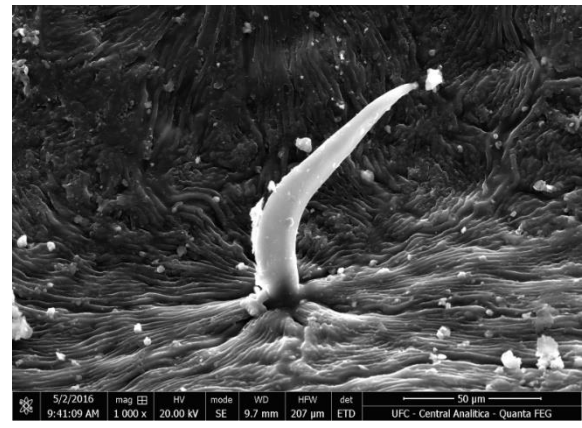
Figura 3 - Microanálise de raios-X das faces inferior e superior de folhas de *E. puniceifolia* oriundas de três diferentes áreas: Savana Costeira (A,B), Floresta Estacional Decídua (C,D) and Arbustaria Densa Decídua (E,F).





Plantas das áreas DDS e SDF apresentaram estruturas semelhantes à tricomas nas folhas (Figura 4), não tendo sido observadas nas amostras obtidas em plantas da área CS.

Figura 4 - Tricomas na superfície adaxial de folhas de *E. puniceifolia* oriundas de duas diferentes áreas: Arbustaria Densa Decídua (A) e Floresta Estacional Decídua (B).



2.4.4 Discussão

Em razão do avançado grau de intemperização em que se encontram os solos tropicais, o Si é encontrado basicamente na forma de quartzo, opala e outras formas não disponíveis às plantas (BARBOSA FILHO et al., 2001), ou formando ainda complexos com

Al, Fe, metais pesados e matéria orgânica (FARMER et al., 2005). O solo da área CS é originário de sedimentos areno-argilosos (JACOMINE et al., 1975), confirmado pela granulometria do solo (Tabela 1). Os menores teores de Si nessa área, quando comparada às demais, podem estar relacionados com as condições específicas do clima da área, como maior intensidade de chuvas e altas temperaturas, o que contribui para a dessilificação, ou seja, maior remoção de Si em função do intenso intemperismo (KORNDÖRFER et al., 2006). Solos das áreas DDS e SDF se diferenciam ainda de CS quanto à profundidade (mais rasos), pH, textura e a concentração de Si disponível no solo (ver Tabela 1). Os valores mais baixos de pH das áreas DDS e SDF ($\text{pH}_{\text{médio}}$ de 4,5) também contribuem para uma maior solubilidade do Si e a redução do número de sítios de adsorção (SAVANT et al., 1999; PULZ et al., 2008). Uma descrição morfológica dos perfis das áreas DDS e SDF mostra que a dessilificação foi menos intensa em comparação com a área CS, possivelmente devido às menores precipitações e temperaturas mais baixas que ocorrem nas áreas da bacia sedimentar. As concentrações de Si em solos mais intemperizados são menores que solos menos intemperizados e mais férteis (SUNMER et al., 1991). Assim, DDS e SDF têm solos mais jovens do que CS e, portanto, maiores teores de Si disponível, como indicado nesse estudo. A própria classificação de solo confirma nossos resultados, uma vez que a ordem dos Ultisols, ordem do solo em CS, é geralmente formada em regiões onde a precipitação excede a evapotranspiração potencial durante alguns períodos do ano, contribuindo para percolação de água e nutrientes no perfil, mantendo baixa a quantidade de bases (SOIL SURVEY STAFF, 2014).

Além do Si, os teores dos demais elementos estudados também são reflexos do material de origem e das condições climáticas do ambiente. Os elementos metais que são considerados tóxicos para as plantas, como Cu, Al, Mo e Ni (DALCORSO et al., 2013; HOSSAIN; KOMATSU 2013; HOSSAIN et al., 2012). A concentração desses elementos no solo pode ser de ocorrência natural ou ser decorrente de interferência antrópica (OVEČKA; TAKÁČ 2014). Solos de rochas básicas, naturalmente mais ricas em metais, apresentam maiores teores desses elementos, quando comparados com aqueles formados sobre granitos, gnaisses, arenitos e siltitos (VALADARES, 1975; ROVERS et al., 1983; TILLER, 1989; OLIVEIRA et al., 1999). A principal constituição do solo sob CS é de sedimentos arenosos recentes (SOUSA et al. 1979). Ademais, assim como para os teores de Si, as condições climáticas favoráveis associadas a maior profundidade do solo, o que confere boa drenagem, acentua os efeitos da lixiviação em CS, contribuindo para perda desses elementos e a ocorrência de solos mais pobres do ponto de vista químico. A lixiviação pode ter contribuído ainda para percolação dos maiores teores de Cu, Mo e Ni observados no perfil, o que nesse caso pode estar

ligado à localização da área, no caso em um centro urbano, onde além da influência do material de origem, o aumento nos teores de metais pesados no solo pode ser decorrente da queima de combustíveis e deposição atmosférica (MOREIRA; SIQUEIRA 2006).

Outros fatores, além do material de origem e da interferência antrópica, podem interferir na disponibilidade de elementos para solução do solo e um deles é a matéria orgânica (MOS). As condições climáticas em SDF podem ter contribuído para maior acúmulo de MOS (16,38 g kg⁻¹ de MOS) que é fonte de muitos nutrientes, como por exemplo, o S. No solo, o S é encontrado predominantemente na forma orgânica. Assim, a capacidade do solo em suprir a demanda da planta pelo nutriente está estreitamente relacionada aos teores de MOS e sua mineralização, que disponibilizará o S na forma de sulfato para a solução do solo, podendo ser absorvido pelas plantas (TIECHER et al., 2012). De modo geral, a MOS evita perdas de nutrientes por lixiviação e é capaz de suprir a solução do solo com agentes ligantes solúveis que previnem a fixação de Cu, Mo, Cl, também elevados nessa área, permitindo que eles fiquem disponíveis para as plantas (MORAGHAN; MASCAGNI 1991; MORTVEDT, 1999; SRIVASTAVA; GUPTA 1996).

Embora não se tenha observado diferenças significativas para os demais elementos, sabe-se que o Si melhora a tolerância das plantas à presença de: Na (KIM et al., 2014), Fe (FU et al., 2012), Zn (GU et al., 2012; SONG et al., 2011) e Cd (TRIPATHI et al., 2012) na solução do solo, podendo beneficiar igualmente as plantas das fitofisionomias aqui estudadas, mesmo que as concentrações destes metais não sejam consideradas tóxicas dentro dos limites estabelecidos.

A área DDS, com maiores teores de Si no solo, também apresenta os maiores teores de Al. A toxicidade do Al em plantas superiores é uma área de investigação muito ativa (RYDER et al., 2003), já que o Al é responsável pela diminuição da produção agrícola em solos tropicais e sub-tropicais (FOY, 1992). As interações entre Si e Al, o alívio exercido pelo Si nos efeitos tóxicos do Al e os possíveis mecanismos envolvidos nesses aspectos são extensamente examinados (LIANG et al., 2007). As interações entre Si e Al reduzem a atividade desse íon metal tóxico no meio e este é um possível mecanismo para redução do efeito tóxico causado pelo Al no solo (SINGH et al., 2011; HIRADATE et al., 1998). Essas interações e a redução dos efeitos tóxicos do Al são apoiados por experimentos agrícolas que constataram que a precipitação de espécies hidroxialuminiosilicatadas inertes parece ser responsável pela diminuição da concentração de Al fitotóxico na solução (BAYLIS et al., 1994; LIANG et al., 2007).

A ação aliviadora do Si na presença do Al em plantas expostas a concentrações tóxicas de Al, semelhantes às observadas em DDS, está associada a redução da inibição do crescimento (principal efeito fitotóxico do Al em plantas), quando Si é adicionado à solução de cultura (BARCELÓ et al., 1993). Tal ação pode uma das responsáveis que facilitam a ocorrência da espécie *E. puniceifolia* na área DDS, mesmo em teores elevados de Al no solo.

Essa afirmação pode ser confirmada quando se observa a maior concentração de Si em folhas das plantas que ocorrem nessa área. De acordo com Ma et al. (2001), o principal órgão de depósito de Si são as folhas, sendo depositado na forma de sílica hidratada ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). A concentração do elemento nas folhas é favorecida pela transpiração e influenciada ainda pela capacidade da planta em absorver o Si (EPSTEIN, 1999). A acumulação de Si nos órgãos de transpiração provoca a formação de uma dupla camada de sílica, o que causa redução da transpiração por diminuir a abertura dos estômatos e limita, assim, a perda de água (KORNDÖRFER et al., 1999, OLIVEIRA; CASTRO, 2002).

Além da presença de elementos como Al no solo, absorção de Si é estimulada pelas condições abióticas como temperaturas elevadas ou variações de temperatura (CURRIE; PERRY, 2007). Em DDS, a temperatura média anual máxima e mínima é de $19,14 \pm 1,78^\circ\text{C}$ e $33,6 \pm 3,85^\circ\text{C}$ respectivamente. Além disso, a localização e a altitude dessa área implicam em variações diárias de até 10°C na temperatura (ROCHA et al., 2002), o que faz desta área com condições climáticas mais adversas dentre as estudadas, podendo ser responsável pelo maior estímulo à absorção de Si por essas plantas.

A concentração de Si em uma planta é ainda dependente da concentração de ácido silícico disponível no substrato (EPSTEIN, 1999), como pode ser constatado pelos maiores teores de Si no solo em DDS. A concentração do Si em solos ácidos é regulada pelo pH do solo, afetando a precipitação e a polimerização do elemento. A absorção do Si pelas raízes das plantas tende a ser maior em valores de pH mais baixo, como os encontrados em DDS, pois ocorrem maiores concentrações do elemento na solução do solo nessas condições (TAVAKKOLI et al., 2011; MEHARG; MEHARG 2015).

No entanto, o maior acúmulo de Si em plantas não resultou em maior produção de biomassa de plantas, como era esperado e amplamente relatado na literatura (SHEN et al., 2010; PEI et al., 2010). A presença Al no solo, por exemplo, pode ter limitado a produtividade e a eficiência biológica de plantas (RIZWAN et al., 2012, SOURI et al., 2021). Essa limitação é resultado da fitotoxidez desse elemento e pode ser associada ao menor crescimento radicular, redução na absorção de nutrientes e distúrbios no metabolismo da planta (DAN et al., 2008). Mesmo com a presença do Si e seus efeitos de alívio a estresses, não se pode deixar de

considerar que a maioria dos estudos com o elemento são decorrentes de experimentos onde as plantas são submetidas a estresses isolados. Em ambientes naturais, as plantas são submetidas a combinação de dois ou múltiplos fatores que condicionam o estresse (nesse estudo, as plantas são submetidas a estresse hídrico, variações na temperatura e metais fitotóxicos). O efeito do Si em plantas submetidas a combinação de estresses é uma questão importante, mas ainda pouco entendida (BALAKHNINA; BORKOWSKA 2012).

É importante ainda entender e investigar fatores como os mecanismos e agentes de dispersão ocorrentes nessa área, que podem determinar fortemente os padrões de distribuição espacial, estrutura e abundância de espécies (KANG et al., 2017). A maior densidade e abundância de *E. puniceifolia* na área de Savana Costeira possivelmente pode ter sido influenciada pela maior absorção e alocação de Si em folhas, corroborando a capacidade do Si em conferir resistência às plantas.

Os padrões de acúmulo de Si estimulado pela combinação de estresses ambientais foi mais uma vez confirmado pelas imagens obtidas em MEV e a microanálise de raios-X. As imagens do MEV mostraram a ocorrência de uma cutícula mais espessa, o que de acordo com Song et al. (2021) é decorrente do maior acúmulo de Si em folhas. Nesse estudo, na área DDS, essa condição pode ter sido estimulada pelas combinações de estresse ambientais a que as plantas dessa área são submetidas. A deposição de Si junto à cutícula das folhas confere proteção às plantas e ameniza os efeitos de estresses de natureza biótica e abiótica (EPSTEIN, 1999). O Si ocorre com maior frequência nas regiões onde a água é perdida em grande quantidade, ou seja, na epiderme foliar junto às células-guarda dos estômatos e outras células epidérmicas e são esses depósitos de sílica nos tecidos foliares que promovem a redução na taxa de transpiração (DAYANANDAM et al., 1983; WANG et al., 2021). Sendo assim, confirmamos nossa hipótese de que as plantas de *E. puniceifolia* se beneficiam do Si presente no solo, desde que o ambiente estimulasse a sua absorção e alocação. A redução da condutância estomática é parcialmente atribuída a essa barreira física de fitólitos nos estômatos. A conjugação entre o ácido silícico e a calose gera células-guarda que são cravejadas com fitólitos de sílica e isso se estende ainda mais nos poros estomáticos para inibir a perda de água (LAW; EXLEY, 2011; UENO; AGARIE, 2005). Além disso, pesquisas recentes indicaram que o silício pode fechar os poros estomáticos mediando o efluxo de K^+ das células-guarda (VANDEGEER et al., 2020).

Não foram investigados teores de irídio no solo, por esse não ser um elemento de ocorrência comum em ambientes naturais. No entanto, observamos a presença desse elemento em folhas. Nesse caso, estudos futuros podem trazer uma investigação mais profunda dos teores

naturais de metais pesados em solo e planta, definindo os mais comuns em solos de região semiárida e estabelecer seus níveis de referência no solo, útil para uma possível avaliação de contaminação.

Além disso, a ocorrência de estruturas semelhantes à tricomas observadas apenas nas folhas das áreas SDF e DDS merece melhor investigação, por possivelmente constituir mais um mecanismo de adaptação dessas plantas aos seus locais de origem. Em plantas de ambientes áridos, a presença de tricomas é comumente observada (FAHN; CUTLER 1992) e está relacionada à baixa disponibilidade de água e elevadas temperaturas (SANDQUIST; EHLERINGER 1997). Isso se deve ao fato de que estes apêndices epidérmicos diminuem a taxa de transpiração, tanto pelo aumento da reflexão da radiação solar, que reduz a temperatura, quanto pelo espessamento da camada de ar retido acima da folha, que funciona como uma barreira à perda de água (SANDQUIST; EHLERINGER 1997). O Si pode estar distribuído em pelos urticantes ou nessas estruturas de tricomas (MUSTAFA, et al. 2018). Esses tipos de biossilificação ou extrusão de Si não foram demonstrados em muitas espécies de plantas e, seria interessante investigar o motivo de uma mesma espécie, como a aqui estudada, apresentar ou não tais estruturas.

2.4.5 Conclusões

As plantas de *E. puniceifolia* absorvem mais Si do solo quando estimuladas pela presença de fatores limitantes, como condições climáticas ou presença de elementos fitotóxicos no solo, refletindo adaptações aos seus locais de origem. A composição química desses solos, além de estar relacionada com o material de origem, também foi influenciada pelas condições climáticas das áreas. Esse estudo permite inferir sobre funções de alívio do Si a estresses em sistemas naturais, confirmando os benefícios do elemento já discutidos para culturas agrícolas, o que nesse caso, possibilitam a ocupação e distribuição das plantas em áreas com limitações abióticas, especialmente quando submetidas à combinação de dois ou mais fatores que condicionem estresse, como demonstrado aqui. No entanto, o efeito do elemento em condições naturais pode não ser tão evidente, pois existe a influência de outros fatores como o porte e distribuição da espécie.

O conhecimento da função de alívio do Si em uma espécie que ocupa distintos ambientes naturais ou submetidas a mais de uma condição de estresse simultaneamente foi iniciado nesse estudo. Mas ainda há a necessidade de compreender melhor a função do Si em um maior número de espécies e em interações mais complexas como as que ocorrem em

ambientes naturais. As diversas funções sugeridas em experimentos agrícolas forneceram evidências dos mecanismos que ocorrem nos sistemas aqui estudados, e, assim, os ecologistas podem ser beneficiar destes recursos para explicar questões ainda não resolvidas.

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3. CONSIDERAÇÕES FINAIS

As informações apresentadas nesse trabalho demonstram a importância do Si, já conhecida para estudos agrícolas, para estudos que envolvam outras áreas do conhecimento como a ecologia.

Inicialmente foi verificado que plantas de uma mesma espécie, que ocupa diferentes fitofisionomias podem apresentar requisitos germinativos que indicam aspectos de aclimação destas ao ambiente de ocorrência. Essa condição reforça a hipótese de que as condições abióticas do meio influenciam diretamente na sobrevivência das plantas. Assim, uma vez submetidas a um ambiente que apresente condições limitantes, as plantas desenvolvem estratégias de convivência com tais condições, o que pode incluir, por exemplo, a maior absorção de Si.

Nesse estudo proveu-se uma perspectiva numérica das pesquisas com o elemento já publicadas em diversas áreas, com diferentes espécies e submetidas a estresses abióticos variados e foi confirmado o efeito positivo de alívio a estresses pelo elemento. No entanto, mesmo depois de muitos estudos, pouco se sabe sobre os mecanismos envolvidos no alívio a estresses pelo elemento e não são apresentados avanços relacionados ao estudo desse elemento, com redução de seu efeito geral ao longo do tempo. Essa condição poderia, portanto, ser revertida se um número maior de espécies fosse incluído nos estudos, não apenas plantas cultivadas, ou se fossem considerados estresses múltiplos, como os que ocorrem em ambientes naturais. Essa maior complexidade de informações poderia ajudar a elucidar os questionamentos que ainda permanecem sobre a relação do Si com estresses adversos.

Quando foram montados experimentos para testar estresses por elevadas temperaturas e déficit hídrico, as plantas repetiram em laboratório o que confirmou-se no campo. Condições abióticas mais extremas implicam em maior absorção do elemento pelas plantas, desde que esse esteja em maior disponibilidade para as mesmas e o resultado disso é, principalmente um aumento na produção de biomassa, já confirmado para plantas cultivadas.

Se as plantas em ambientes naturais estão se beneficiando do Si, informações de estudos que tragam novidades e avanços no conhecimento do Si podem, por exemplo, explicar a distribuição e ocupação de espécies, interações entre espécies vegetais, processos de facilitação, adaptações das espécies a condições limitantes ou ainda direcionar pesquisas e ações futuras, como por exemplo, para regeneração de áreas.

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