UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

CLARISSA BUARQUE VIEIRA

QUINOA (*Chenopodium quinoa* Willd.) AND SPINACH (*Spinacia oleracea* L.) CULTIVATION IN SALINE-SODIC SOILS FROM SEMIARID

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Clarissa Buarque Vieira
Engenheira Agrônoma

Quinoa (*Chenopodium quinoa* Willd.) and spinach (*Spinacia Oleracea* L.) cultivation in saline-sodic soils from semiarid

Tese apresentada ao Programa de Pós-Graduação em Ciência do Solo, da Universidade Federal Rural de Pernambuco, como parte dos requisitos para a obtenção do título de Doutora em Ciência do Solo.

Orientadora:

Profa. Dra. Maria Betânia Galvão dos Santos Freire

Coorientadores:

Prof. Dr. Brivaldo Gomes de Almeida

Profa. Dra. Giselle Monteiro Gomes Fracetto

Prof Dr. Luiz Guilherme Medeiros Pessoa

Supervisores no Exterior:

Dr. Jorge F. S. Ferreira

Dr. Devinder Sandhu

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Profa. Dra. Maria Betânia Galvão dos Santos Freire

Orientadora

Universidade Federal Rural de Pernambuco (UFRPE – SEDE)

BANCA EXAMINADORA

Prof. Dr. Luiz Guilherme Medeiros Pessoa

Universidade Federal Rural de Pernambuco (UFRPE - UAST)

Dr. Jorge F. S. Ferreira

United States Salinity Laboratory (USDA-ARS)

Dr. Josimar Gurgel Fernandes

Instituto Agronômico de Pernambuco (IPA)

Dr. Walter Quadros Ribeiro Junior

EMBRAPA Cerrados

DEDICO

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Quinoa (*Chenopodium quinoa* Willd.) and spinach (*Spinacia Oleracea* L.) cultivation in saline-sodic soils from semiarid

GENERAL ABSTRACT

Soil salinization is one of the factors that reduces productivity in arable lands. Thus, the search for crops tolerant to salinity/sodicity has been intensifying. Quinoa (*Chenopodium quinoa* Willd.) is a facultative halophyte, with high nutritional value capable of mitigating hunger. Spinach (*Spinacia oleracea* L.), that belongs to the same family as quinoa, is a glycophyte with genetic potential to tolerate salinized soils. This work tested two quinoa genotypes (CPAC 09 and CPAC11 - EMBRAPA Cerrados) and spinach (cv. Gazelle), in saline-sodic soils. Two experiments were carried out in a greenhouse, with quinoa (genotype CPAC 09), in the winter and summer seasons in Brazil, in three soils (two saline and one non-saline) found in the semiarid of Pernambuco, under the addition of rice husk biochar – RHB – (0, 10, 20, 40, 60, 80, and 100 t ha^{-1}), in randomized blocks and four replications. Soils chemical and physical attributes were evaluated, and biometric, nutritional, and enzymatic analysis were carried out on plants. RHB reduced pH, soil electrical conductivity (EC_e), and sodium adsorption rate (SAR) in alkaline and saline soils and increased pH in acidic soil. RHB served as a source of K^+, also contributing to the reduction of bulk density (BD) and an increase in saturated hydraulic conductivity (K_{sat}) in sandy soils. The improvement in soils attributes favored the development of quinoa, increasing its biomass and the K^+/Na^+ ratio. For CPAC 09, the phytoextraction potential followed the order of K>Cl>Mg>Ca>Na in winter, and K>Cl>Mg>Na>Ca in summer. In the third experiment, two quinoa genotypes (CPAC 09 and CPAC 11) were evaluated under applications of saline water with electrical conductivity (EC_w) of 2, 25, 40, and 55 dS m^{-1}. Additionally, spinach (cv. Gazelle) was subjected to EC_w of 2 and 25 dS m^{-1}. The experiment was conducted using a randomized block design with four replicates. Chemical analyses were carried out on soils, and biometric, nutritional, and physiological analysis on plants. For quinoa, there was a reduction between 50 and 60% in grain yield between EC_w of 2 and 25 dS m^{-1} and of more than 95% under EC_w of 55 dS m^{-1}. For spinach, shoot biomass reduction was 80% between EC_w of 2 and 25 dS m^{-1}. After treatments, soils and plant tissues showed an increasing concentration of salts, mainly Na and Cl. The crops showed a high salt tolerance potential in saline soils, surviving under EC_e between 25-30 dS m^{-1} (spinach), and more than 65 dS m^{-1} (quinoa). We established that spinach plants, like quinoa, also possess Epidermal Bladder Cells (EBCs). This discovery represents the first report of its kind in the scientific literature. To detect the EBCs in spinach, the spinach varieties Gazelle and Seaside and the quinoa genotype CPAC 09 were used. Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDS) of EBCs allowed for a comparison of the ionic signatures in plants irrigated with waters of low and high salinity. Results demonstrated that spinach EBCs accumulated Na, Cl, and K, whereas quinoa EBCs accumulated K and Cl. In addition, expression analysis of 19 genes known to play important roles in salinity tolerance indicated that certain genes were differentially expressed in EBCs and leaves without bladders. These include genes associated with sodium transport such as *SOS3*, *NHX1*, *NHX2*, and *AKT1*; chloride transport like *NPF2.5*, *SLAH1*, *NPF2.4*, and *ALMT12*; and some additional genes that play roles in regulatory mechanisms for managing salinity stress. Our results indicate that spinach can tolerate high salinity levels and possesses specialized structures similar to those found in quinoa. Based on these findings, we propose reclassifying spinach from a glycophyte to a facultative halophyte, akin to quinoa.

Keywords: Biochar. Salt phytoextraction. Quinoa genotypes. Salt bladders. Facultative halophytes.

Cultivo de quinoa (*Chenopodium quinoa* Willd.) e espinafre (*Spinacia Oleracea* L.) em solos salino-sódicos do semiárido

RESUMO GERAL

A salinização dos solos é um dos fatores de redução da produtividade em terras agrícolas. Assim, a busca por culturas tolerantes à salinidade/sodicidade vem se intensificando. A quinoa (*Chenopodium quinoa* Willd.) é uma halófita facultativa, com alto valor nutricional capaz de auxiliar na mitigação da fome. O espinafre (*Spinacia oleracea* L.), apesar de glicófita, tem potencial genético para tolerar solos salinizados. Este trabalho testou dois genótipos de quinoa (CPAC 09 e CPAC11 - EMBRAPA Cerrados) e o espinafre (cv. Gazelle), em solos salino-sódicos. Dois experimentos foram conduzidos em casa de vegetação, com a quinoa (genótipo CPAC 09), nos períodos de inverno e verão no Brasil, em três solos (dois salinos e um não salino) encontrados no semiárido pernambucano, sob adição de biochar de casca de arroz (0, 10, 20, 40, 60, 80 e 100 t ha^{-1}), em blocos casualizados e quatro repetições. Foram avaliados atributos químicos e físicos dos solos e realizadas avaliações biométricas, nutricionais e enzimáticas nas plantas. O biochar reduziu o pH, a condutividade elétrica do solo (CE_e) e a relação de adsorção de sódio (RAS) em solos alcalinos e salinos e aumentou o pH em solo ácido. Também foi fonte de K^+, sendo também responsável pela redução da densidade do solo (Ds) e aumento da condutividade hidráulica saturada (K_{sat}) em solos arenosos. A melhoria nos atributos químicos e físicos dos solos favoreceu o desenvolvimento da quinoa, aumentando sua biomassa e relação K^+/Na^+. Para o CPAC 09, o potencial de fitoextração seguiu a ordem de K>Cl>Mg>Ca>Na, no inverno e K>Cl>Mg>Na>Ca no verão. No terceiro experimento foram avaliados dois genótipos de quinoa (CPAC 09 e CPAC11) e o espinafre (cv. Gazelle), sendo conduzido a partir da aplicação de águas salinas (2, 25, 40 e 55 dS m^{-1}), em casa de vegetação, com delineamento em blocos casualizados e quatro repetições. O espinafre foi submetido à CE_a de 2 e 25 dS m^{-1}, em quatro repetições. Foram feitas avaliações químicas nos solos e biométricas, nutricionais e fisiológicas nas plantas. Para a quinoa, houve redução de aproximadamente 60% na produtividade de grãos entre as CE_a de 2 e 25 dS m^{-1} e em mais de 95% sob CE_a de 55 dS m^{-1}. Para o espinafre, a redução na produtividade foi de 80% entre as CE_a de 2 e 25 dS m^{-1}. Após os tratamentos, solos e plantas apresentaram uma concentração crescente de sais, principalmente Na e Cl. As culturas apresentaram alto potencial em solos salinos, chegando a sobreviver sob CE_e entre 25-30 dS m^{-1} (espinafre), e mais de 65 dS m^{-1} (quinoa). Foi estabelecido que o espinafre, semelhante à quinoa, possui glândulas epidérmicas especializadas. Esta descoberta está sendo primeiramente reportada neste trabalho. Para a detecção das glândulas, foram utilizadas as variedades Gazelle e Seaside (espinafre) e CPAC 09 (quinoa). O microscópio eletrônico de varredura com espectroscopia de energia dispersiva de Raio-X (MEV-EDS) foi utilizado para comparar as glândulas da quinoa e do espinafre irrigadas com águas salina e não salina. Os resultados mostram que as glândulas do espinafre acumulam Na, Cl e K e as de quinoa apenas K e Cl. Também foi realizada análise de 19 genes relacionados a tolerância à salinidade com expressões distintas nas glândulas e em folhas sem glândulas. Isto inclui genes associados ao transporte de Na^+ como *SOS3*, *NHX1*, *NHX2* e *AKT1*; transporte de Cl^- como *NPF2.5*, *SLAH1*, *NPF2.4* e *ALMT12* e outros genes que participam dos mecanismos regulatórios para manejo do estresse salino. Nossos resultados indicam que o espinafre tolera altas salinidades e possui estruturas especializadas como na quinoa, sendo melhor classificado como halófita facultativa.

Palavras-chave: Biochar. Fitoextração de sais. Genótipos de quinoa. Glândulas de acúmulo de sais. Halófitas facultativas.

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LIST OF ABBREVIATIONS AND ACRONYMS

APX – Ascorbate Peroxidase

BD – Bulk Density

C – Carbon

Ca - Calcium

CAT - Catalase

CEC – Cation Exchangeable Capacity

Ci – Intercellular CO_2 Concentration

Cl – Chloride

C/N – Carbon/Nitrogen

H - Height

PD – Particle Density

TP – Total Porosity

EBCs – Epidermal Bladder Cells

ECe – Soil Electrical Conductivity

ECw – Water Electrical Conductivity

ESP – Exchangeable Sodium Percentage

gs – Stomatal Conductance

K – Potassium

K/Na – Potassium/Sodium ratio

K_{sat} – Saturated Hydraulic Conductivity

MDA - Malondialdehyde

Mg – Magnesium

N - Nitrogen

Na - Sodium

P – Phosphorus

pH - Potential of Hydrogen

pHes – Potential of Hydrogen (saturated extract)

Pn – Net Photosynthetic Rate

RHB – Rice Husk Biochar

SAR – Sodium Adsorption Rate

S - Sulfur

SD – Stem Diameter

SDW – Shoot Dry Weight

SEM/EDS - Scanning Electron Microscopy / Energy Dispersive Spectroscopy

SFW – Shoot Fresh Weight

SOD – Superoxide Dismutase

SPAD - Soil Plant Analysis Development

TOC – Total Organic Carbon

Tr – Transpiration Rate

V –Base Saturation

WUE – Water Use Efficiency

SUMMARY

1. GENERAL INTRODUCTION

Soils affected by salts are a group of soils found on every continent originated by natural or anthropogenic factors. Saline soils have limitations in food production, and they are responsible for the reduction or even unproductiveness in land areas. Countries such as Australia, United States of America, China, and Brazil have a large extension of degraded lands due to salt and sodium accumulation. It is essential to use techniques that allow the soil reclamation or the coexistence of the population with this environmental problem. To minimize problems of salt affected soils, some techniques were emerging to enable the reclamation of these areas, such as the addition of excess irrigation to leach salts, soil amendments and organic compounds application, and phytoremediation. Phytoremediation is characterized as a low-cost technique but requires a long time to reach the final objective.

Scientists are trying to discover new technologies that increase the productivity of hyperaccumulating salt species without causing other environmental impacts. The objectives of this study are to develop remediation methods for saline-affected areas using quinoa (*Chenopodium quinoa* Willd.), and to investigate its potential for phytoremediation. Additionally, the study aims to compare the salinity tolerance of quinoa with that of spinach (*Spinacia oleracea* L.), a related glycophytic species from the same family, Amaranthaceae. Another key aspect of the research involves testing the efficacy of quinoa in soils amended with various doses of biochar to determine how different conditions affect its salt absorption capabilities.

The study focuses on evaluating soil reclamation techniques for removal or immobilization of salts, as well as assessing the salt-tolerance of two species from the Amaranthaceae family: quinoa and spinach. By enhancing the cultivation of these crops, which are of high agronomic interest, in saline and sodic areas, this research aims to facilitate sustainable soil reclamation. This approach not only increases food production but also conserves environmental resources, offering economic benefits to local populations. Additionally, by improving agricultural viability in saline-affected regions, this strategy could help mitigate rural exodus, particularly in northeastern Brazil. This would enable hundreds of families to sustain their livelihoods in the semiarid regions of Brazil and potentially other countries.

1.1. Hypotheses

- Quinoa's (*Chenopodium quinoa* Willd.) genotypes 09 and 11, from EMBRAPA Cerrados - Brazil, are tolerant to salinity and sodicity and develop in different ways under distinct soil types and biochar doses. In addition, these two quinoa genotypes can potentially be used for phytoextraction of salts in soils.
- Quinoa and spinach (*Spinacia Oleracea* cv. Gazelle) can grow in high salinity levels.
- Spinach is considered a facultative halophyte;
- Quinoa cultivation under the application of biochar enables the food production in saline-sodic soils, promoting the development of agriculture in semiarid areas of northeast Brazil.
- Rice husk biochar promotes improvements in soils physical and chemical attributes, allowing the quinoa cultivation in soils with high salinity.

1.2. Objectives

1.2.1. General Objectives

Enabling the production of crops of economic interest such as quinoa and spinach in the Brazilian semiarid region. By assessing their responses to salinity, understanding the mechanisms behind their tolerance, and implementing sustainable agricultural practices, we seek to reclaim areas degraded by salts and sodium. This approach not only supports local agriculture but also promotes the restoration of land affected by salinization.

1.2.2. Specific Objectives

- To evaluate the genetic, physiological, enzymatic, and agronomical response of quinoa (genotypes CPAC 09 and CPAC 11) and spinach (cv. Gazelle) under high levels of salts;
- To compare the salinity responses of quinoa and spinach, highlighting both the differences and similarities in their performance under saline conditions;
- To explore the potential of using rice husk biochar (RHB) and phytoextraction with quinoa for promoting the reclamation of salt-affected soils;
- To identify the optimal biochar dosage that facilitates quinoa cultivation in saline and sodic soils.

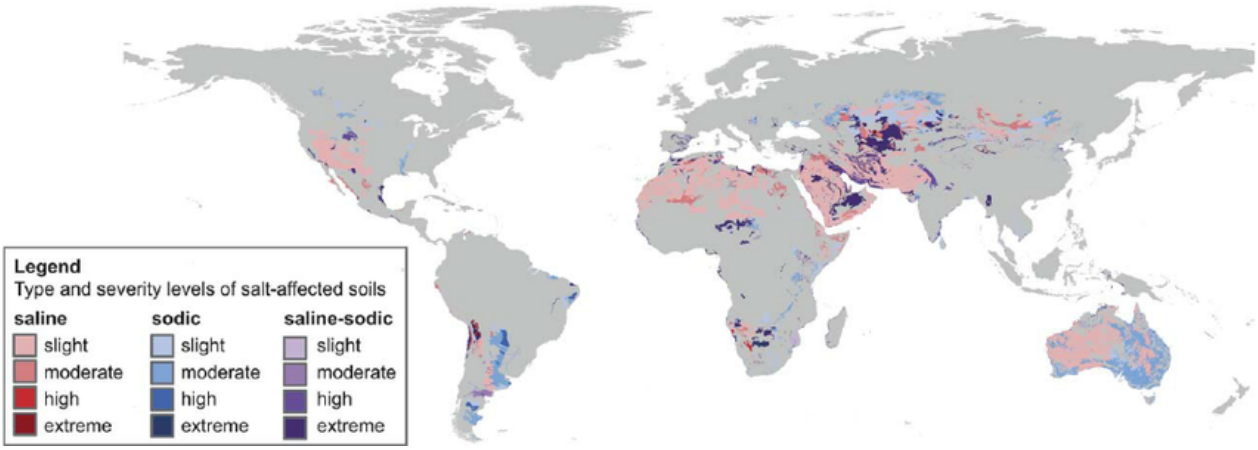
2. BIBLIOGRAPHIC REVIEW

2.1. Saline and sodic soils

Historically, the association between human beings and problems with soil salinity extends over millennia. Throughout civilizations, there has been a noticeable increase in the proportions of salt-affected soils, with documented reports dating back to the Mesopotamia era (SHAHID; ZAMAN; HENG, 2018). This type of soil degradation exists on all continents, and it is present in more than 100 countries nowadays, totaling an area of more than 1 billion hectares (SHAHID; ZAMAN; HENG, 2018). Figure 1 illustrates the extension of saline, sodic, and saline-sodic soils on the World.

Soil degradation due to salts and sodium accumulation has increased significantly in recent decades, especially in regions with arid and semiarid climates. About 7% to 10% of the world's arable land is threatened by salinization and countries such as Australia, China, Pakistan, and the United States of America are some of the locations with extensive saline and/or sodic areas (FAO, 2015). The cause of salinization can be due to natural or anthropogenic factors such as inadequate irrigation management and poor drainage of soils susceptible to salinization. In Brazil, most of these areas are in the so-called drought polygon, which involves a large part of the Northeast of the country, where excessive evaporation and poor natural soil drainage are fundamental factors for the increase of salts and sodium in the soil profile (BARROS et al., 2004; Li et al., 2014; FAO, 2015).

Figure 1 – Distribution of saline and sodic soils. Source: Wicke et al. (2011)



According to FAO (2015), about 20-25% of irrigated areas in Brazil are degraded by salts and sodium. This problem often leads to land abandonment and an intense rural exodus. In addition to the Northeast, Brazil has several regions with salts and sodium accumulation, but in smaller proportions such as in Mato Grosso's Pantanal (swamp) where at certain times of the year, evapotranspiration is higher than precipitation, promoting the appearance of saline, saline-sodic and alkaline soils. In this region, which does not belong to semiarid or arid climate, saline and alkaline lakes appear seasonally, which can intensify soil salinization (COUTO et al., 2017).

Salt and sodium accumulation in soils creates an environmental problem that limits food production. This phenomenon is directly related to poor agricultural management and excessive exploitation of water resources, mainly due to inadequate irrigation management. This leads to damage to plant development and degradation of chemical, physical, and biological soils properties (FREIRE et al., 2003; CUEVAS et al., 2019).

According to the USSL STAFF (1954), a pioneering American group in the study of saline and sodic soils, soils can be classified as detailed in Table 1. These classifications have been used consistently since the publication of the handbook 60 and continue to be used nowadays.

Table 1 – Classification of saline and sodic soils according to USSL STAFF (1954), with reference values of electrical conductivity (EC_e), exchangeable sodium percentage (ESP), and sodium adsorption rate (SAR)

Soil classification	EC_e	ESP	SAR
	dS m^{-1}	%	$(mmol_c\ L^{-1})^{0,5}$
Saline	≥ 4,0	< 15	< 13
Sodic	< 4,0	≥ 15	≥ 13
Saline-Sodic	≥ 4,0	≥ 15	≥ 13

Salt affected soils are known as halomorphic, and reclamation techniques must be adopted to avoid even more severe problems such as reduced productivity, land abandonment, groundwater contamination, and other environmental risks. Continued research is essential for the pedological

identification of the extent of these areas, which will help determine the most effective reclamation strategies for each specific situation (RIBEIRO; RIBEIRO FILHO; JACOMINE, 2016).

2.2. Reclamation of saline and sodic soils

In some areas, salinization has been an obstacle to food production. Therefore, it is necessary to adopt sustainable agricultural practices that allow the reclamation of these soils based on the understanding of the impacts caused by desalination techniques. This understanding has the potential to reduce the risk of land abandonment (SHI et al., 2021). Over the past decades, science has discovered strategies to promote, in a viable way, the reclamation of soils affected by salts, such as use of agricultural amendments, installation and maintenance of efficient soil drainage systems, use of organic compounds, phytoremediation, among other techniques. From these studies, it is evident that the reclamation of saline areas is mainly due to a combination of techniques, which allows this process to be faster, less costly, and more efficient, allowing the resumption of agricultural production in these regions (PEDROTTI et al., 2015; CUEVAS et al., 2019).

Among some forms of reclamation, one of the techniques that has been widely used due to its sustainable bias is phytoremediation, characterized as a practice of implanting salts phytoextractor and hyperaccumulator crops in degraded areas (SILVA et al., 2016). Another way of improving the physical and chemical properties of saline/sodic soils is the use of biochar, which is a solid carbonaceous residue produced under oxygen-free or oxygen-limited conditions at temperatures ranging from 300 to 1000 °C. Biochar can improve crop productivity, increase water availability in the soil, reduce ESP, and increase nutrient uptake by plants (LIN et al., 2015; ALI et al., 2017; SAIFULLAH et al., 2018).

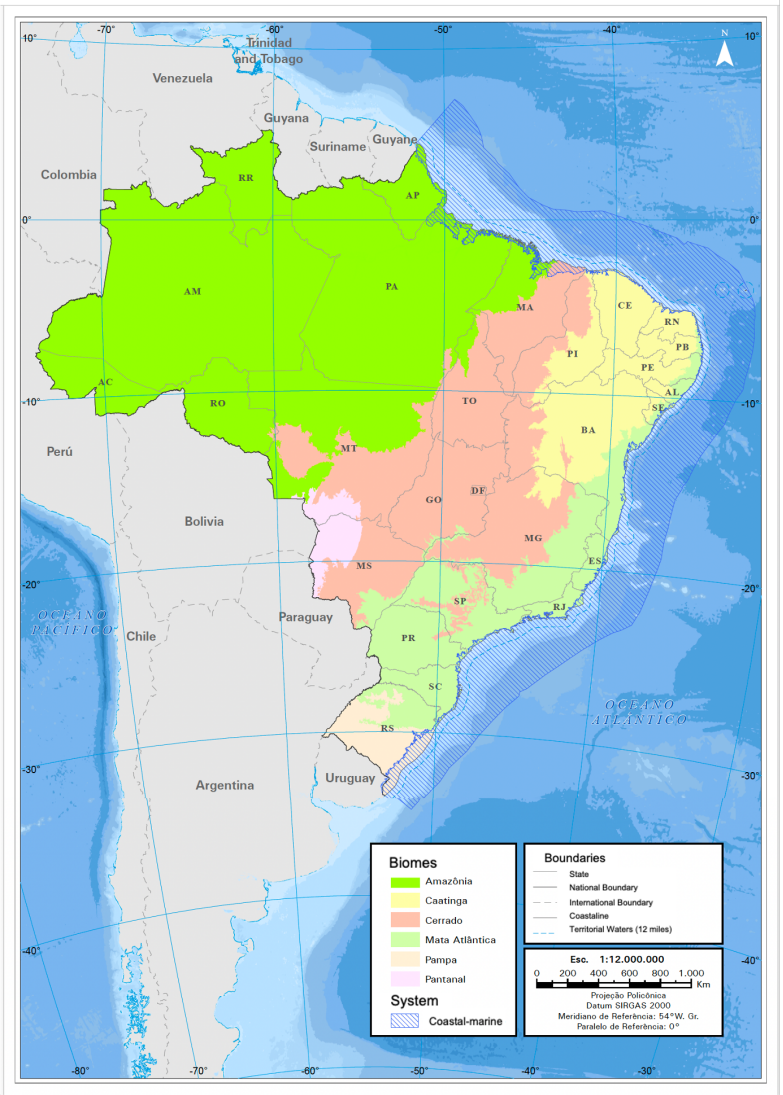
Some researchers are using quinoa (a halophyte plant) as an alternative for food production in salinized areas, with the possibility of recovering or mitigating the damage caused by the accumulation of salts and sodium in soils (JAIKISHUN et al., 2019). From this perspective, biochar and quinoa have the potential in promoting improvements in the chemical, physical, and biological properties of salt affected soils from semiarid regions, especially with the use of acidifying biochar. It may enable the reuse of these areas for crop cultivation of agricultural interest.

2.3. Brazilian Semiarid

According to data reported by the Brazilian National Institute of Semiarid - INS (BRASIL, 2024), the Brazilian semiarid region is an area that covers 1262 cities located in Northeast states (Alagoas, Bahia, Ceará, Maranhão, Pernambuco, Piauí, Rio Grande do Norte, and Sergipe) and Southeast (only in the north of Minas Gerais state), occupying 12% of the national territory and being considered the most populated semiarid region in the world (population around 28 million inhabitants). The main characteristics of this region are annual rainfall of less than 800mm, an aridity index of up to 0.5, and drought levels greater than 60%.

The predominant vegetation is the Caatinga (figure 2), an exclusively Brazilian biome, with an area of approximately 800,000 km^2, located in the so-called “polígono da seca” (drought polygon – English) (ALVES; ARAÚJO; NASCIMENTO, 2008; PINHEIRO et al., 2016). In this region, the soils can vary from shallow to deep, less or highly weathered and with variable degrees of fertility depending on the parent material (crystalline basement rocks, highland sedimentary basins, limestone, and others) (ARAÚJO FILHO et al., 2023). Despite the wide soil types, the most predominant are shallow soils of high fertility, with salinized soils often being found especially in irrigated areas (PESSOA et al., 2022; BRASIL, 2024).

Figure 2 – Brazilian biomes. Adapted from: IBGE, 2019



Despite the high biodiversity present in the Brazilian semiarid region, climate change has been intensifying in this region with the formation of clusters of desertification (SILVA et al., 2023). The semiarid region represents approximately 63% of the Northeast of the country and 11.5% of the national territory. Areas susceptible to desertification in Brazil correspond to 15% of the territory, extending beyond semiarid regions, reaching around 31 million people according to Moreas, Wanderley, and Delgado (2023).

For the first time, Brazil reported the presence of an arid climate within the territory, which indicates the intensification of climate change in the country, with the emergence of desert areas and, consequently, the advancement of drought and salinity. According to Brasil (2023), based on

studies by CEMADEN (National Center for Monitoring and Alerts of Natural Disasters) and INPE (National Institute for Space Research), arid areas are present in the north of Bahia (Brazilian Northeast state). The data was obtained from the analysis of climate variations between the years 1960 and 2020.

Thus, the study of plants resistant to salinity, sodicity, drought, and high temperatures is increasingly essential for food production and reducing food insecurity in Brazil, mainly due to the increase in desertification areas, which causes the rural exodus and the reduction in the country's agricultural lands.

2.4. Halophytes

The classification of plants encompasses several characteristics, one of which is salt tolerance. A plant that survives in a saline environment is called a Halophytes. According to Grigore, Toma, and Boscaiu (2010), the beginning of halophytes definition is not clear, and the current definitions are based on the scientific background of the halophyte specialists. The authors suggested a chronological list starting with Crozier (1892), who defined a halophyte as “a plant containing a large quantity of common salt in its composition, and which thrives best in salty places”.

Chapman (1942) defined halophytes as all plants that can survive and grow under more than 0.5% of sodium chloride (NaCl), and glycophytes as plants that cannot survive in saline environments. The author defined Euhalophytes as a plant that display optimal growth in environments that contains more than 0.5% of NaCl such as species of Salicornia and Rhizophora. Whereas Miohalophytes are the plants that can survive in environments that contains more than 0.5% of NaCl, but its optimal development is in environments with less than 0.5% of NaCl. Flowers, Hajibagheri, and Clipson (1986) defined halophytes as plants that can grow in salt concentration equal to or greater than 200 mM NaCl. Nikalje et al. (2018) classified halophytes as “salt-tolerant plants that have the potential to complete their life cycle under high-salts conditions where survival for glycophytes is not possible”. These authors also divided the halophytes into two groups: obligate halophytes and facultative halophytes. An obligate halophyte needs the salts for their optimum growth, and a facultative halophyte can grow in saline and nonsaline environments. Also, Flowers and Colmer (2015) summarized halophytic plants as the “flora of saline

environments". According to Grigore and Toma (2017) the definitions of halophytes and glycophytes have evolved over time and there is no scientific unanimity about this topic.

One of the most famous facultative halophytes is quinoa, a plant originating in South America with high potential to reduce food insecurity and capable of thriving in saline and sodic soils in areas where other plant species are unable to survive.

2.5. Pseudocereal

Unlike cereals, which are grains from grass species (Poaceae family) such as corn, wheat, rice, barley, sorghum, millet, oats, and rye (KOEHLER; WIESER, 2013), pseudocereals are grains belonging to dicotyledonous plants, gluten-free and with high nutritional value. The three main pseudocereals are quinoa, amaranth, and buckwheat (MARTÍNEZ-VILLALUENGA; PEÑAS; HERNÁNDEZ-LEDESMA, 2020).

The search for more nutritional balanced foods has highlighted pseudocereals as they are richer in proteins, fibers, unsaturated fats, and bioactive compounds (saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, betalains, and bioactive proteins and peptides) than cereals. These sets of compounds found in pseudocereals have been widely associated with anti-inflammatory, anti-hypertensive, anti-cancer, anti-diabetes, and other health properties (MARTÍNEZ-VILLALUENGA; PEÑAS; HERNÁNDEZ-LEDESMA, 2020; NANDAN et al. 2024).

One hypothesis about pseudocereals is that they can correct nutrient imbalances that carbohydrate-rich cereals can cause on human health (NANDAN et al., 2024). Although the benefits, pseudocereal cultivation is still 500 – 4500 times lower than regular cereals such as wheat, rice, and maize that constitute 80% of food consumption in the world (PIRZADAH; MALIK, 2020; NANDAN et al. 2024).

Pseudocereals are being considered as crops of the 21st century by FAO and UNESCO due to their nutritional values compared to cereals and their climate resilience, being cultivated in marginal areas that cereals cannot grow (PIRZADAH; MALIK, 2020).

One of the best examples is quinoa, a pseudocereal with high protein content and resistant to cold, salt, and drought being considered as a "golden grain" (ANGELI et al., 2020). Thus, quinoa importance to world food security and climate changes will be discussed in the following topic.

2.6. Quinoa cultivation in saline soils

Soil salinization is one of the major causes of reduced agricultural productivity in areas with arid and semiarid climates around the world, requiring remediation or the use of tolerant plants to maintain agriculture activities in these areas (QADIR; GHAFOOR; MURTAZA, 2000; HASANUZZAMAN et al. 2014).

In soils with excess of salts, most plants cannot complete their life cycle. Except for halophyte species, which are highly salt tolerant and can survive in extreme conditions. Many plants, known as glycophytes, do not develop at EC and ESP below the limits of 4 dS m^{-1} and 15%, respectively, considered to be the critical limit for saline and sodic soils (USSL STAFF, 1954; HASANUZZAMAN et al. 2014).

The critical factors that limit the survival of plant species on salt affected soils are the reduction in water availability due to the decrease in osmotic pressure, the toxic effect of ions at high concentrations such as Na^+ and Cl^-, and the degradation of soil physical properties caused by high concentrations of exchangeable sodium such as reduced water infiltration. Some species are more tolerant than others, and in recent decades, there has been a general demand to provide improvements in salt tolerance that it is possible to maintain agricultural production fields even in soils in the process of degradation (FREIRE et al., 2003; SCHLEIFF, 2008).

Quinoa emerges as a grain crop of Andean origin highly tolerant to salts and drought. Quinoa is a pseudocereal of great nutritional relevance, high protein quality, low cholesterol and gluten-free, desired by consumers from different regions of the world for both human and animal food (SPEHAR; SANTOS, 2002; OLIVEIRA FILHO, 2017). Quinoa center of origin is the Bolivian and Peruvian Andes, being considered as an oligocentric species originated around the Titicaca Lake. Due to its origin center, quinoa is highly adapted to different arid climates (GARCIA; CONDORI; CASTILLO, 2015).

Quinoa is a versatile crop grown under saline water irrigation in some countries in Mediterranean region and the Middle East (REZZOUK et al., 2020). Wilson, Read, and Abo-Kassem (2002) observed that quinoa increases its leaf area when submitted to irrigation with water with an EC of 11 dS m^{-1}. When they applied water with EC of 19 dS m^{-1}, the authors mention that the nutritional variations of quinoa were minimal, indicating a nutritional balance under saline conditions.

The BRS Piabiru variety was the first recommended for cultivation in Brazil. It has an average height of 190 cm and lacks saponin (which gives the grain a bitter taste). The variety reaches with floral maturation in an average of 145 days and an average grain production of 2.8 t ha^{-1} (SPEHAR; SANTOS, 2002). According to Silva et al. (2021), in recent research, BRS Piabiru reached more than 8 t ha^{-1}, in a water regime of 389 mm in the Brazilian Cerrado. The authors also achieved a grain productivity of 8.21 and 6.8 t ha^{-1} for the genotypes CPAC 09 and CPAC 11 in a water regime of 480 and 389mm respectively, the same genotypes used in this present work, but in acidic soil without salinity problems. In a water regime of just 150 mm in the cycle both quinoa genotypes (CPAC 09 and CPAC 11) reached more than 2 t ha^{-1} of productivity, with these genotypes being considered drought resistant.

Due to quinoa's resistance to salt and drought, one of the objectives of this work is to provide scientific knowledge for the introduction of quinoa in the Brazilian semiarid, especially in saline and sodic areas that other plants, including Caatinga plants, cannot survive. These areas are located in the so-called drought polygon (ALVES; ARAÚJO; NASCIMENTO, 2008). According to Pessoa et al. (2019) the Brazilian Semiarid has halomorphic soils that are controlled by the presence of soluble and exchangeable salts, including sodium. In these soils, quinoa can grow and produce grains to improve food security and reduce rural exodus in Brazilian lands.

2.7. Quinoa mechanisms for salts and heat tolerance

According to Adolf et al. (2013), quinoa is a halophyte plant that can survive in environments with salinity higher than seawater, depending on the variety. Due to its high tolerance to extreme environmental conditions, quinoa has developed several adaptation mechanisms. These include physiological, nutritional, biochemical, enzymatic, and genetic responses that enable tolerance to soil salinity and sodicity. Na^+ sequestration in vacuoles, Na^+ translocation in the xylem, ROS tolerance, K^+ retention, reduction in stomatal conductance, photosynthetic system efficiency, low concentration of ABA and efficient use of water by the plant are some mechanisms used by quinoa varieties that allow its high adaptation in extreme environments (ADOLF et al., 2013; SHABALA et al., 2013; YANG et al., 2018).

The scientific community has been discussing the possibilities of adapting quinoa to extreme environments. Studies about salt tolerance, drought and low and high temperatures have been developed to adapt quinoa to areas with food insecurity. Despite recurrent research on

quinoa under saline, drought, and frost stresses, little is known about the resistance of different quinoa genotypes to high temperature conditions (HINOJOSA et al., 2018; ALVAR-BELTRÁN et al., 2020).

In some studies, at temperatures above 38 °C, quinoa cv Titicaca showed a 50% reduction in germination and a 30% decrease in seed yield, with a high impact on the pollination phase, with the germination and flowering phases being the most critical when quinoa is subjected to high temperatures (ALVAR-BELTRÁN et al., 2020). Hinojosa et al. (2018), in research with two Chilean quinoa genotypes (QQ74 - PI 614886 and 17GR - Ames 13735), state that the reduction in pollination between treatments with day/night temperatures of 22/16 °C (control) and 40/24 °C (thermal stress) was 30 to 70%, but this stress did not significantly influence in seed size and productivity. More research on quinoa under high temperatures is needed to understand the behavior of different quinoa genotypes and their possibilities to use in adverse climatic conditions.

2.8. Quinoa under biochar application

Biochar produced from different raw materials and pyrolysis temperatures has been studied as an alternative conditioner for saline-sodic soils. It has been used mainly by promoting improvements in soil physical chemical, and biological properties, allowing increases in crop productivity by reducing associated stress as drought and toxicity of certain elements (THOMAS et al., 2013). Biochar provides changes in soil physical attributes such as decrease in bulk density; increase in porosity, water retention and infiltration rate; reduces soil resistance to root penetration, and others. This ends up enabling an edaphic environment suitable for maintaining the soil microbiota due to the increase in carbon stock, proportion of micropores and moisture; greater availability of nutrients, favoring the growth of microbial biomass (LEHMANN et al., 2011; BLANCO-CANQUI, 2017; KAVITHA et al., 2018).

Ramzani et al. (2017), Naveed et al. (2020), and Yang et al. (2020), in studies with quinoa and biochar produced from corn cobs, tree twigs, and corn straw, respectively, demonstrated that quinoa has an adequate development in saline soils under biochar application, due to its increasing resistance to drought and salinity. Biochar indirectly promotes increase in photosynthesis, nutrient absorption, water use efficiency, growth, and decrease in oxidative stress.

Biochar application and its use efficiency also vary considerably depending on soil texture and type of biochar. In research with quinoa and peanut shell biochar, Kammann et al. (2011)

concluded that the biochar application in sandy soils promotes satisfactory water retention, lower greenhouse gas emissions and higher carbon sequestration, also helping quinoa in its best development.

Further research is necessary to explore the role of different types of biochar in improving chemical, physical, and biological properties of soils thereby fostering the growth of plant species in saline-sodic soils. This advancement could facilitate the use and even reclamation of soils previously deemed unsuitable for agriculture, potentially improving food security in affected regions.

2.9. Spinach cultivation in saline soils

Spinach (*Spinacia Oleracea* L.) is an annual plant belonging to the Amaranthaceae family and is native to Asia. Its leaves and sprouts are consumed, and they are rich in vitamins A, B2, B6, E, K, as well as manganese; magnesium; folic acid; iron, potassium, omega-3 and dietary fiber (MAEDA et al., 2010; PANDEY; KALLOO, 1993). In its composition, spinach has approximately 91% water, 0.4-0.6% lipids, 2.9% proteins, 2-10% carbohydrates and 2.2% fiber. In addition to its nutritional importance, this crop has been evaluated for cardiovascular protection, anti-obesity, hypoglycemic activity, anti-inflammatory properties, anti-cancer properties, among others (MURCIA et al., 2020).

According to Ors and Suarez (2016), spinach, cultivar Racoon, is considered a moderately salt-tolerant plant, withstanding irrigation water with an EC_w of 9 dS m^{-1} in cold climates, without loss of productivity. The authors state that, with the increase in average temperatures (from 11.9 °C to 20.15 °C), spinach reduces its productivity by around 27% under application of the same water, indicating that a double stress (salinity x temperature) is harmful for the cultivation of this plant species.

Ferreira et al. (2018), in research with spinach under water of 9.4 dS m^{-1}, affirmed that spinach did not show any symptoms of toxicity after 23 days of treatment, where the plants maintained constant nutrient concentration, physiological parameters and antioxidant capacity during the application of saline waters. In Brazilian semiarid, spinach has a potential to grow under saline soil from Caatinga biome, especially during winter season.

As quinoa and spinach belong to the same botanical family, the genetic similarities might suggest that spinach could also possess genes for salinity tolerance. This hypothesis necessitates

comprehensive studies across various spinach genotypes to explore this potential. In the next chapters will be discussed the adaptation of Brazilian quinoa genotypes in saline soils commonly found in northeastern Brazil and also their tolerance to extreme saline environments with the application of waters with an EC similar to seawater. The resistance of spinach varieties in soils under application of saline waters will also be discussed and some physiological, nutritional, morphological, and genetic mechanisms for both spinach and quinoa to tolerate high salinities will also be presented.

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3. CHAPTER II: CHEMICAL AND PHYSICAL ATTRIBUTES OF NATURAL SALINE SOILS OF NORTHEAST BRAZIL AFTER CULTIVATION WITH QUINOA AND RICE HUSK BIOCHAR APPLICATION

Abstract

Soil salinization and sodification have worsened due to climate change and inadequate human activities on agricultural lands. The need to recover these soils, especially in regions with an arid and semiarid climate, is fundamental for the human maintenance in the countryside and the sustainable development of these regions. This work aims to combine two techniques for remediating salt affected soils promoting improvements in chemical and physical attributes of soils using the halophyte species quinoa (*Chenopodium quinoa* Willd.) and rice husk biochar (RHB). Two experiments were set up in a greenhouse in the winter and summer periods in Brazil, in a 3x7 factorial scheme, using three soils (two saline and one non-saline) commonly found in the Brazilian semiarid and seven biochar doses (0, 10, 20, 40, 80, and 100 t ha^{-1}), in a randomized block design, in two quinoa cycles. The soils were previously classified as Cambisol, Fluvisol, and Planosol. At the end of the second cycle, soil samples with disturbed and undisturbed structures were collected to evaluate soil attributes. Because they are chemical and physically distinct soils, the biochar addition promoted different changes in the evaluated soils. For saline soils, increasing biochar doses promoted a reduction in pH, soil electrical conductivity (EC_e), sodium adsorption rate (SAR), cation exchangeable capacity (CEC), exchangeable sodium percentage (ESP), and bulk density (BD) and an increase in exchangeable K^+. For the non-saline soil, biochar was responsible for the increase in pH, CEC, ESP, and exchangeable K^+, and reductions in EC_e, SAR, and BD. Biochar also increased saturated hydraulic conductivity (K_{sat}) for Cambisol and Planosol and decreased it for Fluvisol. Thus, RHB was considered efficient in reducing parameters related to salinity and sodicity of salt affected soils in the Brazilian semiarid, being capable of altering chemical and physical attributes mainly at high doses. In the summer cycle, biochar also reduced the harmful effects of high temperatures on the survival of quinoa plants in saline-sodic soils.

Keywords: Reclamation. Potassium. Sodium. Saturated hydraulic conductivity. Salts.

CHAPTER II: ATRIBUTOS QUÍMICOS E FÍSICOS DE SOLOS NATURALMENTE SALINOS DO NORDESTE DO BRASIL APÓS CULTIVO COM QUINOA E APLICAÇÃO DE BIOCHAR DE CASCA DE ARROZ

Resumo

A salinização e a sodificação dos solos vêm se intensificando devido às alterações climáticas e atividades humanas inadequadas em áreas agrícolas. A recuperação destes solos, principalmente em regiões de clima árido e semiárido, é fundamental para a manutenção da população no campo e para o desenvolvimento sustentável dessas regiões. Este trabalho tem como objetivo combinar duas técnicas de remediação de solos afetados por sais que possam promover melhorias nos atributos químicos e físicos dos solos utilizando a espécie halófita quinoa (*Chenopodium quinoa* Willd.) e biochar de casca de arroz. Foram instalados dois experimentos em casa de vegetação nos períodos de inverno e verão no Brasil, em esquema fatorial 3x7, utilizando três amostras de solos (dois salinos e um não salino) comumente encontrados no semiárido brasileiro e sete doses de biochar (0, 10, 20, 40, 80 e 100 t ha^{-1}), em delineamento de blocos casualizados, em dois ciclos de quinoa. Os solos foram previamente classificados como Cambissolo, Neossolo Flúvico e Planossolo. Ao final do segundo ciclo, foram coletadas amostras dos solos com estruturas deformada e não deformada para avaliação dos atributos do solo. Por serem solos química e fisicamente distintos, a adição de biochar promoveu diferentes alterações nos solos avaliados. Nos solos salinos, o aumento das doses de biochar promoveu redução de pH, condutividade elétrica (CE_e), relação de adsorção de sódio (RAS), capacidade de troca catiônica (CTC), porcentagem de sódio trocável (PST) e densidade do solo (Ds), e aumento de K^+ trocável. No solo não salino, o biochar foi responsável pelo aumento do pH, CTC, PST e K^+ trocável e reduções de CE_e, RAS e Ds. O Biochar também aumentou a condutividade hidráulica saturada (K_{sat}) no Cambissolo e Planossolo e diminuiu no Neossolo Flúvico. Assim, o RHB foi considerado eficiente na redução de atributos relacionados à salinidade e sodicidade de solos afetados por sais no semiárido brasileiro, sendo capaz de alterar atributos químicos e físicos, principalmente em altas doses. No ciclo de verão, o biochar também reduziu os efeitos nocivos das altas temperaturas na sobrevivência das plantas de quinoa em solos salino-sódicos.

Palavras-chave: Remediação. Potássio. Sódio. Condutividade hidráulica saturada. Sais.

3.1. Introduction

Soil salinization and sodification are pedogenetic processes that have been intensifying, especially in regions with arid and semiarid climates. Worldwide, it is estimated that 1.1 billion hectares are degraded or in the process of being degraded by salts and sodium. Of this total, 831 million hectares are present in arable lands. One of the main challenges is the saline soil reclamation and reuse, as this type of degradation has been considered one of the biggest global problems in the environmental and socioeconomic spheres, especially with the visible advance of climate change in the 21st century (HASSANI; AZAPAGIC; SHOKRI, 2021).

In Brazil, soil salinization occurs mainly in the semiarid northeastern region, where evapotranspiration annually exceeds precipitation, and irrigation/drainage management is often inefficient. It is estimated that 20% of arable lands are already degraded by salts and sodium, where 33% of irrigated areas are in the process of salinization/sodification (SHRIVASTAVA; KUMAR, 2015; PESSOA et al., 2016; PESSOA et al., 2022).

Techniques such as salt leaching, addition of conditioning agents, improvement in irrigation systems, and chemical reclamation often become unfeasible and costly. From this perspective, one of the main current research lines is the use of halophyte plants to improve and recover degraded soils. Thus, arises quinoa (*Chenopodium quinoa* Willd.), a facultative halophyte species of Andean origin (South America), highly resistant to saline soils, capable of producing grains in environments with severe stress, allowing soil improvement and economic return for the population in these extreme areas (BAZILE; JACOBSEN; VERNIAU, 2016; ALANDIA et al., 2020; LÓPEZ-MARQUÉS et al., 2020).

Another technique that has been evaluated for soils affected by salts is the addition of biochar, which is a product derived from the pyrolysis of organic feedstock, and it is used as a soil conditioner, capable of reducing bulk density, increasing hydraulic conductivity, and improving the soils chemical and biological attributes. Studies show that the relationship between quinoa and biochar in saline-sodic soils has led to a reduction in sodium toxicity, an increase in crop biomass and crop productivity (ABBAS et al., 2022).

It is possible to produce biochar from different feedstocks and pyrolysis temperatures, one of which is rice husk, as it is one of the most produced by-products in global agriculture, especially in eastern countries such as China. The transformation of rice husk into biochar provides a sustainable purpose for this material that are discarded or burned by farmers (ASADI et al., 2021).

Thus, this work aims to evaluate the effects of rice husk biochar (RHB) on saline soils commonly found in the northeastern region of Brazil cultivated with the CPAC 09 genotype developed by EMBRAPA Cerrados (Brazilian Agricultural Research Corporation), being the first work published with this genotype for salts and sodium resistance in the Brazilian semiarid region.

3.2. Material and methods

The experiments were carried out in a greenhouse at the Agronomic Institute of Pernambuco (IPA), in Recife (PE), Brazil, in the months of Mar/Aug 2022 (Winter) and Nov/Feb 2022/23 (Summer). The genotype CPAC 09 of quinoa (*Chenopodium quinoa* Willd.) from EMBRAPA - Cerrados was selected, which was cultivated under application of increasing doses of biochar, in three soils, one non-saline and two saline-sodic, in two quinoa cycles.

3.2.1. Soils selection

Three soils commonly found in the semiarid region of Pernambuco, Brazil, were selected, according to soil type and salinity levels. The soils were collected in the cities of Cabrobó-PE, Parnamirim-PE, and Caruaru-PE, being classified as Cambisol, Fluvisol, and Planosol, respectively. Soil samples were collected in the 0-20 cm layer, air-dried, grinded, and sieved through a 2 mm mesh to characterize the soil.

3.2.2. Soils characterization

To characterize the soils, physical and chemical analyzes were carried out (Table 2). The physical properties evaluated were texture, with analysis of particle size composition and clay dispersed in water, using the pipette method as proposed by Ruiz (2005); particle density, using the volumetric ring method; and bulk density using the clod method (EMBRAPA, 2017). With the results of total clay and clay dispersed in water, the degree of soil flocculation was calculated; and total porosity was calculated with bulk and particle densities values.

For the chemical characterization we analyzed the soil pH in water (1:2.5); pH, soil electrical conductivity (EC_e), soluble cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and Cl^- in the saturated paste extract; exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) by the ammonium acetate method, and potential acidity by the calcium acetate method. K^+ and Na^+ were measured by flame emission photometry, Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometry, Cl^- by titration from the reaction of potassium chromate with silver nitrate, and potential acidity by

titration from the sodium hydroxide. The methods adopted are described by EMBRAPA (2017) and USSL STAFF (1954). The base saturation (V), exchangeable sodium percentage (ESP), cation exchange capacity (CEC), and sodium adsorption ratio (SAR) were calculated (Table 2).

3.2.3. Biochar characterization

The biochar feedstock was rice husk, and the pyrolysis temperature was 400 °C. To characterize the biochar (Table 3), grinding and sieving through a 0.200 mm mesh were carried out. The elements Ca, Mg, K, P, and Cl were determined using the portable-XRF, S1 TITAN model 800. The organic carbon, hydrogen, and nitrogen of the biochar were determined via dry combustion, using a CHN628 elemental analyzer (LECO), enabling the calculation of the C/N ratio. The biochar pH and EC were determined at a ratio of 1:10, according to Singh et al. (2017).

Table 2 – Chemical and physical characterization of three soils from Brazilian Semiarid

Attribute	Soil		
	Cambisol	Fluvisol	Planosol
Physical attributes			
Total sand (g kg^{-1})	592.0	349.03	714.89
Coarse sand (g kg^{-1})	25.29	95.15	471.2
Fine sand (g kg^{-1})	566.7	253.9	243.7
Silt (g kg^{-1})	344.36	468.53	222.33
Clay (g kg^{-1})	63.64	182.43	62.77
WDC (g kg^{-1})	82.28	59.26	70.91
BD (g cm^{-3})	1.51	1.60	1.71
PD (g cm^{-3})	2.57	2.58	2.50
TP (%)	41.24	37.98	31.60
Texture	Sandy Loam	Loam	Loamy Sandy
Chemical attributes			
pH (H_20)	8.09	6.35	5.68
pH (EPS^5)	8.12	7.58	6.35
ECe (dS m^{-1})	30.99	22.59	0.88
Soluble cations			
Ca^{2+} ($mmol_c$ L^{-1})	40.08	32.83	1.84
Mg^{2+} ($mmol_c$ L^{-1})	46.35	55.68	6.25
Na^{+} ($mmol_c$ L^{-1})	152.24	118.65	3.03
K^{+} ($mmol_c$ L^{-1})	1.67	1.02	2.30
SAR ($mmol_c$ $L^{-1})^{-0.5}$	23.26	18.73	1.50

Exchangeable cations			
Ca^{2+} ($cmol_c\ kg^{-1}$)	25.16	7.48	0.44
Mg^{2+} ($cmol_c\ kg^{-1}$)	6.63	7.49	0.93
Na^{+} ($cmol_c\ kg^{-1}$)	2.09	1.70	0.40
K^{+} ($cmol_c\ kg^{-1}$)	0.30	0.28	0.42
H + Al ($cmol_c\ kg^{-1}$)	0.23	0.87	2.78
CEC ($cmol_c\ kg^{-1}$)	34.42	17.82	4.97
V (%)	99.33	95.12	44.02
ESP (%)	6.07	9.54	8.04

WDC: Water dispersible clay; BD: Bulk density; PD: Particle density; TP: Total porosity; ECe: Soil electrical conductivity, TOC: Total organic carbon; SAR: Sodium adsorption ratio; CEC: Cation exchangeable capacity; V: Base saturation; ESP: Exchangeable sodium percentage.

Table 3 – Chemical characterization of rice husk biochar (RHB)

RHB	
pH	7.25
EC ($dS\ m^{-1}$)	0.22
C (%, w/w)	47.67
N (%, w/w)	1.4
H (%, w/w)	6.32
C/N	34.05
Ca ($g\ kg^{-1}$)	1.52
Mg ($g\ kg^{-1}$)	0.71
Na ($g\ kg^{-1}$)	-
K ($g\ kg^{-1}$)	5.53
P ($g\ kg^{-1}$)	1.33
Cl ($g\ kg^{-1}$)	0.41

3.2.4. Tap water characterization

In both experimental cycles, tap water with high salinity (table 4) was add to the pots to simulating the irrigation conditions commonly found in Brazilian semiarid regions, characterized by frequently saline-sodic soils with available water of poor chemical quality for irrigation, generally saline, sodic, or saline-sodic.

During the first cycle, 6.9 liters of water per pot were added to the soil after transplanting until flowering began. In the second cycle, due to the high temperatures, a total of 9.05 liters was added in the pots. After the two quinoa cycles, approximately 48.9 mg kg^{-1} of Ca^{2+}, 44.2 mg kg^{-1} of Mg^{2+}, 145.52 mg kg^{-1} of Na^{+}, 13.05 mg kg^{-1} of K^{+} and 403.8 mg kg^{-1} of Cl^{-} was added to the soils through irrigation water.

Table 4 – Tap water characterization used during quinoa cycles

TAP WATER	
Color	5
Turbidity	0.9
EC (dS m^{-1})	1.465
pH	7.8
Total dissolved solids	944
Hydroxide Alkalinity	0.00
Carbonate Alkalinity	0.00
Bicarbonate Alkalinity	62
Total Alkalinity	62
Total Hardness	87.02
Ca^{2+} (mg L^{-1})	45.69
Mg^{2+} (mg L^{-1})	41.33
Na^{+} (mg L^{-1})	136.00
K^{+} (mg L^{-1})	12.20
Cl^{-} (mg L^{-1})	377.41
SO_4^{2-} (mg L^{-1})	31.17
*Water classification	C3S1

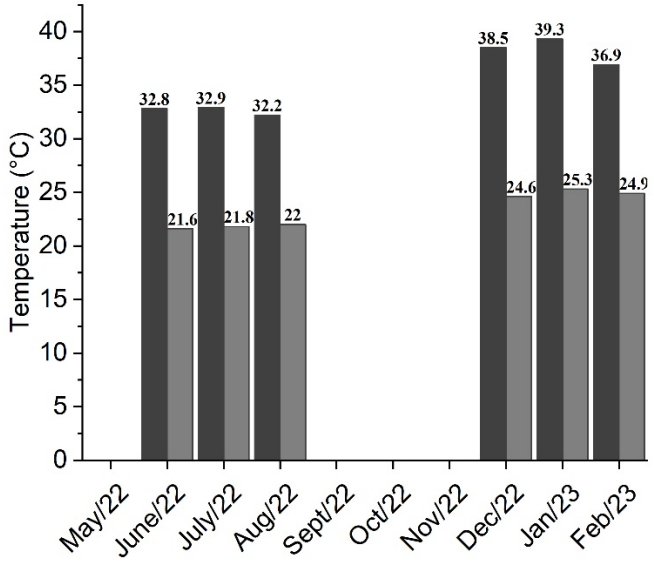
*Water classification according to USSL Staff (1954).

3.2.5. Average temperature in the quinoa cycles

The temperature inside the greenhouse was constantly measured, with minimum and maximum temperatures being evaluated daily. After the daily measurements, a temperature variation graph was created in the winter and summer periods in Brazil during the three months of quinoa evaluation, in each cycle, in the greenhouse (June-August 2022 and December-February

2022/ 2023) totaling 6 months of evaluation. The months from June to August 2022 were characterized as those with the lowest temperatures and from December to February, the months with the highest temperatures (Figure 3).

Figure 3 – Maximum and minimum temperatures during first quinoa cycle (June-Aug – Brazil winter) and second quinoa cycle (Dec-Feb – Brazil summer) in greenhouse



3.2.6. Experimental setup and design

The experiment consisted of a 3 x 7 factorial with three soil types and seven biochar doses (0, 10, 20, 40, 60, 80 and 100 t ha^{-1}), in a randomized block design, with four replications, totaling 84 experimental units. The experiment was conducted in two quinoa cycles, the second cycle being cultivated in the same pots as in the first cycle, respecting the treatments configuration.

The experiment was conducted in 20 L pots and 15 kg of soil was placed in each pot. The biochar was previously grinded until the particle size of 0.300-0.850 mm according to Liu et al. (2016), being classified as a medium texture biochar. Biochar was incorporated into the soil total mass, according to each dose.

After the experiment setup, the plants were irrigated with saline tape water. The experiment was conducted over a period of 6 months, with two cycles of 90 days each, a period necessary for the plants flowering.

3.2.7. Soil analysis

3.2.7.1. Physical analysis

The physical properties, evaluated at the end of the second cycle, were bulk density using the volumetric ring method, particle density, and soil saturated hydraulic conductivity using the vertical column permeameter and constant load method (EMBRAPA, 2017). With the bulk density and particle density values the total porosity was calculated.

3.2.7.2. Chemical analysis

The chemical properties, also evaluated at the end of the second cycle, were soil pH in water (1:2.5); pH, soil electrical conductivity (EC_e), soluble cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and Cl^- by the saturated paste extract; exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) by the ammonium acetate method and cation exchange capacity by the index cation method (USSL STAFF, 1954).

K^+ and Na^+ were measured by flame emission photometry, Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry, and Cl^- by titration based on the reaction of potassium chromate with silver nitrate. The methods adopted are described by EMBRAPA (2017) and USSL STAFF (1954). Base saturation (V), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) values of the soils were calculated.

3.2.8. Data analysis

The results obtained were initially subjected to normality tests (Shapiro-Wilk, $p < 0.05$) and homoscedasticity (Levene Test, $p>0.05$). After these procedures, analysis of variance (ANOVA, $p<0.05$) was performed and the average values of the results obtained were compared between soils using Tukey test ($p<0.05$). Regression analyzes were also carried out to verify the effects of biochar doses on soil and plant attributes, selecting the models that presented the best coefficient of determination (adjusted R^2) and significance of 5% using the t test.

3.3. Results

3.3.1. Quinoa survival rate as a function of biochar doses

Quinoa had a survival percentage in the first cycle (winter) of 100% in all treatments regardless of the salinity level and biochar doses (Table 5). In the second cycle (summer), plants with lower biochar doses in saline soils were affected by double stress (salinity x high

temperatures). At Cambisol, only 25% of the plants survived without biochar addition. From a dose of 40 t ha^{-1}, 75 – 100% of the plants survived. The double abiotic stress strongly affected plants on Fluvisol, with a survival rate of 25% at biochar dose of 60 t ha^{-1} and 50% at doses of 80 and 100 t ha^{-1}. For Planosol, all plants survived under high temperature, due to its low salinity.

Table 5 – Quinoa survival rate under biochar doses in two cycles (winter and summer)

Biochar dose (t ha^{-1})	Plant survival in each treatment after 90 days (%)		
	Cambisol	Fluvisol	Planosol
	First cycle (winter)		
0	100%	100%	100%
10	100%	100%	100%
20	100%	100%	100%
40	100%	100%	100%
60	100%	100%	100%
80	100%	100%	100%
100	100%	100%	100%
	Second cycle (summer)		
0	25%	25%	100%
10	50%	25%	100%
20	50%	25%	100%
40	100%	25%	100%
60	75%	25%	100%
80	100%	50%	100%
100	100%	50%	100%

3.3.2. Soil chemistry properties after RHB application and quinoa cultivation

Chemical attributes of the evaluated soils were measured after two quinoa cycles and one year of biochar doses application. For attributes related to soil salinity and sodicity, biochar significantly reduced soluble Na^+ (Table 6), soil electrical conductivity (EC_e), and SAR for Cambisol and Planosol soils (Figures 5 and 6). From the data, it was possible to obtain regression

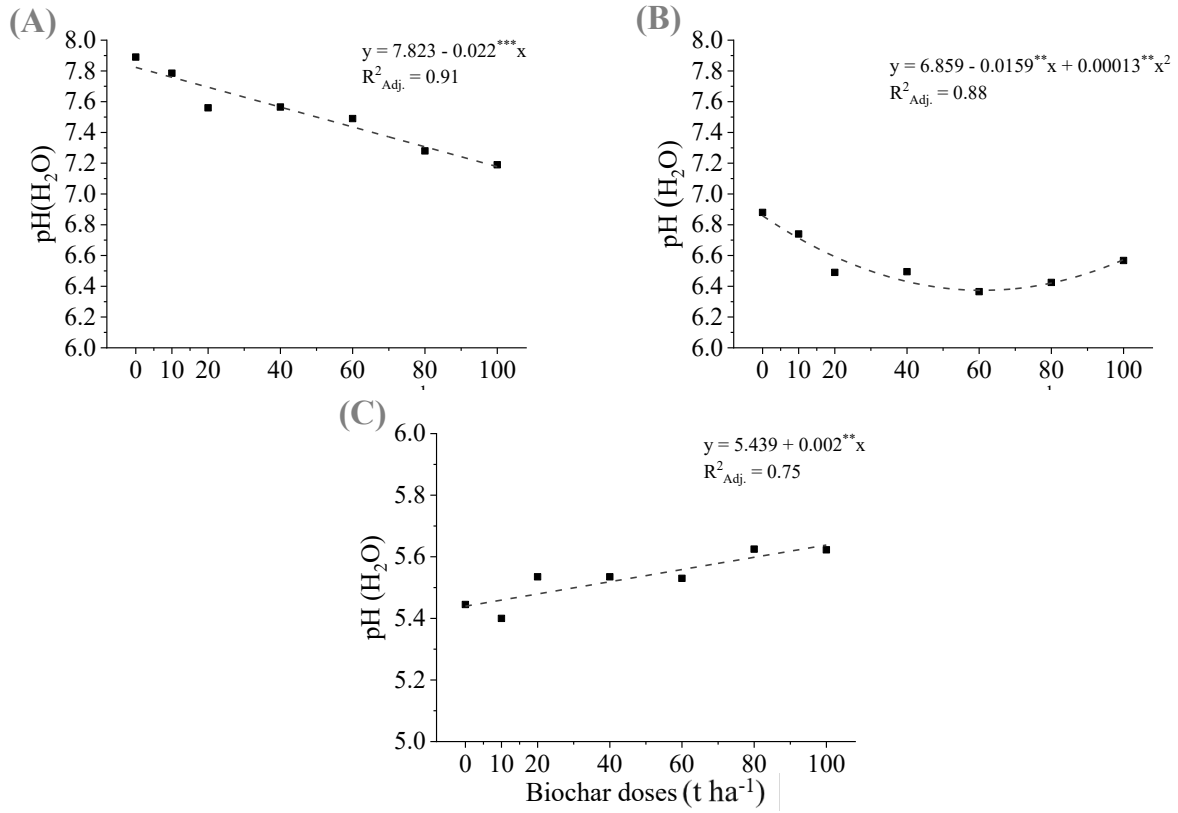
adjustments for the variables pH (H_2O), EC_e, SAR, and exchangeable K^+, as a function of biochar doses (Figures 4, 5, 6, and 7).

Due to the slightly basic biochar pH (Table 3), the RHB was able to reduce the pH of alkaline (Cambisol) and raise the pH of acidic soil (Planosol) as shown in figure 4. For Cambisol, the reduction in pH between the control and 100 t ha^{-1} dose was 8.87% and, for Fluvisol, the most pronounced reduction was at the biochar dose of 60 t ha^{-1} (7.47%). For Planosol, there was an increase in soil pH of 3.12% (control to 100 t ha^{-1}) as shown in the figure 4C.

For electrical conductivity (EC_e), biochar was responsible for significantly reducing the EC_e of Cambisol and Planosol, with a decrease of 37.23% and 26.83%, respectively, between the first and last treatments. For Fluvisol EC_e, the result was not significant by the Tukey test at 5% probability.

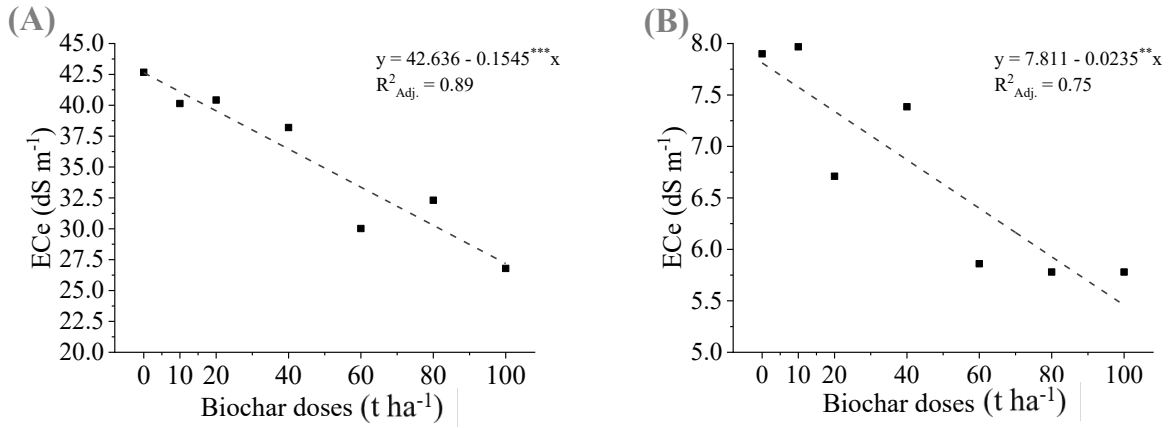
Soil SAR was significantly modified by RHB in Cambisol and Fluvisol. In both soils, SAR was reduced when biochar doses were applied as shown in figure 6. In Cambisol, the SAR reduction was about 34.88% and in Fluvisol it was 8.02%.

Figure 4 – Soils pH as a function of RHB doses for Cambisol (A), Fluvisol (B), and Planosol (C)



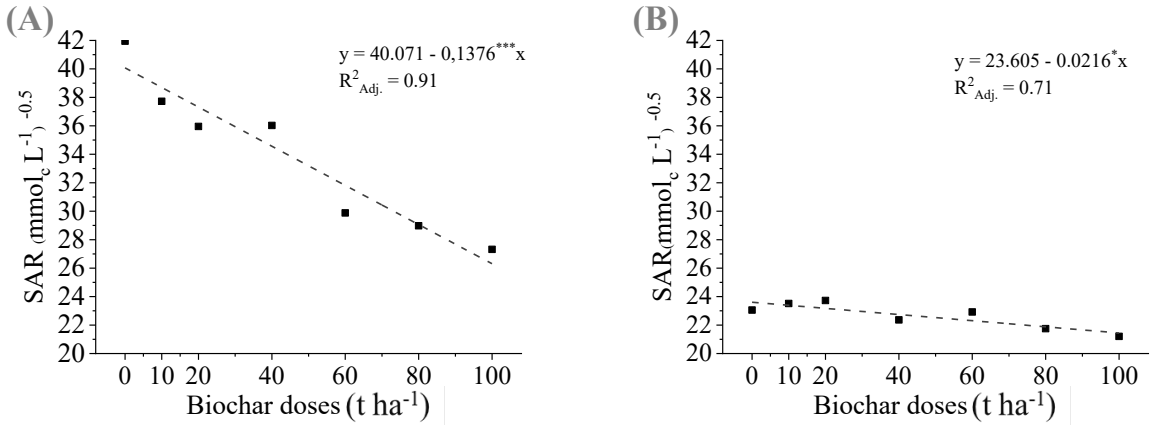
***0.1%; **1% significance in t-test.

Figure 5 – ECe as a function of RHB doses for Cambisol (A) and Planosol (B)



***0,1%; **1% significance in t-test.

Figure 6 – SAR as a function of RHB doses for Cambisol (A) and Fluvisol (B)



***0,1%; *5% significance in t-test.

In general, RHB promoted reduction in Cambisol and Fluvisol exchangeable cations, but with a significant increase in K^+ (Table 7). Potential acidity (H + Al) was not directly affected in Cambisol and it had a significant increase for Fluvisol. CEC and ESP had a significant reduction with the biochar doses in Cambisol. As for Planosol, there was an increment in exchangeable Na^+ and K^+, with a significant increase also in potential acidity, CEC, and ESP. RHB was responsible for the increment in exchangeable K^+ in all evaluated soils (figure 7). The increment was higher in Planosol (143.56%) between doses of 2 and 100 t ha^{-1} than in the other two soils.

Table 6 – Mean values for soil soluble cations (Ca^{+2}, Mg^{+2}, Na^{+}, and K^{+}) and soluble Cl^{-}

Biochar doses (t ha^{-1})	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}
			$mmol_c\ L^{-1}$		
			Cambisol		
0	73.77 a	93.81 a	385.98 a	4.27 a	381.2 a
10	80.29 a	83.32 a	346.63 ab	4.22 a	365.0 a
20	85.77 a	87.02 a	334. 78 ab	4.36 a	373.7 a
40	82.21 a	84.76 a	334.89 ab	4.26 a	356.2 a
60	73.92 a	61.51 a	250.22 ab	4.26 a	266.2 a
80	87.39 a	73.24 a	255.96 ab	5.56 a	338.7 a
100	75.89 a	61.51 a	211.80 b	5.90 a	273.7 a
CV (%)	19.84	35,75	33,80	22,98	34,3
			Fluvisol		
0	100.58 a	78.38 a	217.83 a	1.63 ab	342.5 a
10	106.38 a	85.58 a	228.15 a	1.72 ab	370.0 a
20	86.62 a	59.66 a	214.24 a	1.59 b	311.2 a
40	107.57 a	88.66 a	221.30 a	1.77 ab	365.0 a
60	104.01 a	93.26 a	227.10 a	2.03 a	390.0 a
80	98.43 a	80.02 a	204.67 a	1.86 ab	368.7 a
100	81.70 a	61.10 a	185.43 a	1.75 ab	335.0 a
CV (%)	22.39	30.2	16.9	11.92	19.59

			Planosol		
0	15.55 a	17.10 a	49.16 a	1.180 b	158.3 a
10	14.80 a	16.40 a	47.63 a	1.814 ab	120.0 a
20	12.40 a	12.75 a	35.04 a	1.115 b	129.1 a
40	14.12 a	12.84 a	37.76 a	1.430 ab	116.2 a
60	10.44 a	9.21 a	32.43 a	1.559 ab	106.5 a
80	9.29 a	10.62 a	30.43 a	1.507 ab	91.2 a
100	10.11 a	11.95 a	35.52 a	2.653 a	102.5 a
CV (%)	34.11	37.42	26.9	47.79	26.87

Lowercase letters compare means in the column in each soil. Means compared using the Tukey test at 5% of probability.

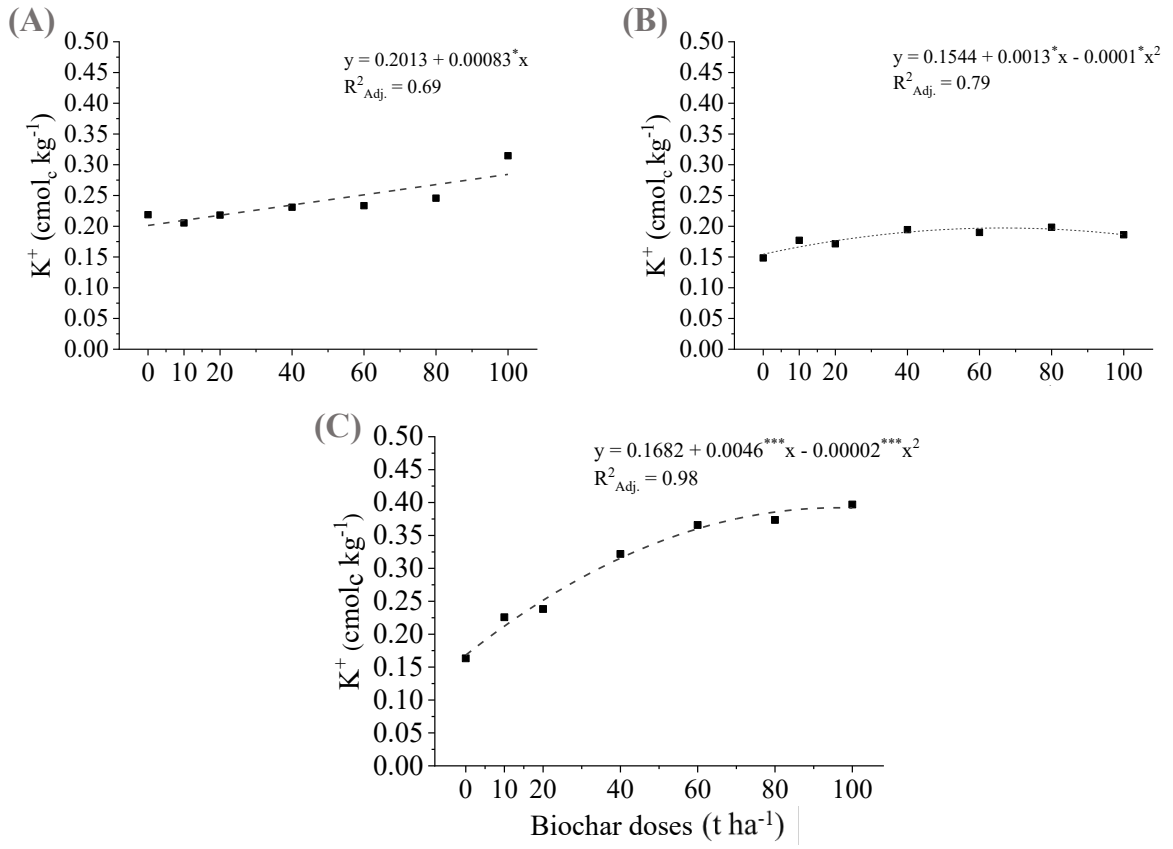
Table 7 – Mean values for exchangeable cations, CEC (cations exchange capacity), bases saturation (V), and ESP (exchangeable sodium percentage)

Biochar dose (t ha^{-1})	Exchangeable cations							
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	H + Al	CEC	V (%)	ESP
	$cmol_c\ kg^{-1}$						%	
	Cambisol							
0	24.76 a	6.42 a	1.97 a	0.22 b	0.70	34,06 a	97.96 a	5.79 a
10	23.92 a	5.42 ab	1.81 a	0.21 b	0.70	32.27 a	97.66 ab	5.64 a
20	24.15 a	5.51 ab	1.71 ab	0.22 b	0.70	32.27 a	97.85 ab	5.28 a
40	24.40 a	5.42 ab	1.49 bc	0.23 b	0.70	32.23 a	97.84 ab	4.63 bc
60	21.49 b	4.69 b	1.20 cd	0.23 b	0.70	28.30 b	97.54 b	4.23 c
80	21.57 b	4.67 b	1.08 cd	0.25 b	0.70	28.25 b	97.54 b	3.80 c
100	21.20 b	4.78 b	1.32 d	0.31 a	0.70	28.30 b	97.54 b	4.66 ab
CV (%)	7.53	14.06	22.64	18.09	0.00	8.51	0.23	16.96
	Fluvisol							
0	9.78 a	8.22 a	3.03 a	0.15 b	0.95 d	22.02 a	95.64 a	11.68 a
10	9.47 ab	8.92 a	3.10 a	0.18 ab	1.13 cd	22.80 a	95.05 ab	12.16 a
20	9.30 ab	8.71 a	3.15 a	0.17 ab	1.26 bc	22.60 a	94.42 bcd	12.81 a
40	9.19 ab	8.71 a	2.99 a	0.19 ab	1.17 bcd	22.08 a	94.71 abc	11.27 a
60	9.33 ab	8.95 a	3.07 a	0.19 ab	1.43 ab	23.07 a	93.78 cd	12.62 a
80	8.76 b	8.81 a	2.99 a	0.20 a	1.43 ab	22.22 a	93.56 de	12.79 a
100	8.96 b	9.26 a	2.70 a	0.19 ab	1.69 a	22.80 a	92.58 e	10.85 a

CV (%)	5.70	8.12 a	6.94	11.81	19.46	4.16	1.13	7.44 a
				Planosol				
0	1.37 a	1.09 a	0.21 c	0.16 c	1.08 e	3.91 c	71.93 a	5.27 c
10	1.36 a	1.10 a	0.27 bc	0.22 cb	1.17 de	4.12 c	71.44 a	6.44 abc
20	1.36 a	1.04 a	0.27 bc	0.24 cb	1.43 cd	4.30 c	66.68 ab	6.16 bc
40	1.47 a	1.08 a	0.37 ab	0.32 ab	1.61 bc	4.85 b	66.80 ab	7.67 ab
60	1.51 a	1.19 a	0.46 a	0.37 a	1.82 ab	5.35 a	65.83 ab	8.63 a
80	1.53 a	1.15 a	0.46 a	0.37 a	1.76 ab	5.28 a	66.54 ab	8.74 a
100	1.53 a	1.20 a	0.40 a	0.40 a	1.94 a	5.46 a	64.59 b	7.28 abc
CV (%)	8.78	12.77	30.55	33.02	21.42	13.60	6.07	20.73

Lowercase letters compare means in the column in each soil. Means compared using the Tukey test at 5% of probability.

Figure 7 – Exchangeable K^+ as a function of RHB doses for Cambisol (A), Fluvisol (B), and Planosol (C)



***0,1%; *5% significance in t-test.

3.3.3. Soil physical attributes after RHB application and quinoa cultivation

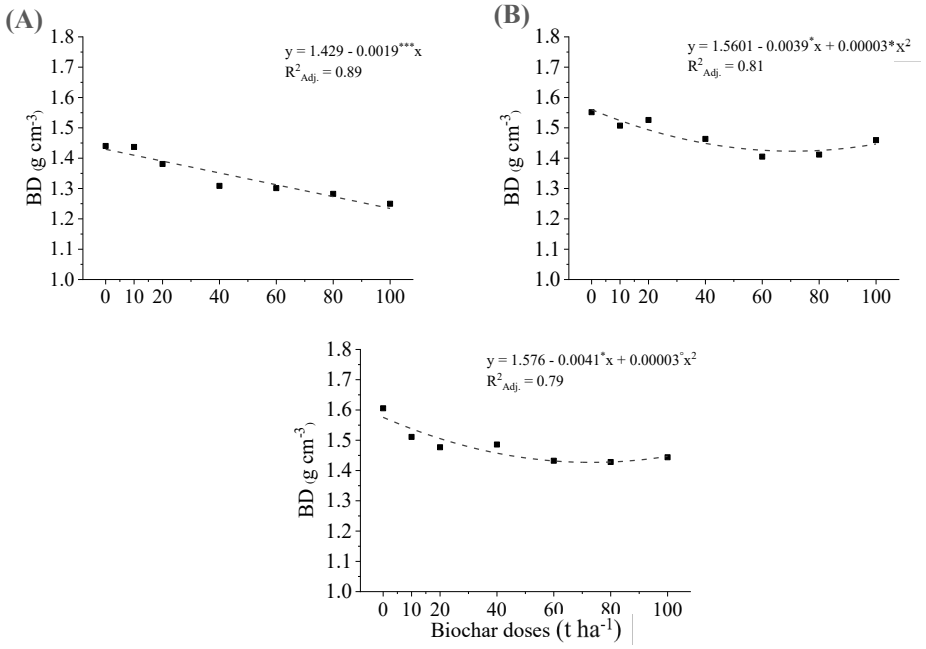
Similar to chemical attributes, the physical attributes were also measured after two quinoa cycles and one year of RHB application. For the three evaluated soils, bulk density (BD) decreased significantly with increasing biochar doses. Saturated hydraulic conductivity (K_{sat}) increased for Cambisol and Planosol and reduced for Fluvisol. Particle density (PD) only had a significant reduction for Planosol and, for total porosity, there was a significant increase for Cambisol as shown in table 8.

From the data, it was possible to obtain regression adjustments for the variables BD and K_{sat} as a function of biochar doses (Figures 8 and 9). For the three evaluated soils there was a significant decay in BD, with a tendency towards stabilization from doses of 20 to 40 t ha^{-1} of

biochar in the Fluvisol and Planosol, as represented in figure 8. In Cambisol, the decrease in BD presented a linear character, with the lowest value obtained at the highest dose of biochar (100 t ha^{-1}).

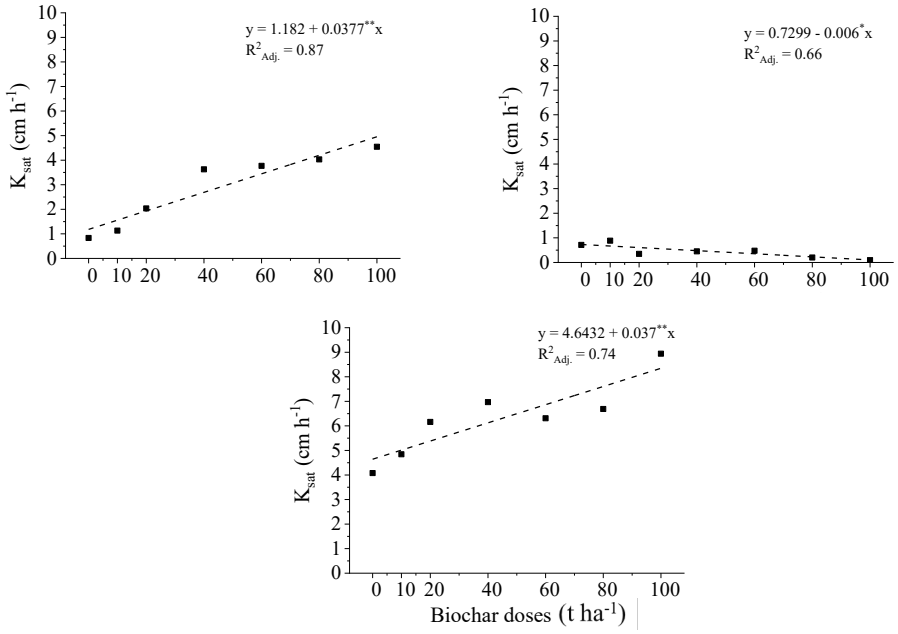
For K_{sat}, Cambisol increased it from 0.83 cm h^{-1} in the treatment without RHB to 4.55 cm h^{-1} for the dose of 100 t ha^{-1}. In Planosol there was the opposite trend, where K_{sat} was reduced between doses of 0 and 100 t ha^{-1} (0.71 to 0.09 cm h^{-1}, respectively). As for Planossol, there was a linear increase between the first and last treatment with an increment in K_{sat} from 4.08 to 8.97 cm h^{-1} as shown in figure 9.

Figure 8 – Bulk density (BD) as a function of RHB doses for Cambisol (A), Fluvisol (B), and Planosol (C)



***0,1%, **1%; *5%, °10% significance in t-test.

Figure 9 – Saturated hydraulic conductivity (Ksat) as a function of RHB doses for Cambisol (A), Fluvisol (B) e Planosol (C)



***0,1%, **1%; *5% significance in t-test.

Table 8 – Soil physical attributes after biochar application in two quinoa cycles. Mean values for particle density (PD) and total porosity (TP)

Biochar dose (t ha^{-1})	PD g cm^{-3}	TP %
	Cambisol	
0	2.57 a	44.8 ab
10	2.56 a	44.0 ab
20	2.52 a	41.4 b
40	2.56 a	48.8 ab
60	2.54 a	48.8 ab
80	2.55 a	48.8 ab
100	2.55 a	51.0 a
CV (%)	1.30	8.77

	Fluvisol	
0	2.58 a	39.8 a
10	2.56 a	41.8 a
20	2.58 a	40.8 a
40	2.59 a	43.2 a
60	2.49 a	43.5 a
80	2.63 a	44.8 a
100	2.51 a	41.7 a
CV (%)	5.22	8.04
	Planosol	
0	2.50 ab	35.9 a
10	2.51 ab	39.7 a
20	2.53 a	41.7 a
40	2.42 ab	37.1 a
60	2.50 ab	43.1 a
80	2.31 b	37.9 a
100	2.33 ab	37.9 a
CV (%)	4.73	10.17

Lowercase letters compare in the column, in each solo. Means compared using Tukey test at 5% of probability.

3.4. Discussion

3.4.1. Effects of increasing RHB doses on salinity/sodicity and K^+ availability in Brazilian semiarid soils

According to the results, RHB promoted different effects depending on the soil type. Under saline tap water addition to all soils, there was an increment in EC_e, soluble cations, and SAR compared to the soils characterization before the experiment set up. In addition to increasing the solubilization of salts in the soils, irrigation with saline tap water was also responsible for the increment of salts, favoring an increase in soils salinity/sodicity, similar to what occurs naturally in irrigated lands in the Brazilian semiarid.

For Cambisol, characterized as a saline-sodic soil, alkaline, with high CEC, and with sandy loam texture, the biochar promoted a reduction in pH; EC_e; soluble Na^+; exchangeable Ca^{2+}, Mg^{2+}, and Na^+; SAR; bases saturation (V%); CEC, and ESP with a significant increase in exchangeable K^+ (Table 6 and 7).These significant reductions in soil bases are related to the concentration of these elements in the biochar. As it is a material poor in bases and slightly alkaline, its application in soil with highly alkaline pH, high CEC, and saline-sodic characteristic, the dilution effect occurs. As RHB is an organic material rich in K^+, its additions promoted a significant increase in exchangeable K^+.

The increment in K^+ concentration in soil is positive for quinoa, given the plant's high demand for this nutrient, especially in environments with intense abiotic stresses such as high temperatures, salinity, and drought. According to TURCIOS; PAPENBROCK; TRÄNKNER (2021), the uptake of K^+ by quinoa increases significantly in saline-sodic soils. The authors affirm that this high K^+ uptake is due to the plant's need to regulate the K^+/Na^+ ratio as a way of reducing osmotic stress, favoring quinoa growth in environments with saline stress. Thus, K^+ addition in soil due to biochar application is essential for the maintenance and good quinoa development in salt affected soils.

For Cambisol, biochar doses were considerably beneficial for reducing salinity and sodicity parameters (pH, EC_e, SAR, and ESP), especially at high RHB doses such as 60, 80, and 100 t ha^{-1}. Similar results were reported by PHUONG et al. (2020) that, in saline soils in Vietnam cultivated with rice, the authors observed significant decrease in pH, ESP, and exchangeable Na^+ of the evaluated soil, increasing K^+ concentration after the addition of RHB. Zhang et al. (2019), using rice straw biochar, also observed reduction in EC_e and exchangeable Na^+, alleviating salt stress for plants.

For Fluvisol, the biochar effect was significantly evident in reducing pH and exchangeable Ca^{2+}, with an increase in exchangeable K^+ similar to Cambisol. Although not significant, there was a downward trend in parameters related to soil salinity/sodicity such as SAR and Na^+ (soluble and exchangeable). As Fluvisol is a soil with a high silt concentration (approximately 50% according to Table 2) and it was collected in Brazilian semiarid, this soil has a strong tendency for physical degradation than other soils, resulting in a reduction in K_{sat} after the addition of biochar doses. The decrease in K_{sat} results in difficulties in water infiltration and, therefore, favors the salts

accumulation in soil upper layers, which would be a justification for the RHB does not significantly reduce the parameters related to salts accumulation in Fluvisol.

As for Planosol, soil pH increased with the addition RHB doses. This increment in pH was due to the difference between the soil pH (5.68) and the biochar pH (7.25). It is important to note the multiple effect that RHB has on the soil pH in the acidic and alkaline range. Because it has a relatively lower pH than other biochar types, RHB made under 400°C pyrolysis can reduce the pH of highly alkaline soils and increase the pH of acidic soils, being recommended for both situations as shown in figure 4. The increase in K^+ is also evident in Planosol, proving that, regardless of the soil, RHB increases the concentration of K^+ available in soils, mainly favoring plants with a high nutritional need for this element such as quinoa.

Zhang et al. (2020), using biochar made from sugarcane bagasse, orange bagasse, and corn cob feedstocks, observed that both biochar from sugarcane and orange were efficient in reducing EC_e, SAR, and ESP in saline-sodic soil cultivated with corn. However, treatments with biochar made from corn cobs were not efficient in reducing parameters related to soil salinity/sodicity, which corroborates the idea that the biochar's feedstock type and pyrolysis temperature directly influence the biochar efficiency in acting as a remediator for salt affected soils.

Comparing to Brazilian semiarid soils, RHB has low concentration of Ca^{+2} and Mg^{+2} in its composition (Table 3). Although several authors state that biochar has the potential to make Ca^{+2} and Mg^{+2} available to soils (AKHTAR; ANDERSEN; LIU, 2015), this pattern becomes ineffective for highly saline soils, such as Cambisol and Fluvisol, where biochar had a diluting effect on these salts when applied in high concentrations (from 60 t ha^{-1}). It was possible to observe in table 7, a significant reduction in exchangeable Ca^{+2} and Mg^{+2} mainly for Cambisol (highly saline soil), confirming the dilution concentration idea of these salts when high RHB doses are applied on saline soils.

In Cambisol there was a reduction in exchangeable Ca^{+2} from 24.76 $cmol_c$ kg^{-1} in the control to 21.20 $cmol_c$ kg^{-1} at a dose of 100 t ha^{-1}. For Mg^{+2}, this reduction was from 6.42 to 4.78 $cmol_c$ kg^{-1} under the same conditions. The decrease in Ca^{+2} concentration was also significant in Fluvisol, where the control presented concentrations of 9.78 $cmol_c$ kg^{-1} and the 100 t ha^{-1} dose was 8.96 $cmol_c$ kg^{-1}. Planosol is a loamy sandy soil (total sand = 714.89 g kg^{-1}) with the lowest salts concentration (EC_e = 0.88 dS m^{-1}), so RHB acted as a source of cations increasing them in the exchangeable phase especially Na^+ and K^+.

The effect of biochar doses is evident on plant survival throughout the experiment, especially in saline/sodic soils. Due to the increment in maximum and minimum temperatures during the second quinoa cycle, associated to salinity and sodicity of Cambisol and Fluvisol, the addition of high biochar doses allowed the survival of 75-100% of plants among dose of 40 t ha^{-1} and 100 t ha^{-1} for Cambisol and 50% for Fluvisol between doses of 80 t ha^{-1} to 100 t ha^{-1} (Table 5). This survival was possibly due to the reduction of parameters related to the salt's accumulation in soil and to the increase in the K^+ concentration in the soils, favoring the reduction of the double effect (temperature x salinity) during the summer. Without the addition of biochar, only 25% of the plants in saline soils survived to the high temperature characteristic of summer months in Brazil, among December and February (Table 5).

3.4.2. Effects of increasing RHB doses on saturated hydraulic conductivity (K_{sat}) and bulk density in Brazilian semiarid soils

After two quinoa cycles (winter and summer) with the addition of increasing biochar doses, we observed that some soil physical parameters such as saturate hydraulic conductivity (K_{sat}), bulk density, particle density, and total porosity were modified depending on the soil type.

For Cambisol, with the addition of increasing biochar doses, there was an increment in K_{sat} (Figure 9A). As it is a predominantly sandy soil with a low clay concentration, biochar possibly helped to structure the soil along with a significant reduction in bulk density, allowing better water infiltration through the soil layers. The reduction in SAR and exchangeable Na^+ also favors soil restructuring and possibly a reduction in clay dispersion, improving parameters related to soil permeability.

Biochar application together with reduction in soil SAR promoted a 58.4% increase in K_{sat} in a saline-alkali soil in an experiment with wheat straw biochar (1% w/w) and gypsum developed by Zhang et al. (2020). The authors state that the application of gypsum to reduce sodicity together with biochar favored an increase in water infiltration in the evaluated soil.

A similar result was demonstrated by Ouyang et al. (2013), in which the authors, working with two soils (silty clay and a sandy loam soils) with the addition of 2% (w/w) of dairy manure biochar, observed an increase in soil K_{sat}. The authors attributed this increase to the formation of macroaggregates mainly in sandy soil, stating that in silty soil this increase was less noticeable.

The same did not occur for Fluvisol, where there was an opposite trend of K_{sat} (Figure 9B). As it is a predominantly silty soil with a clay concentration three times greater than Cambisol, biochar may have caused clogging of the soil pores, mainly due to the interaction with silt and clay, reducing water infiltration in the soil. Furthermore, there was no significant reduction in parameters related to soil sodicity (SAR and ESP), favoring the dispersion of soil colloidal particles (clays), and consequently a reduction in soil permeability. As RHB is less dense than soil, the density of Fluvisol was also significantly reduced with the addition of biochar doses.

For Planosol, RHB increased soil K_{sat} (Figure 9C) and reduced bulk and particle densities. It is a predominantly sandy soil with low natural fertility, and the increment in the biochar doses (organic matter) promoted clay flocculation, improving the water infiltration in the soil in the same way as occurred in Cambisol.

Zhang; Chen; You (2016), using four different types of biochar, observed an opposite trend to that demonstrated in this work when biochar was applied to sandy soils. The authors attributed the reduction in soil K_{sat} to the difference between the hydraulic properties of the soil and biochar, and to the particle size of the biochar. According to these authors, the reduction in the biochar size particles favored the reduction in the soil hydraulic properties. Thus, the interaction of the RHB particles with the three soils may have been different, because the particle's size and composition of each soil (Table 2).

The reduction on bulk density under biochar application was also verified by TOKOVÁ et al. (2020) in a silty loam soil. The authors observed a 12% reduction in bulk density when applied 20 t ha^{-1} of mixture of paper fiber sludge and grain husks biochars (1:1). Organic materials act on bulk density because they have low density values and promote the organization of colloids, increasing porosity.

According to Barnes et al. (2014), the addition of biochar to soils can increase or decrease soil drainage depending mainly on the soil type, biochar amendment rate, and biochar properties. Therefore, studies on the action of different biochar types in soils with different chemical and physical characteristics must be further developed to adapt the needs of the soil, plant, and farmer, always aiming at environmentally sustainable and economically viable practices.

3.5. Conclusion

The addition of increasing doses of RHB was effective in remediating salt affected soils cultivated with quinoa due to the reduction in pH, ECe, ESP, SAR, V, and BD. Depending on the soil, RHB, made from pyrolysis at 400 °C, has an amphoteric character, reducing the pH in basic soils and increasing it in acidic soils. As RHB is a material rich in K^+, biochar increased the availability of this nutrient in all soils, allowing quinoa to resist the double stress of temperature versus salinity. In sandy soils, biochar promoted an increase in saturated hydraulic conductivity (K_{sat}) in both saline and non-saline soils. In silty soil, biochar drastically reduced K_{sat}, negatively influencing water infiltration into the soil. Studies on the biochar dynamics in saline soils should advance mainly based on the evaluation of different feedstocks and pyrolysis temperatures, considering the divergence and diversities of each soil.

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4. CHAPTER III: NUTRITIONAL, BIOMETRIC, AND ENZYMATIC RESPONSE OF BRAZILIAN QUINOA (*Chenopodium quinoa* Willd.) CULTIVATED UNDER SALINE SOILS, HIGH TEMPERATURE, AND APPLICATION OF RICE HUSK BIOCHAR

Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a species with high potential for human and animal nutrition. In Brazil, its cultivation is limited, and research has been carried out to adapt this crop to the country's different climatic conditions. As it is a facultative halophyte, quinoa has great potential for adaptation to the Brazilian semiarid region, as the semiarid region has low precipitation, soils with high salinity levels, and a population with food insecurity. Biochar is an organic material made from the slow pyrolysis of organic feedstocks, with the potential to reduce the harmful effects of high concentrations of salts and sodium. To evaluate quinoa adaptation under doses of rice husk biochar (RHB) in saline soils commonly found in the Brazilian semiarid, two experiments were set up in a greenhouse, with the quinoa genotype CPAC 09, developed by EMBRAPA Cerrados. The first one was carried out in the winter season and the other in the summer, in a 3x7 factorial, with three soils (classified as Cambisol, Fluvisol, and Planosol) and seven biochar doses (0, 10, 20, 40, 80, and 100 t ha^{-1}). To evaluate quinoa adaptation to saline soils under RHB application, biometric, nutritional, and enzymatic analyzes were carried out at the end of the experiment. In the winter cycle, the plants showed good development in all evaluated soils, with an increase in shoot fresh and dry weight with the addition of RHB. Biochar also promoted greater K uptake by plants, alleviating salt stress and mitigating the effects of Na on plants. In the summer cycle, plants under low RHB doses, especially in saline soils, were highly affected by high temperatures. With the increase in biochar doses, the plants showed a survival rate above 75% for Cambisol and 50% for Fluvisol. Planosol plants (low salinity) developed despite high temperatures, due to the high absorption of K in the highest RHB doses. The increase in K and decrease in Na favored the reduction of H_2O_2 in plant cells, due to the high activity of the ascorbate peroxidase (APX) enzyme. EMBRAPA's CPAC 09 genotype showed a high potential for adaptation to saline environments. Therefore, quinoa cultivation in the semiarid region must be associated with months with moderate temperatures and application of RHB doses from 40 t ha^{-1} to mitigate the negative effects of salinity on plants.

Keywords: Potassium concentration. Phytoremediation. Sodium absorption. Quinoa CPAC 09 genotype.

CHAPTER III: RESPOSTAS NUTRICIONAIS, BIOMÉTRICAS E ENZIMÁTICAS DE QUINOA (*Chenopodium quinoa* Willd.) BRASILEIRA CULTIVADA SOB SOLOS SALINOS, ALTAS TEMPERATURAS E APLICAÇÃO DE BIOCHAR DE CASCA DE ARROZ

Resumo

A quinoa (*Chenopodium quinoa* Willd.), é uma espécie com alto potencial para nutrição humana e animal. No Brasil, seu cultivo é limitado e pesquisas vêm sendo desenvolvidas para adequação desta cultura às diversas condições climáticas do país. Por ser uma halófita facultativa, a quinoa possui grande potencial de adaptação ao semiárido brasileiro, por ser uma região com baixa precipitação, solos com altos índices de salinidade e população com insegurança alimentar. O biochar é um material orgânico feito a partir da pirólise lenta de biomassas vegetais, com potencial de redução dos efeitos deletérios das altas concentrações de sais e sódio nos solos. Para avaliar a adaptação da quinoa sob efeito de doses de biochar de casca de arroz em solos salinos comumente encontrados no semiárido brasileiro, foram montados dois experimentos em casa de vegetação, com o genótipo de quinoa CPAC 09, desenvolvido pela EMBRAPA Cerrados. O primeiro foi no período de inverno e o outro no verão, em fatorial de 3x7, sendo três solos (classificados como Cambissolo, Neossolo Flúvico e Planossolo) e sete doses de biochar (0, 10, 20, 40, 80 e 100 t ha^{-1}). Para avaliar a adaptação da quinoa em solos salinos sob efeito de doses do biochar, foram feitas análises biométricas, nutricionais e enzimáticas ao final do experimento. No ciclo de inverno, as plantas apresentaram um bom desenvolvimento em todos os solos avaliados, com aumento no peso fresco e seco com a adição das doses de biochar. O biochar também promoveu uma maior absorção de K pelas plantas, aliviando o estresse salino e atenuando os efeitos do Na. No ciclo de verão, as plantas sob baixas doses de biochar, principalmente nos solos salinos, foram altamente afetadas pelas altas temperaturas. Com o aumento das doses de biochar, as plantas apresentaram taxa de sobrevivência acima de 75% para o Cambissolo e de 50% para o Neossolo Flúvico. As plantas do Planossolo (baixa salinidade) se desenvolveram apesar das altas temperaturas, devido à alta absorção de K nas doses mais altas de biochar. O aumento no K e diminuição no Na favoreceu a redução de H_2O_2 nas células vegetais, a partir da alta atividade da enzima ascorbato peroxidade (APX). O genótipo CPAC 09 da EMBRAPA apresentou um alto potencial de adaptação a ambientes salinos. Assim, o cultivo da quinoa no semiárido deve ser associado a meses com temperaturas amenas e aplicação de doses de biochar a partir de 40 t ha^{-1} para atenuar os efeitos negativas da salinidade nas plantas.

Palavras-chave: Concentração de potássio. Fitorremediação. Absorção de sódio. Genótipo quinoa CPAC 09.

4.1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a halophyte species considered by FAO (2011) to be one of the promising crops for mitigating hunger in the world. Because it has high nutritional value and contains all the essential aminoacids for the human diet, quinoa has been gaining prominence in research, mainly because it is a tolerant species to adverse conditions such as drought, salinity, low temperatures, and can also survive in latitudes with high temperatures (BHARGAVA; SHUKLA; OHRI, 2006; BAZILE et al., 2016; BAZILE; JACOBSEN; VERNIAU, 2016; BECKER et al., 2017; GARCIA-PARRA et al., 2020).

Depending on the genotype, quinoa tolerates extreme environments such as 200 mm of precipitation in sand soil associated with salinity of 40 dS m^{-1} (or even more) in some varieties. Quinoa can develop and be used as a tool in the phytoremediation of salt affected soils, having a high potential for use in regions with water scarcity and advancing salinization, especially in areas where other crops cannot survive (JACOBSEN; MU-JICA; JENSEN, 2003; RUIZ et al., 2016, IQBAL et al., 2019). In saline and sodic soils, quinoa has adaptation and survival mechanisms such as osmotic adjustment, osmoprotection, Na^+ storage and transportation, ROS tolerance, K^+ retention, and stomatal control (ADOLF; JACOBSEN; SHABALA, 2013; RUIZ et al., 2016).

As it is a crop of high altitudes and cold climates, quinoa has limitations for cultivation in environments with high temperatures (TOVAR et al., 2020). Although surviving for approximately 4 hours at temperatures around -8°C, studies show that above 32°C quinoa can suffer physiological damage that affects its development (JACOBSEN; MUJICA; JENSEN, 2003; TOVAR et al., 2020). Regardless of these limitations, research states that some quinoa genotypes have high plasticity to adapt to environments with high temperatures and, despite the reduction in pollen viability at temperatures of 40°C, there were no changes in terms of quinoa productivity and development under this temperature according to HINOJOSA; MATANGUIHAN; MURPHY (2019).

Thus, this work aims to evaluate nutritional, biometric, and enzymatic parameters of quinoa, in two periods of the year (summer and winter in Northeast Brazil), in saline-sodic soils commonly found in the Brazilian semiarid region.

4.2. Material and methods

The experiments were carried out in a greenhouse at the Agronomic Institute of Pernambuco (IPA), in Recife (PE), Brazil, in the months of Mar/Aug 2022 (Winter) and Nov/Feb 2022/23 (Summer). The genotype CPAC 09 of quinoa (*Chenopodium quinoa* Willd.) from EMBRAPA Cerrados was selected, which was cultivated under application of increasing biochar doses, in three soils, one non-saline and two saline-sodic soils, in two quinoa cycles.

4.2.1. Soils selection

Three soils commonly found in the semiarid region of Pernambuco, Brazil, were selected, according to soil type and salinity levels. The soils were collected in the cities of Cabrobó-PE, Parnamirim-PE, and Caruaru-PE, and classified as Cambisol, Fluvisol, and Planosol, respectively. Soil samples were collected in the 0-20 cm layer, air-dried, grinded, and sieved through 2 mm mesh to characterization.

4.2.2. Soils characterization

To characterize the soils, physical and chemical analysis were carried out (Table 9). Physical properties evaluated were texture, by analysis of particle size composition and water dispersed clay, using the pipette method as proposed by Ruiz (2005); particle density, using the volumetric method; and bulk density using the clod method (EMBRAPA, 2017). With the results of total clay and water dispersed clay, the degree of soil flocculation was calculated; and total porosity was estimated by bulk and particle densities values.

For chemical characterization we analyzed soil pH in water (1:2.5); pH, soil electrical conductivity (EC_e), soluble cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and Cl^- in the saturated paste extract; exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) by the ammonium acetate method; and potential acidity by the calcium acetate method. K^+ and Na^+ were measured by flame emission photometry, Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometry, Cl^- by titration from the reaction of potassium chromate with silver nitrate, and potential acidity by titration from the sodium hydroxide. The methods adopted were described by EMBRAPA (2017) and USSL STAFF (1954). Base saturation (V), exchangeable sodium percentage (ESP), cation exchange capacity (CEC), and sodium adsorption ratio (SAR) were calculated (Table 9).

Table 9 – Chemical and physical characterization of three soils from Brazil Semiarid

Attribute	Soil		
	Cambisol	Fluvisol	Planosol
		Physical attributes	
Total sand (g kg^{-1})	592.0	349.03	714.89
Coarse sand (g kg^{-1})	25.29	95.15	471.2
Fine sand (g kg^{-1})	566.7	253.9	243.7
Silt (g kg^{-1})	344.36	468.53	222.33
Clay (g kg^{-1})	63.64	182.43	62.77
WDC (g kg^{-1})	82.28	59.26	70.91
BD (g cm^{-3})	1.51	1.60	1.71
PD (g cm^{-3})	2.57	2.58	2.50
TP (%)	41.24	37.98	31.60
Texture	Sandy Loam	Loam	Loamy Sandy
		Chemical attributes	
pH (H_20)	8.09	6.35	5.68
pH (EPS)	8.12	7.58	6.35
ECe (dS m^{-1})	30.99	22.59	0.88
TOC (g kg^{-1})			
		Soluble cations	
Ca^{2+} ($mmol_c$ L^{-1})	40.08	32.83	1.84
Mg^{2+} ($mmol_c$ L^{-1})	46.35	55.68	6.25
Na^{+} ($mmol_c$ L^{-1})	152.24	118.65	3.03
K^{+} ($mmol_c$ L^{-1})	1.67	1.02	2.30
Cl^{-} ($mmol_c$ L^{-1})			
SAR ($mmol_c$ $L^{-1})^{-0.5}$	23.26	18.73	1.50
		Exchangeable cations	
Ca^{2+} ($cmol_c$ kg^{-1})	25.16	7.48	0.44
Mg^{2+} ($cmol_c$ kg^{-1})	6.63	7.49	0.93
Na^{+} ($cmol_c$ kg^{-1})	2.09	1.70	0.40
K^{+} ($cmol_c$ kg^{-1})	0.30	0.28	0.42
H + Al ($cmol_c$ kg^{-1})	0.23	0.87	2.78
CEC ($cmol_c$ kg^{-1})	34.42	17.82	4.97
V (%)	99.33	95.12	44.02
ESP (%)	6.07	9.54	8.04

WDC: Water dispersible clay; BD: Bulk density; PD: Particle density; TP: Total porosity; ECe: Soil electrical conductivity, TOC: Total organic carbon; SAR: Sodium adsorption ratio; CEC: Cation exchangeable capacity; V: Base saturation; ESP: Exchangeable sodium percentage.

4.2.3. Biochar characterization

The biochar feedstock was rice husk (RHB), and the pyrolysis temperature was 400 °C. To characterize the biochar (Table 10), it was grinded and sieved through 0.200 mm mesh. Ca, Mg, K, P, and Cl were determined by portable-XRF, S1 TITAN model 800. Organic carbon, hydrogen, and nitrogen of the biochar were determined by dry combustion, using a CHN628 elemental analyzer (LECO), enabling the calculation of C/N ratio. Biochar pH and EC were determined at a ratio of 1:10, according to Singh et al. (2017).

Table 10 – Chemical characterization of rice husk biochar (RHB)

RHB attributes	
pH	7.25
EC (dS m^{-1})	0.22
C (%, w/w)	47.67
N (%, w/w)	1.40
H (%, w/w)	6.32
C/N	34.05
Ca (g kg^{-1})	1.52
Mg (g kg^{-1})	0.71
Na (g kg^{-1})	-
K (g kg^{-1})	5.53
P (g kg^{-1})	1.33
Cl (g kg^{-1})	0.41

4.2.4. Tap water characterization

In both experimental cycles, tap water (Table 11) with high salinity was add to the pots to simulating the irrigation conditions commonly found in Brazilian semiarid, characterized by some saline-sodic soils with available water of poor chemical quality for irrigation, generally saline, sodic, or saline-sodic.

During the first cycle, 6.9 liters of water per pot were added to the soil after transplanting until flowering began. In the second cycle, due to the high temperatures, a total of 9.05 liters were

added in the pots. After two quinoa cycles, approximately 48.9 mg kg^{-1} of Ca^{2+}, 44.2 mg kg^{-1} of Mg^{2+}, 145.52 mg kg^{-1} of Na^{+}, 13.05 mg kg^{-1} of K^{+} and 403.8 mg kg^{-1} of Cl^{-} were added to the soils through irrigation.

Table 11 – Tap water characterization used during quinoa cycles

Tap water attributes	
Color	5
Turbidity	0.9
EC (dS m^{-1})	1.47
pH	7.80
Total dissolved solids	944.00
Hydroxide alkalinity	0.00
Carbonate alkalinity	0.00
Bicarbonate alkalinity	62.00
Total alkalinity	62.00
Total hardness	87.02
Ca^{2+} (mg L^{-1})	45.69
Mg^{2+} (mg L^{-1})	41.33
Na^{+} (mg L^{-1})	136.00
K^{+} (mg L^{-1})	12.20
Cl^{-} (mg L^{-1})	377.41
SO_4^{2-} (mg L^{-1})	31.17
*Water classification	C3S1

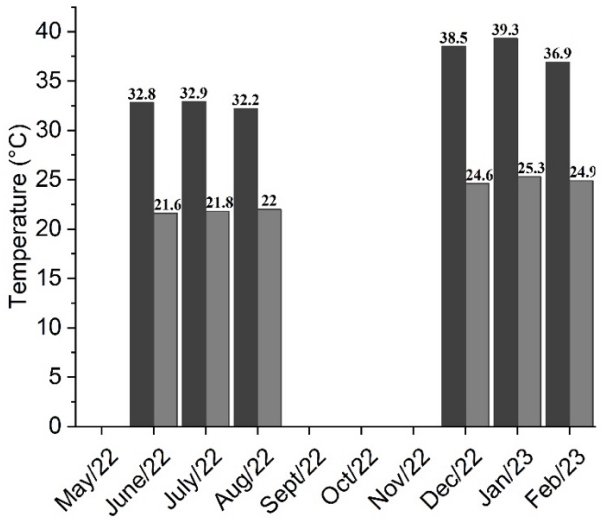
*Water classification according to USSL Staff (1954).

4.2.5. Average temperature in the quinoa cycles

Temperature inside the greenhouse was constantly measured, with minimum and maximum temperatures being evaluated daily. After the daily measurements, a temperature variation graph was created in the winter and summer seasons in this region of Brazil during the three months of quinoa evaluation, in each cycle, in the greenhouse (June-August 2022 and December-February 2022/ 2023) totaling six months of evaluation. The months from June to August 2022 were

characterized as those with lower temperatures and from December to February, the months with higher temperatures (Figure 10).

Figure 10 – Maximum and minimum temperatures during first quinoa cycle (June-Aug – Northeast of Brazil winter) and second quinoa cycle (Dec-Feb – Northeast of Brazil summer)



4.2.6. Experimental setup and design

The experiment consisted of a 3x7 factorial with three soil types and seven biochar doses (0, 10, 20, 40, 60, 80, and 100 t ha^{-1}), in a randomized block design, with four replications, totaling 84 experimental units. The experiment was conducted in two quinoa cycles, the second cycle being cultivated in the same pots as in the first cycle, respecting the treatments configuration.

The experiment was conducted in 20 L pots and 15 kg of soil was placed in each pot. Biochar was previously grinded until the particle size of 0.300-0.850 mm according to Liu et al. (2016), being classified as a medium texture biochar. Biochar was incorporated into the soil total mass, according to each dose.

Sowing was carried out in trays, where the plants were adapted to salinity conditions. For seedlings adaptation to the salinity, saline water applications were carried out from the 10^{th} day after sowing. Water was prepared by a mixture of the salts NaCl, $Ca(NO_3)_2$, $CaCl_2$ with Ca/Na in the proportion of 3/1. For five days, water with EC_w of 2 dS m^{-1} was applied; another five days with EC_w of 4 dS m^{-1}; and, finally, water with an EC_w of 8 dS m^{-1} was applied in the last four days. Transplanting was carried out after the opening of the 6^{th} definitive leaf.

After implementing the experiment, the plants were irrigated with saline tap water (table 3). The experiment was conducted over a period of six months, with two cycles of 90 days each, a period necessary for the plants flowering.

4.2.7. Plant sample preparation

The plants were evaluated by biometric measurements such as height and stem diameter. Completely expanded young leaves from the plants middle third were collected approximately 80 days after germination, during the flowering period, for enzymatic evaluations. Leaves were macerated in liquid nitrogen and kept in ultrafreezer at -80 °C to preserve their components. Aerial part (shoot) of the plants was collected 90 days after sowing, in both cycles, dried in a forced circulation oven at 65 °C, and grinded for nutritional analysis.

4.2.8. Biometrical analysis

Each plant in the experimental units was measured for shoot height and stem diameter (at 2 cm from the ground). Measurements were taken 70 days after transplanting.

When the plants reached approximately 90 days, shoots were collected and weighed to determine shoot fresh weigh (SFW) and shoot dry weight (SDW).

4.2.9. Plant nutritional analysis

Dry plant samples were ground and subject to digestion by HNO_3 1 mol L^{-1} to determine N, Ca, Mg, Na, K, P, and Cl. Ca and Mg were measured by atomic absorption spectrophotometry, Na and K by flame photometry, and P by molybdenum blue spectrophotometry. Total N content was determined by titration by Kjeldahl method and, Cl was determined by titration with silver nitrate according to EMBRAPA (2009).

Plants nutritional composition and soil chemical data were used to estimate the phytoextraction potential to evaluate quinoa's salts phytoextraction in saline-sodic soils. Phytoextraction data were expressed in kg ha^{-1} and in g $plant^{-1}$ to provide discussion between the ways of evaluating these data in controlled greenhouse environments with plants grown in pots.

4.2.10. Enzymatic analysis

For the biochemical evaluation were determined: lipidic peroxidation (MDA) by method described by Heath and Packer (1968) and peroxide (H_2O_2) determined by Alexieva et al. (2001). The determination of plant antioxidant enzymes was carried out: superoxide dismutase (SOD) using the method described by Giannopolitis and Ries (1977); Catalase activity (CAT) determined by the consumption of H_2O_2 and decay of absorbance at 240 nm according to Havir and Michale (1987) and modified by Azevedo et al. (1998), and ascorbate peroxidase (APX), using the monobasic potassium phosphate buffer and determined by the spectrophotometer at the length of 290 nm wave (NAKANO; ASADA, 1981).

4.2.11. Data analysis

The results obtained were initially subjected to normality tests (Shapiro-Wilk, $p < 0.05$) and homoscedasticity (Levene, $p > 0.05$). After these procedures, analysis of variance (ANOVA, $p<0.05$) was performed and the average values of the results were compared among soils using the Tukey test ($p<0.05$). Pearson's linear correlation was also performed between plant and soil attributes.

4.3. Results

4.3.1. Quinoa tropical winter cycle

4.3.1.1. Biometric analysis

For biometric parameters, the plants grown in Planosol obtained the highest averages, indicating a better quinoa development in this soil (Table 12). For plant height, the highest averages were observed in Cambisol and Planosol (88.43 and 113.29 cm respectively). There was a double interaction among biochar doses and soils for stem diameter (SD), where Planosol plants presented the highest averages. In Cambisol there was an increment in SD with increasing biochar doses, with values of 5.03 cm in the control dose (0 t ha^{-1}) and 5.90 cm in the last dose (100 t ha^{-1}).

There was also a double interaction for the shoot fresh weight (SFW), with the highest averages observed for plants in Planosol. Among the biochar doses, Cambisol plants showed a significant increase in SFW from 47.06 to 80.36 g $plant^{-1}$ between the control and the last dose. As for Fluvisol, there was an increase from 41.7 to 59.4 g $plant^{-1}$ between the first and last doses. For

Planosol plants, there was no significant difference in SFW among biochar doses, with a general average for plants grown in this soil of 70.92 g plant^{-1}.

In general, plants grown in Cambisol and Planosol showed a greater accumulation of shoot dry weight (SDW), with average of 19.80 and 19.24 g plant^{-1}. The lowest SDW average was observed in Fluvisol (15.83 g plant^{-1}). Analyzing the Tukey test at 5% probability, it is clear that the 40 t ha^{-1} biochar dose presented better potential for field application, as it does not differ significantly from the higher RHB doses, especially among the SD, SFW, and SDW parameters.

Table 12 – Biometric parameters in the first quinoa cycle (tropical winter) as function of soils and RHB doses. Plant height (H), stem diameter (SD), shoot fresh weight (SFW), and shoot dry weight (SDW)

Biochar Dose (t ha^{-1})	H				SD				SFW				SDW			
	cm				cm				g plant^{-1}				g plant^{-1}			
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	82.50	76.25	111.25	90.00	5.03Bb	4.78Ab	6.20Aa	5.34	47.06Bb	41.7ABb	71.97Aa	53.60	15.58	14.99	18.57	16.38B
10	86.50	81.50	113.50	93.83	6.00Aa	4.44Ab	6.65Aa	5.70	68.72Aa	41.16Bb	70.87Aa	60.25	18.84	14.53	19.00	17.50AB
20	93.50	74.25	105.2	91.00	5.63ABb	5.03Ab	6.60Aa	5.75	68.83Aa	43.5ABb	70.38Aa	60.91	19.4	14.66	19.00	17.70AB
40	91.25	84.25	106.00	93.83	6.46Aa	5.14Ab	5.95Aa	5.85	70.12Aa	50.6ABb	73.51Aa	64.75	21.05	16.06	20.22	19.11A
60	90.00	84.00	115.75	96.58	6.15Aa	5.11Ab	6.56Aa	5.94	78.63Aa	57.0ABb	72.29Aa	69.31	21.90	16.81	20.32	19.67A
80	87.75	84.25	117.75	96.58	6.01 Ab	5.15Ac	6.80Aa	5.98	79.41Aa	59.4Ab	73.62Aa	70.81	20.58	17.45	21.12	19.71A
100	87.50	82.50	123.50	97.83	5.90ABb	5.02Ac	6.79Aa	5.90	80.36Aa	56.46ABb	76.3Aa	71.04	21.14	16.35	16.44	17.9AB
Mean	88.43b	81.00c	113.29a		5.88	4.95	6.51		70.45	49.99	70.92		19.80a	15.83b	19.24a	
ANOVA	F				F				F				F			
Soil	65.82***				89.92***				63.73***				32.6***			
Dose	0.87^{NS}				3.09*				7.43***				4.42***			
Soil x Dose	0.74^{NS}				2.37*				2.05*				0.9^{NS}			
CV (%)	11.7				7.55				12.88				11.28			

Uppercase letters compare means in the column, and lowercase letters compare means in the line. Means compared using the Tukey test at 5% probability. ***, **, *, and ^{NS} are equal to 0.1, 1, 5% of probability and non-significant, respectively.

4.3.1.2. Nutritional analysis

According to nutritional analysis (Table 13), plants in Cambisol and Fluvisol accumulated more salts in their biomass than in Planosol. Ca concentration in shoot biomass was 11.15, 11.47, and 9.42 g kg^{-1} in Cambisol, Fluvisol, and Planosol, respectively, with no significant difference among the biochar doses. The same occurred for Mg, with concentrations of 16.26, 17.4, and 12.5 g kg^{-1} among the plants on these three soils, respectively.

There was a double interaction among biochar doses and soils for the monovalent ions (Na and K). For Na, the increment in biochar doses reduced it concentration in quinoa shoot in all evaluated soils. In Cambisol, the Na concentration in shoot ranged from 6.43 to 1.57 g kg^{-1} between the control and 100 t ha^{-1} doses. For Fluvisol, the averages went from 4.42 to 1.89 g kg^{-1} between the first and last treatments.

For K, there was an increment in its concentration after biochar application, mainly in Fluvisol plants (69.2 to 89.75 g kg^{-1} between the first and last treatments). It is possible to observe, in figure 11, that the proportion of Na in relation to K decreases with the RHB addition. The graphs in figure 11 were constructed from the sum of Na and K concentrations (g kg^{-1}) in quinoa shoot, where this sum was considered to be 100%. For Cambisol plants, the relative proportion between Na and K went from 7.4 and 92.6% at a dose of 0 t ha^{-1} to 1.7 and 98.3% at a dose of 100 t ha^{-1}. For Fluvisol plants, this proportion changed from 5.2 and 94.8% to 2.3 and 97.7%. In Planosol plants, Na and K proportions changed from 1.5 and 98.5% to 1.2 and 98.8% under the same RHB doses.

Regarding N, there was an interaction among soils and biochar doses, with a significant reduction in its concentration in plants grown in Cambisol and Planosol (Table 13). The highest averages were obtained in Cambisol and Fluvisol plants (32.4 and 31.9 t ha^{-1}, respectively) and the lowest average in Planosol plants (22.4 t ha^{-1}).

Like N, P was also higher in Cambisol and Fluvisol plants (12.11 and 13.9 g kg^{-1}) than in Planosol plants (8.9 g kg^{-1}). Considering only the biochar doses, P showed an increase between 0 and 100 t ha^{-1} treatments, with averages varying from 9.9 to 12.42 g kg^{-1}. As for Cl, there was no significant difference between both treatments (soil and biochar doses).

For nutritional parameters, the 40 t ha^{-1} biochar dose was also considered the best one as it does not differ significantly from higher RHB doses, especially among the N, Na, and K.

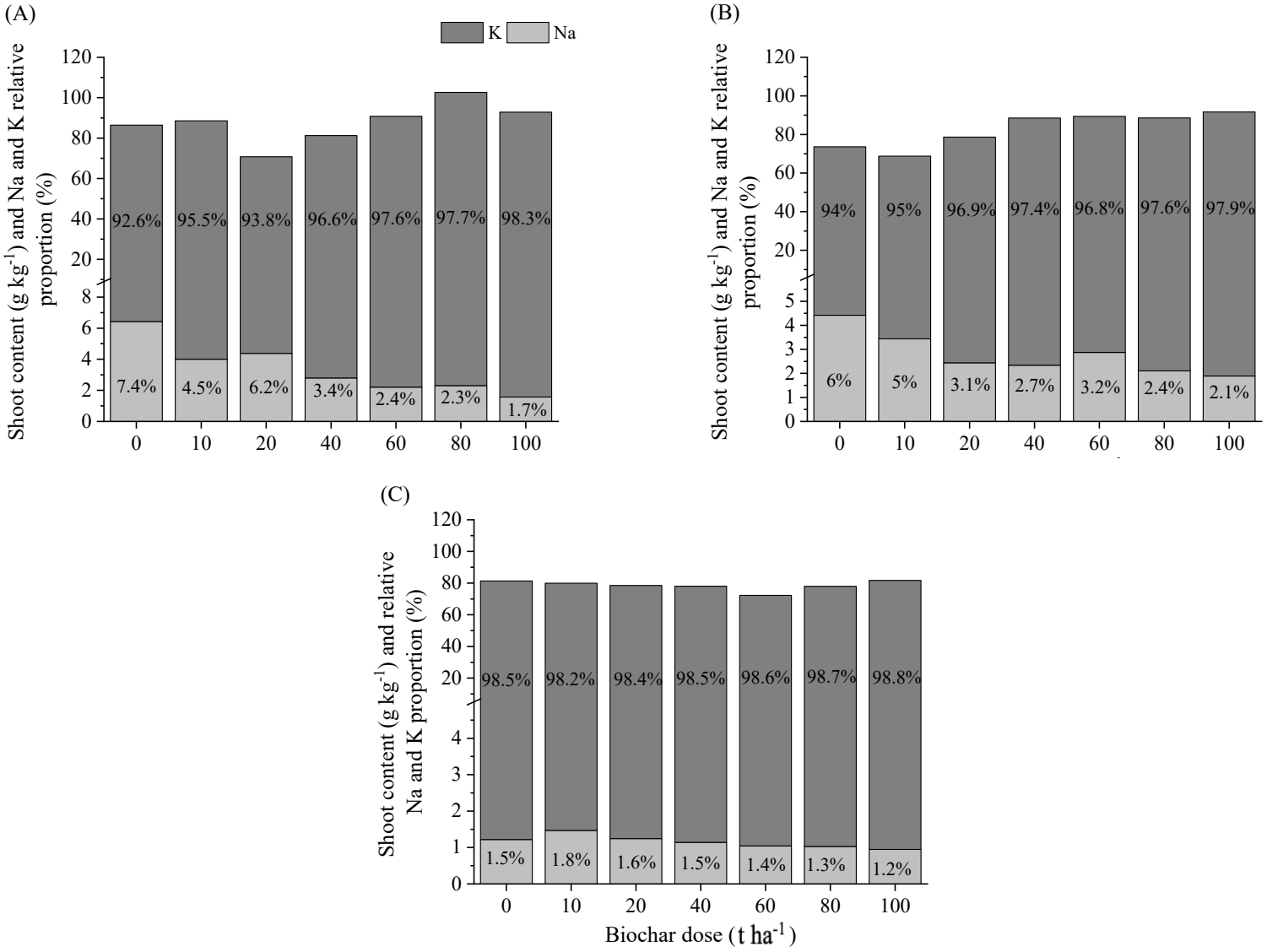
Table 13 – Nutritional parameters for the first quinoa cycle (tropical winter) as function of soils and RHB doses. Mean values for nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K)

Biochar dose (t ha^{-1})	N				P				Ca				Mg			
	g kg^{-1}															
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	34.06ABa	32.17Aa	25.71Ab	30.64	9.62	12.41	7.67	9.90C	11.38	12.94	9.65	11.32	18.85	18.93	14.23	17.34A
10	36.26Aa	30.70Ab	22.00ABc	29.66	11.22	13.74	8.14	11.03BC	11.41	11.44	9.32	10.72	17.05	18.03	13.45	16.18ABC
20	28.67Ca	30.35Aa	23.60ABb	27.54	11.54	14.62	8.35	11.50AB	11.75	11.54	10.29	11.19	17.40	18.70	13.28	16.46AB
40	31.90ABCa	34.91Aa	22.90ABb	29.93	13.40	14.39	8.78	12.19AB	11.68	11.67	9.30	10.88	16.40	17.65	11.90	15.3ABCD
60	31.40BCa	31.03Aa	20.00Bb	27.48	12.62	14.24	9.41	12.09AB	10.81	11.69	8.69	10.40	16.03	17.63	11.50	15.05BCD
80	33.00ABCa	32.29Aa	20.51Bb	28.61	13.21	13.83	9.65	12.23AB	10.56	10.47	9.24	10.09	14.98	16.20	11.20	14.13CD
100	31.40BCa	32.01Aa	22.21ABb	28.54	13.16	13.82	10.29	12.42A	10.43	10.53	9.44	10.13	13.13	14.68	11.90	13.23D
Mean	32.4	31.9	22.4		12.11b	13.9a	8.9c		11.15a	11.47a	9.42b		16.26a	17.4a	12.5b	
ANOVA	F				F				F				F			
Soil	189.67***				168.11***				24.73***				56.03***			
Dose	3.64***				9.24***				2.11				7.23***			
Soil x Dose	3.06**				1.63^{NS}				0.71^{NS}				0.58^{NS}			
CV (%)	7.57				8.84				10.99				11.80			

	Na				K				Cl			
	g kg^{-1}											
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	6.43Aa	4.42Ab	1.22Ac	4.02	80.00BCa	69.20CBa	80.13Aa	76.44	37.75	40.75	39.75	39.42
10	4.00BCa	3.44ABa	1.47Ab	2.97	84.5ABCa	65.30Cb	78.45Aab	76.12	36.00	33.00	38.75	35.92
20	4.38Ba	2.44BCb	1.25Ac	2.69	66.40Ca	76.20ABCa	77.25Aa	73.28	34.75	35.75	39.00	36.50
40	2.79CDa	2.34BCa	1.14Ab	2.09	78.4BCa	86.20ABa	76.88Aa	80.51	34.50	39.00	33.50	35.67
60	2.20Dab	2.87BCa	1.04Ab	2.04	88.6ABa	86.50ABab	71.20Ab	82.11	36.00	40.75	38.50	38.42
80	2.31Da	2.1BCab	1.03Ab	1.81	100.33Aa	86.50ABab	76.95Ab	87.92	37.75	35.25	37.00	36.67
100	1.57Da	1.89Ca	0.95Aa	1.47	91.25ABa	89.75Aa	80.75Aa	87.25	35.00	40.50	34.25	36.58
Mean	3.38	2.79	1.16		84.22	69.96	77.37		35.96	37.85	37.25	
ANOVA	F				F				F			
Soil	78.44***				4.12*				1.42^{NS}			
Dose	18.84***				4.69***				1.23^{NS}			
Soil x Dose	5.85***				3.06**				1.32^{NS}			
CV (%)	28.15				11.21				11.83			

Uppercase letters compare means in the column, and lowercase letters compare means in the line. Means compared using the Tukey test at 5% probability. ***, **, * are equal to 0.1, 1, and ^{NS} are equal to 0.1, 1, 5% of probability and non-significant, respectively.

Figure 11 – Na and K relative proportion in quinoa shoot in the first cycle (tropical winter). (A) Plants in Cambisol; (B) Plants in Fluvisol;(C) Plants in Planosol



Observing table 14, which considers the Person`s correlation among soil chemical attributes and biometric and nutritional plant parameters, it is possible to state that, for plants grown in Cambisol, under RHB, the attributes related to soil salinity and sodicity such as pH, EC, SAR, and ESP were negatively correlate with the SFW, SDW, and P absorption by plants. In general, the correlation among soil soluble and exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) with the same cations absorbed by plants was positive. For exchangeable K, it is possible to note that the correlation with sodium absorption by quinoa plants is negative, inferring that the more the plant absorbs K, the less Na is accumulated in the shoot biomass.

Table 14 – Person`s linear correlation among soil chemical attributes, biometric and nutritional parameters in quinoa first cycle

Soil attribute	Biometric parameters				Nutritional parameters						
	H	SD	FW	DW	N	P	Ca	Mg	Na	K	Cl
					Cambisol						
pH	-0.10	***-0.38***	***-0.69***	***-0.61***	0.32	**-0.62**	0.20	***0.63***	***0.71***	***-0.45***	0.05
pHes	-0.03	0.23	0.24	0.08	-0.26	0.36	***0.39***	-0.16	-0.17	0.28	0.24
EC	-0.03	-0.35	***-0.57***	***-0.43***	0.16	***-0.42***	0.01	***0.54***	***0.50***	***-0.54***	-0.30
Soluble cation											
Ca	-0.13	-0.06	-0.05	-0.02	0.12	-0.01	-0.23	0.09	0.02	-0.16	***-0.40***
Mg	-0.05	-0.32	***-0.46***	-0.37	0.15	-0.35	-0.11	***0.42***	0.37	***-0.49***	-0.30
Na	-0.03	-0.35	***-0.59***	***-0.43***	0.15	***-0.45***	0.00	***0.52***	***0.51***	***-0.55***	-0.31
K	-0.05	-0.04	0.07	0.09	0.03	0.18	***-0.38***	-0.27	-0.24	0.14	-0.29
SAR	-0.08	***-0.41***	***-0.68***	***-0.52***	0.17	***-0.53***	0.05	***0.54***	***0.62***	***-0.55***	-0.26
Cl	-0.19	-0.26	-0.32	-0.32	0.21	-0.27	-0.16	0.30	0.33	-0.29	-0.29
Exchangeable cation											
Ca	0.07	-0.18	***-0.57***	-0.36	0.16	***-0.50***	0.21	***0.45***	***0.69***	***-0.55***	-0.10
Mg	-0.02	-0.36	***-0.48***	***-0.38***	0.17	***-0.61***	0.05	***0.40***	***0.78***	-0.36	0.10
Na	-0.04	***-0.40***	***-0.54***	***-0.51***	0.19	***-0.60***	0.31	***0.46***	***0.72***	***-0.41***	0.16
K	0.02	-0.11	0.14	0.16	-0.10	0.21	-0.27	-0.37	***-0.46***	0.12	-0.30
SB	0.04	-0.27	***-0.59***	***-0.41***	0.18	***-0.58***	0.19	***0.46***	***0.76***	***-0.52***	-0.03
CEC	0.03	-0.26	***-0.59***	***-0.41***	0.19	***-0.58***	0.20	***0.46***	***0.76***	***-0.52***	-0.03
ESP	-0.06	***-0.37***	***-0.41***	***-0.44***	0.14	***-0.51***	0.32	0.37	***0.58***	-0.29	0.22
V	0.07	-0.35	***-0.46***	-0.30	0.04	***-0.45***	0.09	***0.50***	***0.74***	***-0.47***	-0.02
					Fluvisol						
pH	-0.04	-0.34	***-0.58***	-0.33	-0.11	***-0.43***	0.36	0.09	***0.47***	***-0.64***	-0.12
pHes	0.02	-0.24	-0.29	-0.29	-0.07	0.15	0.20	0.06	0.15	***-0.38***	-0.26
EC	0.00	-0.01	-0.27	-0.25	***0.42***	0.13	-0.02	0.14	0.22	-0.08	-0.13
Soluble cations											
Ca	0.09	0.06	-0.23	-0.20	***0.47***	0.10	-0.06	0.12	0.21	-0.08	-0.18
Mg	0.06	0.18	-0.13	-0.04	***0.41***	0.13	0.05	0.17	0.13	0.02	-0.05
Na	-0.01	0.07	-0.36	-0.30	0.28	0.15	-0.01	0.18	0.18	-0.13	-0.24

K	0.14	-0.01	0.02	0.06	0.36	0.21	-0.33	-0.05	-0.07	0.31	-0.20
SAR	0.02	-0.20	***-0.47***	***-0.38***	-0.05	-0.04	0.13	0.29	0.22	***-0.46***	***-0.47***
Cl	0.09	0.08	-0.07	-0.08	***0.41***	0.14	-0.17	-0.06	0.09	0.07	-0.12
Exchangeable cations											
Ca	-0.08	-0.19	-0.26	-0.01	***-0.39***	-0.37	0.25	0.12	0.22	***-0.39***	0.04
Mg	0.01	-0.32	0.14	-0.12	0.05	0.25	***-0.38***	-0.16	-0.06	0.13	-0.14
Na	-0.12	-0.08	-0.27	-0.27	0.05	0.23	0.07	0.20	0.10	-0.21	-0.27
K	0.26	0.25	***0.38***	0.30	0.10	0.19	-0.15	-0.17	***-0.65***	***0.45***	-0.25
SB	-0.05	***-0.40***	-0.08	-0.14	-0.22	0.06	-0.15	0.00	0.09	-0.18	-0.19
H + Al	0.15	0.29	***0.51***	0.34	-0.15	0.18	-0.36	***-0.46***	***-0.65***	***0.65***	0.16
CEC	-0.01	-0.30	0.06	-0.04	-0.25	0.10	-0.24	-0.12	-0.09	0.01	-0.14
ESP	-0.12	0.10	-0.31	-0.25	0.19	0.17	0.21	0.26	0.14	-0.21	-0.21
V	-0.17	***-0.38***	***-0.53***	-0.37	0.11	-0.17	0.33	***0.45***	***0.67***	***-0.68***	-0.18
					Planosol						
pH	0.04	0.31	0.13	0.17	-0.19	***0.54***	0.08	***-0.48***	***-0.54***	0.02	-0.14
pHes	-0.08	0.16	0.19	-0.14	0.02	0.31	0.32	-0.15	-0.11	***0.43***	-0.04
EC	-0.03	0.00	-0.03	0.01	***0.38***	-0.25	0.26	0.27	0.20	0.02	-0.03
Soluble cations											
Ca	-0.15	-0.06	-0.08	-0.08	***0.45***	-0.32	0.28	0.37	0.24	0.03	0.15
Mg	-0.07	0.03	-0.19	-0.17	***0.40***	-0.23	0.28	0.31	0.05	0.19	0.09
Na	-0.04	-0.06	0.05	-0.15	0.37	-0.22	0.17	***0.41***	0.20	0.09	0.08
K	0.34	***0.38***	0.24	0.04	-0.01	***0.50***	-0.02	-0.05	-0.11	0.09	-0.08
SAR	-0.04	-0.06	0.28	0.01	0.23	-0.14	0.06	0.33	0.36	-0.09	0.03
Cl	-0.10	-0.26	0.08	-0.07	***0.45***	-0.18	0.21	***0.45***	0.32	0.04	0.10
Exchangeable cations											
Ca	0.15	0.22	0.10	0.21	-0.35	***0.56***	-0.07	**-0.42**	**-0.58**	-0.07	-0.06
Mg	0.33	0.31	0.06	-0.01	-0.19	0.35	-0.10	-0.18	***-0.47***	-0.05	-0.17
Na	0.17	0.12	0.24	0.36	***-0.65***	***0.62***	-0.34	***-0.59***	***-0.51***	-0.11	-0.24
K	0.35	0.37	0.20	0.27	***-0.45***	***0.72***	-0.19	***-0.58***	***-0.54***	-0.04	-0.35
SB	0.30	0.31	0.16	0.23	***-0.47***	***0.63***	-0.21	***-0.51***	***-0.61***	-0.08	-0.22
H + Al	0.26	0.25	0.05	0.20	***-0.47***	***0.69***	-0.26	***-0.69***	***-0.49***	-0.11	-0.29
CEC	0.32	0.32	0.13	0.24	***-0.53***	***0.75***	-0.26	***-0.67***	***-0.63***	-0.11	-0.29
ESP	0.07	0.01	0.29	***0.39***	***-0.65***	***0.48***	-0.34	***-0.48***	-0.35	-0.13	-0.21
V	-0.09	-0.07	0.08	-0.08	0.24	-0.35	0.18	***0.46***	0.16	0.06	0.17

H – Shoot height; SFW – Shoot fresh weight; SDW – Shoot dry weight; pHes – pH in the paste extract; ECe – Soil electric conductivity; SAR – Sodium adsorption ratio; CEC – Cation exchange capacity; ESP – Exchangeable sodium percentage; V – Bases saturation. Numbers in bold are significant at $p < 0.05$.

In Fluvisol, the Person's correlation was also negative between pH and SFW, and SAR and SFW. The negative correlation between exchangeable K and Na absorption by the crop is also noted. The correlation is also negative between the soil potential acidity (H + Al) and the accumulation of Mg, Na, and K in the plant shoot. In Planosol, the negative correlation between the levels of K in the soil and Na in the plant also stands out.

4.3.1.3. Salt phytorremediation potential

In general, quinoa was able to phytoextract salts in the order of K > Cl > Mg > Ca > Na. Tables 15 and 16 show two different ways of expressing phytoextraction potential data. In table 15, the averages are expressed in kg ha^{-1}, where the calculation was based on the pots area where the plants were grown (0.07 m^2) and the data were extrapolated to hectare (10,000 m^2). In table 16, the data are expressed in g $plant^{-1}$, considering the real data without extrapolations.

According to table 15, there was a double interaction for the phytoextraction potential of K and Na. For K, the higher the biochar dose, the greater the phytoextraction in all soils evaluated. Among the soils, K phytoextraction was in the order of Cambisol > Planosol > Fluvisol. For Na, the phytoextraction potential was reduced with the RHB doses. The total quinoa phytoextraction potential (Ca + Mg + Na + K + Cl) for Cambisol was 425.33 kg ha^{-1}, for Fluvisol it was 336.16 kg $ha^{-1}$and for Planosol it was 376.29 kg ha^{-1}.

Similar trend is observed in table 16. The quinoa phytoextraction potential was 0.22, 0.18, and 0.18 g $plant^{-1}$ for Ca, 0.32, 0.27, 0.25 g $plant^{-1}$ for Mg, 0.05, 0.07, and 0.02 g $plant^{-1}$ for Na, 1.67, 1.26, and 1.49 g $plant^{-1}$ for K, 0.71, 0.60, 0.64 g $plant^{-1}$ for Cl in Cambisol, Fluvisol, and Planosol, respectively. Thus, the total salt phytoextraction potential by quinoa in Cambisol, Fluvisol and Planosol was 2.97, 2.38, and 2.58 g $plant^{-1}$, respectively.

4.3.1.4. Enzymatic analysis

By enzymatic evaluation (Table 17), lipid peroxidation (expressed in MDA) was higher in plants from Fluvisol and Cambisol (9.89 and 8.20 µmol g^{-1}) than in Planosol (7.70 µmol g^{-1}), and H_2O_2 was higher in Planosol and Fluvisol plants (1.20 and 1.13 µmol g^{-1}) than in Cambisol plants (1.02 µmol g^{-1}) according to table 17. For the enzyme superoxide dismutase (SOD), the highest activity occurred in Fluvisol plants (567.15 µmol g^{-1}) and for ascorbate peroxidase, the highest values were found in plants grown in Planosol (965.80 µmol g^{-1}). For catalase (CAT), there were no significant results despite the increasing trend between biochar doses and soils.

Table 15 – Salt phytorremediation potential during quinoa first cycle (tropical winter), in kg ha^{-1}, as function of soils and RHB doses. Mean values for phytoextraction of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K)

Biochar dose (t ha^{-1})	Ca				Mg				Na			
	kg ha^{-1}											
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	24.69	27.25	25.34	25.76	41.48	39.69	35.81	38.99	14.52Aa	9.07Ab	2.89Ac	8.83
10	30.44	23.57	25.10	26.37	45.35	37.19	36.29	39.61	10.66ABCa	6.98ABb	3.98Ab	7.21
20	32.28	23.93	27.65	27.95	48.09	38.75	35.72	40.85	12.25ABa	5.16ABb	3.35Ab	6.92
40	35.00	26.55	24.95	28.83	48.59	40.03	32.59	40.40	8.80BCDa	7.05ABa	3.30Ab	6.38
60	33.64	27.75	24.88	28.76	52.18	41.87	35.08	43.04	6.86CDa	6.86ABa	2.97Ab	5.56
80	30.42	25.82	25.71	27.32	43.52	39.89	33.24	38.89	6.77CDa	5.23ABab	3.42Ab	5.14
100	31.43	24.37	25.52	27.11	39.34	33.97	36.35	36.55	4.69Da	4.39Ba	3.06Aa	4.05
Mean	31.13a	25.60b	25.59b		45.51a	38.77b	35.01b		9.22	6.39	3.28	
ANOVA		F				F				F		
Soil		16.50***				19.99***				63.74***		
Dose		0.93^{NS}				1.21^{NS}				7.51***		
Soil x Dose		1.22^{NS}				0.83^{NS}				3.77***		
CV (%)		15.15				15.83				31.26		

	K				Cl			
	kg ha^{-1}							
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	175.78Ca	153.16ABa	197.49Aa	175.48	83.18	85.74	104.26	91.06
10	225.87BCa	134.06Bb	211.05Aa	190.33	95.96	67.85	104.52	89.44
20	181.33Ca	159.92ABa	207.52Aa	182.92	95.17	73.87	104.75	91.26
40	234.81BCa	195.49ABa	197.28Aa	209.19	103.00	88.74	95.54	95.76
60	274.89ABa	203.81Ab	203.54Ab	227.42	106.58	96.33	110.07	104.33
80	316.35Aa	210.4Ab	226.68Ab	251.17	106.30	87.16	123.57	105.68
100	272.42ABa	207.59Ab	213.85Ab	231.29	104.62	93.63	86.75	95.00
Mean	240.21	180.64	208.20		99.26a	84.76b	104.21a	
ANOVA		F				F		
Soil		25.17***				9.45***		
Dose		9.64**				1.68^{NS}		
Soil x Dose		2.69**				1.21^{NS}		
CV (%)		15.00				18.11		

Uppercase letters compare means in the column and lowercase letters compare means in the line. Means compared using the Tukey test at 5% probability. ***, **, and ^{NS} are equal to 0.1, 1% of probability and non-significant, respectively.

Table 16 – Salt phytorremediation potential during quinoa first cycle (tropical winter), in g planta^{-1}, as function of soils and RHB doses. Mean values for phytoextraction of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K)

Biochar dose (t ha^{-1})	Ca				Mg				Na			
	g plant^{-1}											
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	0.17	0.19	0.18	0.18	0.29	0.28	0.25	0.28	0.10Aa	0.06Ab	0.02Ac	0.06
10	0.22	0.17	0.18	0.19	0.32	0.26	0.26	0.28	0.08ABCa	0.05ABb	0.03Ab	0.05
20	0.23	0.17	0.20	0.20	0.34	0.27	0.25	0.29	0.09ABa	0.04ABb	0.02Ab	0.05
40	0.25	0.19	0.18	0.20	0.34	0.28	0.23	0.29	0.06BCa	0.05ABa	0.02Ab	0.04
60	0.24	0.20	0.18	0.20	0.35	0.30	0.25	0.30	0.05CDa	0.05ABa	0.02Ab	0.04
80	0.21	0.18	0.18	0.19	0.31	0.28	0.23	0.27	0.05CDa	0.04ABa	0.02Aa	0.04
100	0.22	0.17	0.18	0.19	0.28	0.24	0.26	0.26	0.03Da	0.03Ba	0.02Aa	0.03
Mean	0.22a	0.18b	0.18b		0.32a	0.27b	0.25b		0.07	0.05	0.02	
ANOVA		F				F				F		
Soil		15.8***				17.34***				66.60***		
Dose		0.89^{NS}				0.91^{NS}				7.33***		
Soil x Dose		1.15^{NS}				0.67^{NS}				4.11***		
CV		15.24				16.28				30.48		

	K				Cl			
	g plant^{-1}							
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	1.24Cab	1.08ABb	1.49Aa	1.27	0.59	0.61	0.74	0.64
10	1.60BCa	0.95Bb	1.49Aa	1.35	0.68	0.48	0.74	0.63
20	1.28Ca	1.13ABa	1.47Aa	1.29	0.67	0.52	0.74	0.65
40	1.66ABCa	1.38ABa	1.39Aa	1.48	0.73	0.63	0.68	0.68
60	1.94ABa	1.37ABb	1.44Ab	1.58	0.80	0.68	0.78	0.75
80	2.06Aa	1.46Ab	1.60Ab	1.71	0.78	0.62	0.87	0.76
100	1.93ABa	1.47Ab	1.51Ab	1.63	0.74	0.66	0.61	0.67
Mean	1.67	1.26	1.49		0.71a	0.60b	0.74a	
ANOVA		F				F		
Soil		27.26***				10.31***		
Dose		8.40***				2.13^{NS}		
Soil x Dose		2.81**				1.20^{NS}		
CV		14.13				17.88		

Uppercase letters compare means in the column and lowercase letters compare means in the line. Means compared using the Tukey test at 5% probability. ***, **, and ^{NS} are equal to 0.1, 1% of probability, and non-significant, respectively.

Table 17 – Enzymatic analysis in quinoa first cycle (tropical winter) as function of soils and RHB doses. Mean values for lipidic peroxidation (MDA), peroxid (H_2O_2), superoxid dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)

Biochar Dose (t ha^{-1})	MDA				H_2O_2				SOD			
	μmol g^{-1} FW											
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	10.80Aa	11.70Aa	7.89ABb	10.13	0.98	0.97	1.22	1.06	253.60	658.01	421.95	444.52
10	7.48Bb	10.84Aa	8.11ABb	8.81	1.09	1.04	1.21	1.11	281.62	703.41	432.75	472.59
20	6.85Bb	9.29Aa	8.55Aab	8.23	1.07	1.09	1.17	1.11	287.23	556.25	405.17	416.22
40	7.33Ba	9.10Aa	7.28ABa	7.91	1.05	1.19	1.10	1.12	238.47	539.26	327.68	368.47
60	7.83ABa	9.03Aa	8.33Aa	8.40	0.96	1.19	1.20	1.12	295.39	559.80	282.49	379.23
80	7.82Ba	8.91Aa	8.42Aa	8.38	1.05	1.20	1.23	1.16	340.95	424.39	245.28	336.87
100	9.32ABa	10.34Aa	5.32Bb	8.32	0.92	1.19	1.26	1.12	262.46	528.94	248.05	346.48
Mean	8.20	9.89	7.70		1.02b	1.13a	1.20a		279.96b	567.15a	337.63b	
ANOVA		F				F				F		
Soil		19.24***				9.14***				41.24***		
Dose		3.32**				0.42^{NS}				1.99^{NS}		
Soil x Dose		2.91**				0.97^{NS}				1.06^{NS}		
CV		16.07				14.30				31.70		

	APX				CAT			
	μmol g^{-1} FW							
	Cambisol	Fluvisol	Planosol	Mean	Cambisol	Fluvisol	Planosol	Mean
0	587.46	715.71	551.57	618.25	108.95	77.31	96.08	94.11A
10	635.25	648.90	752.02	678.72	118.63	77.94	90.80	95.79A
20	853.24	651.47	981.01	828.57	95.97	72.59	95.59	88.05A
40	901.41	883.83	1073.29	952.84	113.72	96.76	92.98	101.15A
60	926.50	640.61	1215.12	927.41	152.76	131.81	91.46	125.35A
80	929.97	653.04	1086.61	889.87	125.75	137.23	177.05	146.68A
100	928.43	690.12	1100.94	906.50	107.56	129.83	135.27	124.22A
Mean	823.18ab	697.67b	965.80a		117.62	103.35	111.32	
ANOVA		F				F		
Soil		5.34**				0.62^{NS}		
Dose		2.16^{NS}				2.43*		
Soil x Dose		0.69^{NS}				0.74^{NS}		
CV		37.05				43.3		

Uppercase letters compare means in the column and lowercase letters compare means in the line. Means compared using the Tukey test at 5% of probability. ***, **, and ^{NS} are equal to 0.1, 1% of probability, and non-significant, respectively.

4.3.2. Quinoa tropical summer cycle

4.3.2.1. Biometric analysis

In the second quinoa cycle, most of the plants in Cambisol and especially in Fluvisol did not survive. For Cambisol, the highest percentage of surviving plants occurred from the RHB dose of 40 t ha^{-1} (Table 18), where descriptive statistics were performed, with mean values and standard deviation, to evaluate the development trend, nutritional status, phytoextraction potential, and enzymatic activity of plants.

In Fluvisol, quinoa plants had a survival rate of 25% up to a dose of 60 t ha^{-1} (1 plant per treatment) and from a dose of 80 t ha^{-1}, 50% of the plants survived (2 plants per treatment). Due to the low survival rate on Fluvisol in all treatments, it was not possible to perform descriptive statistics.

Despite survival of over 75% from a dose of 40 t ha^{-1} in Cambisol, the plants showed heterogeneous growth and development. For Planosol, all plants survived in the second cycle, making it possible to perform ANOVA and Tukey test at 5% probability. In Cambisol plants, for the elements Ca, Mg, Na, and Cl there was a tendency to reduce their concentration between the doses of 40 and 100 t ha^{-1}. As for K, the trend was upward.

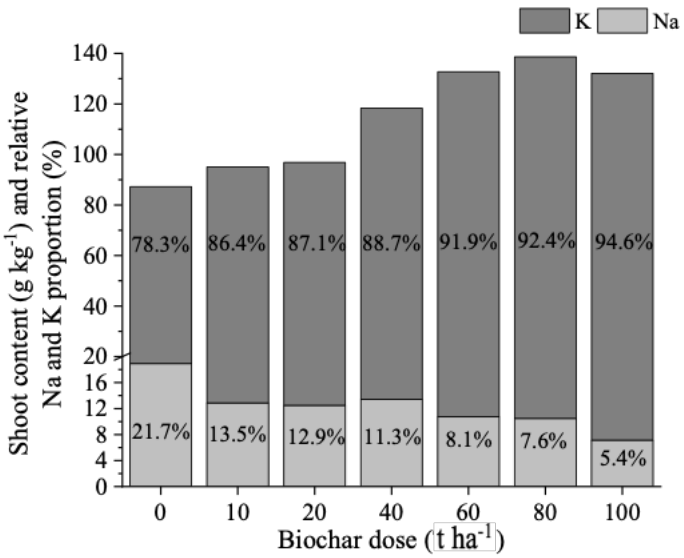
In Planosol, there was a significant reduction in the concentrations of Ca, Mg, and Na in quinoa shoot with increasing biochar doses according to Table 18. For fresh weight, dry weight, and K concentration, there were a significant increase among biochar doses. According to figure 12, the relative proportion of Na in relation to K in quinoa plants among biochar doses was significantly reduced. At the control dose, the Na concentration in the shoot was 21.7%, reducing to 5.4% at 100 t ha^{-1}, while K increases from 78.3 to 94.6%.

Table 18 – Biometric parameters in the second quinoa cycle (tropical summer) as function of soils and RHB doses. Plant height (H), stem diameter (SD), shoot fresh weight, and shoot dry weight

Biochar Dose (t ha^{-1})	H	SD	SFW	SDW	N	Ca	Mg	Na	K	Cl
	cm		g		g kg^{-1}					
					Cambisol					
0	*	*	*	*	*	*	*	*	*	*
10	**	**	**	**	**	**	**	**	**	**
20	**	**	**	**	**	**	**	**	**	**
40	37.00±17.40	4.40±0.86	21.16±6.84	9.37±1.19	34.44±3.00	10.20±1.25	19.25±1.65	17.16±11.75	73.85±12.80	74.25±22.40
60	48.00±10.40	4.16±0.40	21.30±5.42	9.42±1.05	31.96±3.02	10.83±1.36	18.93±0.97	19.45±10.68	89.00±6.16	70.33±18.04
80	38.75± 17.70	4.08±0.53	18.69±4.52	9.10±0.83	32.69±0.90	10.58±1.05	18.17±1.28	16.32±10.19	92.35±6.00	71.00±11.70
100	42.75± 16.07	4.47±1.06	23.26±13.60	9.57±2.01	30.73±2.77	9.06±0.69	14.60±3.04	12.12±6.40	93.90±25.70	70.00±12.90
					Fluvisol					
0	*	*	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*	*	*
20	*	*	*	*	*	*	*	*	*	*
40	*	*	*	*	*	*	*	*	*	*
60	*	*	*	*	*	*	*	*	*	*
80	**	**	**	**	**	**	**	**	**	**
100	**	**	**	**	**	**	**	**	**	**
					Planosol					
0	74.75	5.03A	30.83B	11.05D	29.12	10.12A	18.63	18.93A	68.30C	75.75
10	74.75	5.02A	32.90B	11.15CD	30.45	9.24AB	17.90	12.88AB	82.20BC	75.25
20	72.50	5.22A	36.39B	11.79BCD	29.26	8.74AB	16.80	12.51AB	84.35BC	84.00
40	80.25	5.94A	43.76AB	12.44BCD	28.25	8.25ABC	17.13	13.41AB	104.90ABC	85.25
60	79.50	5.81A	48.68AB	13.65ABC	30.10	6.81BC	15.43	10.72AB	122.00AB	82.75
80	83.25	6.00A	55.55A	15.13AB	27.72	5.76C	14.43	10.48AB	128.15A	84.25
100	81.75	5.93A	55.00A	14.24A	29.58	5.93C	13.40	7.15B	124.95AB	83.75
CV(%)	10.84	12.38	28.28	14.96	8.70	23.80	16.20	38.84	27.58	9.04
p	NS	0,036	0,0006	0,0001	NS	0,0001	NS	0,021	0,0007	NS

Uppercase letters compare means in the column. Means compared using Tukey test at 5% of probability for Planosol and described statistics for Cambisol. NS is equal to non-significant.

Figure 12 – Na and K relative proportion in quinoa shoot in the second cycle (tropical summer) for Planosol



According to table 19, there were negative Person's correlation among EC_e, shoot height, stem diameter, shoot fresh weight, and shoot dry weight in the second quinoa cycle in Planosol. The presence of Ca, Mg, and Na in the soil solution also negatively influenced the biometric parameters of quinoa plants. Soil exchangeable K positively correlates with stem diameter, fresh weight, and dry weight and negatively correlates with the absorption of Ca, Mg, and Na by the plants. It is possible to note in the table 19 that the concentration of soil soluble Cl correlates negatively with stem diameter, fresh weight, and dry weight.

Table 19 – Person`s linear correlation among soil chemical attributes, biometric and nutritional parameters in quinoa second cycle

Soil attribute	Biometrical parameters				Nutritional parameters					
	H	SD	SFW	SDW	N	Ca	Mg	Na	K	Cl
					Planosol					
pH	-0.02	0.13	0.35	0.26	-0.06	***-0.46***	***-0.45***	-0.21	***0.51***	0.25
pHes	0.22	0.35	0.32	0.26	0.02	-0.20	-0.21	-0.16	0.21	0.18
EC	***-0.54***	***-0.50***	***-0.49***	***-0.58***	0.09	0.34	0.08	***0.54***	***-0.40***	-0.24
Soluble cations										
Ca	***-0.46***	***-0.57***	***-0.57***	***-0.63***	0.09	***0.38***	0.17	***0.53***	***-0.57***	-0.33
Mg	-0.37	***-0.62***	***-0.54***	***-0.62***	0.11	0.35	0.16	***0.43***	***-0.49***	-0.37
Na	***-0.46***	***-0.64***	***-0.50***	***-0.59***	0.16	0.32	0.13	0.36	***-0.41***	***-0.43***
K	-0.14	-0.13	0.17	0.07	0.11	***-0.40***	***-0.44***	-0.25	0.21	-0.03
RAS	-0.30	-0.26	-0.16	-0.24	-0.08	0.16	0.02	0.16	-0.10	-0.07
Cl	-0.29	***-0.58***	***-0.51***	***-0.50***	0.24	0.22	0.03	***0.51***	***-0.45***	***-0.49***
Exchangeable cations										
Ca	0.13	0.01	0.33	0.18	0.02	***-0.39***	-0.28	-0.10	***0.53***	0.23
Mg	-0.08	-0.20	0.22	0.05	0.16	-0.31	-0.28	-0.02	***0.44***	0.04
Na	0.31	***0.41***	***0.69***	***0.64***	-0.09	***-0.68***	***-0.54***	***-0.46***	***0.80***	0.25
K	0.12	***0.40***	***0.66***	***0.54***	0.00	***-0.76***	***-0.68***	***-0.45***	***0.78***	0.33
SB	0.13	0.16	**0.53**	**0.38**	0.02	***-0.60***	***-0.49***	-0.27	***0.73***	0.25
H+Al	0.32	***0.59***	***0.65***	***0.63***	-0.01	***-0.74***	***-0.52***	***-0.61***	***0.70***	***0.47***
CEC	0.24	***0.40***	***0.66***	***0.56***	0.00	***-0.75***	***-0.57***	***-0.48***	***0.82***	***0.40***
ESP	0.30	0.37	***0.62***	***0.61***	-0.13	***-0.57***	***-0.47***	***-0.40***	***0.68***	0.16
V	-0.28	**-0.60**	***-0.41***	***-0.48***	0.05	***0.44***	0.24	***0.54***	-0.32	***-0.39***

H – Shoot height; SFW – Shoot fresh weight; SDW – Shoot dry weight; pHes – pH in the paste extract; ECe – Soil electric conductivity; SAR – Sodium adsorption ratio; CEC – Cation exchange capacity; ESP – Exchangeable sodium percentage; V – Bases saturation. Numbers in bold are significant at $p < 0.05$.

4.3.2.2. Phytorremediation potencial

For the quinoa phytoextraction potential in the second cycle, the quinoa plants phytoextracted the elements in the order of K > Cl > Mg > Na > Ca (table 20) for both Cambisol and Planosol. For Planosol there was a significant difference in the phytoextraction of K and Cl among biochar doses. For K, there was an increase from 106.49 to 252.64 kg ha^{-1} between the first and last doses, when observing phytoextraction in kg ha^{-1}. As for Cl, the significant increase was from 118.2 to 179.03 kg ha^{-1} between biochar doses of 0 and 100 t ha^{-1}. The total phytoextraction (Ca + Mg + Na + K + Cl), in kg ha^{-1}, was 402.82.

Evaluating table 20, related to phytoextraction in g $plant^{-1}$, the trend is the same as described in the previous paragraph. The total phytoextraction (Ca + Mg + Na + K + Cl) was 2.83 g $plant^{-1}$.

Table 20 – Salt phytorremediation potential during quinoa second cycle (tropical summer), in kg ha^{-1} and g planta^{-1}, as function of soils and RHB doses. Mean values for phytoextraction of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl)

Biochar Dose (t ha^{-1})	Ca	Mg	Na	K	Cl	Ca	Mg	Na	K	Cl
	kg ha^{-1}					g plant^{-1}				
					Cambisol					
0	*	*	*	*	*	*	*	*	*	*
10	**	**	**	**	**	**	**	**	**	**
20	**	**	**	**	**	**	**	**	**	**
40	13.38±0.72	25.30±1.19	23.95±17.68	98.44±22.9	96.67±21.46	0.09±0.005	0.17±0.008	0.16±0.12	0.69±0.16	0.68±0.15
60	14.30±0.63	25.13±1.59	24.85±10.6	118.50±14.49	91.94±13.49	0.10±0.004	0.17±0.01	0.17±0.07	0.83±0.10	0.64±0.09
80	13.59±1.33	23.31±1.18	20.41±11.8	119.22±15.6	90.56±8.5	0.09±0.009	0.16±0.008	0.14±0.08	0.84±0.11	0.64±0.06
100	12.31±2.94	19.98±6.81	18.11±10.4	127.46±40.22	93.77±20.83	0.08±0.02	0.14±0.05	0.12±0.07	0.90±0.28	0.66±0.14
					Fluvisol					
0	*	*	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*	*	*
20	*	*	*	*	*	*	*	*	*	*
40	*	*	*	*	*	*	*	*	*	*
60	*	*	*	*	*	*	*	*	*	*
80	**	**	**	**	**	**	**	**	**	**
100	**	**	**	**	**	**	**	**	**	**
					Planosol					
0	15.85A	29.26	29.13	106.49B	118.20B	0.11A	0.21	0.21	0.75B	0.84B
10	14.55A	28.34	19.94	130.79B	118.69B	0.10A	0.20	0.14	0.92B	0.84B
20	14.53A	27.89	20.95	140.20B	140.26AB	0.10A	0.20	0.15	0.99B	0.99AB
40	14.34A	29.78	23.77	185.03AB	150.20AB	0.10A	0.21	0.17	1.31AB	1.06AB
60	13.00A	29.62	20.24	235.31A	160.55AB	0.09A	0.21	0.14	1.66A	1.13AB
80	12.29A	30.71	23.04	271.57A	180.55A	0.09A	0.22	0.16	1.92A	1.28A
100	11.97A	27.23	13.76	252.64A	179.03A	0.08A	0.19	0.10	1.79A	1.15AB
CV	15.5	13.7	33.7	37.7	20.4	15.5	13.7	33.7	37.7	20.7
p	0,04	NS	NS	<0,001	0,002	0,04	NS	NS	<0,001	0,002

Uppercase letters compare means in the column. Means compared using the Tukey test at 5% of probability for Planosol and described statistics for Cambisol.

4.3.2.3. Enzymatic analysis

According to table 21, there was a significant increase in lipidic peroxidation (MDA) and reduction in H_2O_2 with the addition of increasing RHB doses in plants grown in Planosol. In the second cycle, there was a significant reduction in superoxide dismutase (SOD) activity with mean values at the control and 100 t ha^{-1} doses from 858.53 to 487.4 µmol g^{-1}. For the enzymes ascorbate peroxidase (APX) and catalase (CAT), there was no significant difference among treatments.

Table 21. Enzymatic analysis in quinoa second cycle (tropical winter) as a function of soils and RHB doses. Mean values for lipidic peroxidation (MDA), peroxide (H_2O_2), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)

Biochar Dose (t ha^{-1})	MDA	H_2O_2	SOD	APX	CAT
	µmol g^{-1} FW				
	Cambisol				
0	*	*	*	*	*
10	**	**	**	**	**
20	**	**	**	**	**
40	6.61±3.04	5.82±1.93	811.66±176.9	1097.1±344.71	137.57±31.78
60	8.77±2.91	7.45±3.63	-	642.26±176.30	82.5±4.63
80	9.70±1.56	4.34±2.2	835.25±297.1	1052.05±674.82	115.69±22.72
100	8.61±1.75	-	926.21±246.6	532.09±191.37	125.17±32.02
	Fluvisol				
0	*	*	*	*	*
10	*	*	*	*	*
20	*	*	*	*	*
40	*	*	*	*	*
60	*	*	*	*	*
80	**	**	**	**	**
100	**	**	**	**	**
	Planosol				
0	8.94B	6.56A	858.53AB	858.27	102.54
10	8.90B	4.33AB	970.94A	1086.93	95.72
20	11.26AB	4.87AB	772.54AB	858.05	103.20
40	13.00AB	4.61AB	539.91B	1011.80	72.48
60	15.35A	4.28AB	645.77AB	967.01	70.06
80	16.48A	3.44B	489.86B	908.34	70.82
100	16.97A	3.36B	487.40B	870.60	62.27
CV (%)	31.04	29.03	34.26	25.1	37.7
p	<0.001	0.0057	0.0055	NS	NS

Uppercase letters compare means in the column. Means compared using the Tukey test at 5% of probability for Planosol and described statistic for Cambisol. FW – Fresh weight.

4.4. Discussion

4.4.1. Biometric, nutritional, and enzymatic effects of quinoa under biochar application in winter cycle in Northeastern Brazil

Considering that the experiments were set up in chemically and physically different soils, quinoa plants grew in divergent ways among the soils and biochar doses. According to biometric parameters, mainly stem diameter (SD), shoot fresh weight (SFW), and shoot dry weight (SDW), quinoa grew similarly in Cambisol and Planosol, differing in Fluvisol (Table 12). It is important to highlight that, despite the statistically similar trend, in relation to the development of quinoa, Cambisol and Planosol differ strongly in their chemical compositions, with the first one being a highly saline soil and the second having low salinity (Table 9). This is indicative of the high tolerance of the quinoa CPAC 09 genotype to salinity, as the plants demonstrated convergent results among saline and non-saline soils.

In addition to saline characteristic, Fluvisol has high levels of silt in its granulometric composition (Table 9). High concentrations of silt in the soil can generate physical degradation, mainly in water infiltration and consequently in permeability (NAZARI et al., 2018). Reduction in water infiltration causes aeration problems, limiting roots development. High salinity and soil physical degradation may have caused a reduction in the growth parameters of quinoa crop in Fluvisol.

The RHB doses significantly changed quinoa SD and SFW, with the interaction among biochar doses and soils. From table 12, the higher the biochar doses, the greater the SD and SFW, especially in Cambisol and Fluvisol plants. In this way, biochar may have alleviated salinity stresses in quinoa in these two soils.

Similar results were found by Yang et al. (2020), using corn straw biochar made at 500 °C in saline soils cultivated with quinoa. The authors observed an increase in growth, photosynthetic parameters, and nutrient absorption by plants, serving to alleviate saline stress in quinoa in semiarid regions.

Evaluating nutritional parameters (Table 13), quinoa showed similar trend when grown in Cambisol and Fluvisol, both extremely saline soils. The concentrations of N, P, Ca, Mg, and Na did not differ significantly between the two soils, however differing from Planosol, as it has a low salinity. Therefore, it is possible to state that the greater the contribution of salts to the soil, the greater the absorption of these elements by quinoa. This is an indication of the salt phytoextractive potential, considering that, according to Spehar and Santos (2002), this crop has a short cycle (average of 4 months), high phytomass, and high planting density (good qualities for phytoremediation).

For K, the greatest absorption by plants was in Cambisol, followed by Planosol, and Fluvisol. The Cl concentration did not differ significantly in any of the applied treatments, whether soil or biochar, throughout the first quinoa cycle. We can observe, in table 13, that there was a interaction among soils and biochar doses for the variables Na and K. It is evident, in figure 11, that, in all soils, the relative proportion of Na^+ to K^+ is reduced with increasing biochar doses. This trend indicates a greater supply of K from the RHB, as it is a material rich in K (Table 10).

Possibly, the high concentration of silt in the Fluvisol reduced the diffusion of O_2 in this soil, which may have reduced the release of K^+ into the soil. In addition, RHB also had the ability to alleviate the stress caused by Na. The effect of reducing Na relative to K is strongly evident in saline soils (Cambisol and Fluvisol), where Na concentrations reached very high and potentially toxic values for crops (Table 9), which can be assessed from the SAR and ESP of these soils. As Planosol has a higher concentration of sand, compared to the other two soils, it is less prone to the accumulation of salts, which leads to a low proportion of Na^+.

Similar results were observed by Ferreira et al. (2020), where the authors evaluated K absorption in spinach plants. As quinoa and spinach belong to the same botanical family (Amaranthaceae), they have some similar mechanisms of nutrient uptake and tolerance to salinity stress. According to the authors, the greater the availability of K in the soil, the greater the absorption of this nutrient by spinach, even in saline soils. This result corroborates with the findings in this present research for quinoa.

From Person`s linear correlation among soil chemical attributes, plant growth, and nutrition parameters (Table 14), it is possible to observe that parameters related to soil salinity and sodicity such as pH, EC_e, SAR, and ESP negatively influenced the development of quinoa, mainly in Cambisol. Despite being a facultative halophyte plant, high levels of salt in the soil can cause some deleterious effects on plant biomass. Despite this negative correlation, quinoa has shown its high potential for development in saline environments, where almost all agricultural crops would not be able to survive.

In all soils evaluated, Na absorption by plants has a negative correlation with K concentrations in the soil, indicating a preference for K absorption compared to Na, especially in saline soils. With these results, it is clear that quinoa uses K as a way to minimize the impacts caused by Na. This mechanism of tolerance to saline environments was also described by Turcios, Papenbrock and Tränkner (2021), in a study with K absorption in saline soils by quinoa. The authors concluded that a high K concentration in soil increase quinoa biomass in saline and sodic soils by the regulation in K^+/Na^+ ratio inside the cells, alleviating the stress

caused by salinity through osmotic regulation and also reduction in plant's stomatal conductance, allowing greater quinoa tolerance to saline environments.

According to tables 15 and 16, the CPAC 09 genotype has a moderate phytoextraction potential for K and Cl and low phytoextraction potential for Na. In this work, two ways of evaluating the phytoextraction potential of these plants in pots in a greenhouse are presented. In general, phytoextraction data are expressed in kg ha^{-1}, based on field work. Little is discussed about how these data can be expressed in controlled situations. Often, the extrapolation of greenhouse data, g $plant^{-1}$ to kg ha^{-1}, can result in an over or underestimation of the data, as it would be impossible to predict the real trend of a crop when added other factors such as biotic and abiotic adversity.

Therefore, in greenhouse experiments, it would be recommended to express the data in g $plant^{-1}$ to evaluate the salts phytoextractive potential. Thus, quinoa phytoextracted salts in the order of K > Cl > Mg > Ca > Na, considering the genotype used and the experimental conditions. In general, quinoa was able to extract 2.97, 2.38, 2.58 g $plant^{-1}$ of salts in Cambisol, Fluvisol, Planosol, respectively. The difference between Fluvisol and Planosol is due to the higher concentration of K in the latter soil, despite the low salinity. Extrapolating these data to the field situation (table 15), the increment in biochar doses also favored an increase in the phytoextraction potential, mainly due to the greater absorption of K by the plants.

Moura et al. (2019), in a study with *Atriplex nummularia* L., considered as one of the most salt-extracting species recorded, reported that this plant phytoextracted 971.21 kg ha^{-1} $year^{-1}$ of salts, mainly Na and Cl ions. Comparing Atriplex with quinoa and considering that in a period of approximately 90 days the salt extraction was more than 350 kg ha^{-1} (depending on the soil), it is possible to infer that consecutive quinoa cycles in field, by year, has a phytoextraction potential similar to Atriplex. The main difference between quinoa and atriplex is that the first one extracts more K^+ and the second one more Na^+, making it difficult to use quinoa as a phytoextractor plant for sodic soil reclamation in these conditions. Also considering the nutritional, food, and forage potential of quinoa, we can assume that, depending on the soil and climate conditions, in addition to the high extraction of salts per year, especially Cl, quinoa would also enter the Brazilian semiarid food market as a potential crop with high commercial value and high nutritional interest for the population.

For enzymatic data (Table 17), plants grown in Cambisol and Fluvisol showed high MDA concentrations, indicating high lipid peroxidation by reactive oxygen species (ROS). The presence of ROS such as H_2O_2 in quinoa plants (Puna genotype) in saline soils was described by Abbas et al. (2022). The authors state that the increase in enzyme activity such as superoxide

dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) is one of the mechanisms used by quinoa to alleviate oxidative stress and eliminate ROS. The highest SOD activity was recorded in Fluvisol, and ascorbate peroxidase (APX) in Planosol.

Although Planosol was a non-saline soil at the beginning of the experiment, the high enzymatic activity of APX on quinoa plants in this soil may be related to the fact that Planosol has a high concentration of sand (Table 9), which favors less water retention in the soil, which may have caused a slight water stress on plants grown in this soil, especially on days with elevated temperatures. In general, the addition of biochar did not significantly influence the enzymatic activity during the winter cycle.

Due to the slightly mild climate among the months of June and August, considering the tropical climate of Brazilian Northeast (Figure 10), no visual changes were observed among the plants due to temperature, with minimum averages varying from 21.8 to 22 °C and maximum values ranging from 32.2 to 32.9 °C.

For the parameters analyzed in this work, the best dose of RHB for quinoa (genotype CPAC 09) was 40 t ha^{-1}, allowing improvement in biometric, nutritional, and enzymatic effects. In general, doses of 40 t ha^{-1} did not differ statistically from higher doses, always being better in relation to the control.

4.4.2. Biometric, nutritional, and enzymatic effects of quinoa under biochar addition in summer cultivation in Northeastern Brazil

Quinoa summer cycle was marked by problems with plant development and survival. At the end of the experiment, plants in saline soils with low biochar doses (Cambisol and Fluvisol) showed a survival rate between 25 and 50% as seen in table 18. Plants in Fluvisol were strongly affected by the heat with a survival rate of 25% up to a dose of 60 t ha^{-1}, and 50% at the doses of 80 and 100 t ha^{-1}. In Planosol, as it is a less saline soil, all the plants survived, but with more compromised development than in the winter cycle.

The minimum average temperatures for summer (December to February, figure 10) ranged from 24.6 to 25.3 °C and the maximum from 36.9 to 39.3 °C with peaks above 40 °C. High temperatures and salinity caused the death of many plants in saline soils. Based on the survival data, we can assume that high RHB doses enabled the survival of more than 75% of the plants, mainly in Cambisol, and could serve as a relief from heat stress in this crop. This is also visible in plants grown in Planosol which significantly increased fresh and dry weight of quinoa shoot with the increment in biochar doses.

Alvar-Beltrán et al. (2020), working with quinoa (cv. Titicaca) under heat stress, concluded that at temperatures of 38 °C, there is a loss of over 30% in seed yield and 50% in seed germination. The authors also indicate that quinoa sowing should be planned so that the hot season is avoided, especially during the plant's flowering period. Quinoa, despite being highly resistant to abiotic stresses such as drought, salinity, and freezing, is relatively sensitive to high temperatures.

With the increment in biochar doses, there was a strong increase in K absorption by quinoa, even if we compare it with the winter cycle, suggesting that for both stresses (saline and heat) quinoa extremely enhances the absorption of K to the detriment of other elements, mainly Na (Figure 12). K content in the shoot reached values above 120 g kg^{-1}.

K is a macronutrient with diverse functions, mainly acting on cell expansion, such as the opening of stomata. K also regulates the pH of the cytoplasm and enzymatic activity in cells. In general, K remains as a soluble ion in cell cytoplasm and can contribute up to 10% of plant dry matter (RAGEL et al., 2019).

In saline environments, quinoa increases the absorption of K for osmotic adjustment, stomatal regulation, and maintenance of an adequate K/Na ratio inside cells, reducing damage caused by salinity stress, increasing its productivity (ADOLF; JACOBSEN; SHABALA, 2013; TURCIOS; PAPENBROCK; TRÄNKNER, 2021).

By the Person's correlation (Table 19), in Planosol, the high exchangeable K content in soil positively correlated with plant growth parameters such as stem diameter (SD), shoot fresh weight (SFW), and shoot dry weight (SDW). The Increase in EC_e negatively correlates with the same parameters mentioned above.

Comparing K concentration in quinoa shoot (Planosol) between the first and second quinoa cycles (77.37 g ka^{-1} and 92.80 g ka^{-1}, respectively), it is possible to observe an increment in 19.94% in K uptake between cycles, indicating greater absorption of this nutrient by quinoa under hot temperatures. The decomposition rate of RHB may also have contributed to a better release of K^+ in the soil during the second cycle, favoring the absorption of K by quinoa.

In the summer cycle, the order of salt phytoextraction was K > Cl > Mg > Na > Ca and the total phytoextraction was 2.83 g $plant^{-1}$ in Planosol (table 20), which would represent 402.82 t ha^{-1}. This represents an increase of 7.05% in salts phytoextraction if we compare the first and second quinoa cycles (Table 16).

In enzymatic evaluation (table 21), for plants grown in Planosol, there was a significant reduction in the concentration of H_2O_2 with the increase in biochar doses. The reduction in H_2O_2 concentration in quinoa plants was also observed by Turcios, Papenbrock, and Tränkner

(2021), when K doses were applied during quinoa cultivation. This indicates that K has a fundamental role in eliminating ROS in plant cells and maintaining enzymatic activity to alleviate oxidative stress. High levels of APX and low level of CAT were also explained by the aforementioned authors, where they state that there is a balance between CAT and APX inside quinoa, as generally when one enzyme has greater activity the other reduces its activity.

The increase in lipidic peroxidation (MDA) with the increase in biochar doses is possibly related to the accumulation of other ROS species in the plant such as superoxides (O_2^-), hydroxyl radicals (OH^+) and singlet oxygen ($^1O_2$) (MITTLER, 2017).

Thus, it is possible to infer that the increase in temperature in the summer cycle, together with the high salinities of Cambisol and Fluvisol, had a deleterious effect on the development of quinoa plants.

4.5. Conclusion

In general, the addition of increasing doses of biochar favored the better development of quinoa, alleviating the harmful effect of Na and increasing the absorption of K. One of the main tolerance mechanisms of quinoa in saline environments is the superabsorption of K. High temperatures with high soil salinity have a negative effect on quinoa survival, which can be a limiting factor for the implementation of this crop in hot seasons in the Brazilian Northeast.

The CPAC 09 genotype from EMBRAPA Cerrados has high adaptability to an extreme salinity environment, with satisfactory plant development when compared to low saline soils, indicating its potential for land use and salt phytoextraction in soils affected by salts in the Brazilian semiarid region. The Na phytoextraction potential is relatively low for this genotype in comparison to the K and Cl extraction, being considered a plant that hyperaccumulates K and Cl, especially in an environment with intense abiotic stress.

For tropical winter cultivation, the dose of 40 t ha^{-1} of RHB presented the best benefits for quinoa, not differing significantly from higher biochar doses, promoting improvements in the crop compared to lower doses. For summer cultivation, doses from 60 t ha^{-1} would be the most appropriate.

Therefore, this work indicates that research on the adaptation of quinoa to saline soils in the Brazilian semiarid region should be intensified, suggesting that the sowing of this species be carried out in the months with the lowest temperatures (May to August).

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5. CHAPTER IV: SALT RESISTANCE OF TWO BRAZILIAN QUINOA GENOTYPES (CPAC 09 AND CPAC 11) AND SPINACH (*Spinacia oleracea* cv Gazelle): NUTRITIONAL, PHYSIOLOGICAL ASPECTS, AND SOIL EVALUATION AFTER HIGHLY SALINE WATERS IRRIGATION

Abstract

The increase in the world's average temperature influences soil quality and the survival of agricultural crops. It is estimated that more than 30% of arable land are in the process of degradation and one of the main factors is salinity/sodicity. Quinoa (*Chenopodium quinoa* Willd.) and spinach (*Spinacia Oleracea* L.) are members of a botanical family notable for species tolerant to abiotic stresses (Amaranthaceae family). This has led to increased research focus on their tolerance to salts and sodium. Due to quinoa and spinach nutritional values, researchers are interested in these species as part of human diet, especially for socially vulnerable and food-insecure populations. Therefore, this work assessed the performance of quinoa and spinach under highly saline conditions. In the first experiment, two quinoa genotypes (CPAC 09 and CPAC 11- EMBRAPA Cerrados) were submitted to salinities of 2, 25, 40, and 55 dS m^{-1}, in randomized blocks, in four replications, with salts concentration propoetional that of seawater. In the second experiment involving spinach cv. Gazelle, two salinities were applied (2 and 25 dS m^{-1}), with four replications each. With increase in salinity, both species increased the Na and Cl concentrations and reduced K concentration in leaves. There was a loss in approximately 60% in quinoa productivity between salinity of 2 dS m^{-1} and 25 dS m^{-1}. Shoot biomass was reduced by 60% when quinoa genotypes were under EC_w of 25 dS m^{-1} and by 95% at 55 dS m^{-1}. For spinach, the reduction was approximately 80% under EC_w of 25 dS m^{-1} compared to the control. The reduction in crop biomass and grain yield were influenced by the increase in Na and Cl concentration in detriment of K. With the saline water application, there was a significant increase in soil EC_e, SAR, and ESP. High salts concentration in soil promoted a reduction in pH and an increase in soluble Na^+ and Cl^- and exchangeable Ca^{2+}, Mg^{2+}, and Na^+. The two quinoa genotypes were highly tolerant to extreme salinity. Although not tolerant as quinoa, spinach has also shown a significant salt tolerance. Both crops have the potential to be cultivated in lands degraded by salts, to reduce food insecurity in communities in arid and semiarid regions.

Keywords: Salinity. Sodicity. Food production. Agricultural crops. Food insecurity.

CHAPTER IV: RESISTÊNCIA A SAIS DE DOIS GENÓTIPOS BRASILEIRO DE QUINOA (CPAC 09 E CPAC 11) E ESPINAFRE (*Spinacia oleracea* cv Gazelle): ASPECTOS NUTRICIONAIS E FISIOLÓGICOS E AVALIAÇÃO DE SOLO APÓS IRRIGAÇÃO COM ÁGUAS ALTAMENTE SALINAS

Resumo

O aumento na temperatura média no planeta causa efeitos na qualidade dos solos e na sobrevivência das culturas agrícolas. Estima-se que mais de 30% dos solos agricultáveis estão em processo de degradação e um dos principais fatores é a salinidade/sodicidade. A quinoa (*Chenopodium quinoa* Willd.) e o espinafre (*Spinacia Oleracea* L.), por pertencerem a uma das famílias botânicas com mais indivíduos resistentes a estresses abióticos (Amaranthaceae), vêm se destacando nas pesquisas sobre tolerância a sais e sódio. Seus valores nutritivos chamam atenção dos pesquisadores para que façam parte da alimentação, especialmente de pessoas em vulnerabilidade social e insegurança alimentar. Assim, este trabalho avaliou a quinoa e o espinafre sob salinidade extrema. No primeiro experimento, duas variedades de quinoa (CPAC 09 e CPAC 11 – EMBRAPA Cerrados) foram submetidas às salinidades de 2, 25, 40 e 55 dS m^{-1}, em quatro repetições, com concentração de sais proprorcionais à água do mar. No segundo experimento, com o espinafre cv. Gazelle, foram utilizadas duas salinidades (2 e 25 dS m^{-1}), com quatro repetições. Ambas as espécies tiveram aumento na concentração de Na e Cl nas folhas e redução de K com o aumento da salinidade. A produtividade da quinoa diminuiu aproximadamente 60% entre as salinidades de 2 e 25 dS m^{-1}. A biomassa da parte aérea foi reduzida em aproximadamente 60% quando os genótipos de quinoa foram submetidos à salinidade de 25 dS m^{-1} e em 95% quando submetidos ao tratamento de 55 dS m^{-1}. Para o espinafre, a redução na biomassa foi de aproximadamente 80% quando irrigado com água de 25 dS m^{-1}. Os fatores que influenciaram na redução da biomassa e produtividade das culturas foi o aumento nos teores de Na e Cl em detrimento ao K e consequente diminuição em alguns parâmetros fisiológicos. Com a aplicação das águas, houve aumento significativo na EC_e, SAR e ESP do solo, tornando-o extremamente salino-sódico. A alta concentração de sais no solo promoveu redução no pH e aumento na concentração de Na e Cl solúveis e Ca, Mg e Na trocáveis. As duas variedades de quinoa se mostraram altamente resistentes a salinidades extremas. Apesar de menos resistente que a quinoa, o espinafre também se mostrou resistente a altos níveis de sais. Ambas as culturas têm potencial para serem cultivadas em solos em processo de degradação por sais, para redução da insegurança alimentar de comunidades em regiões áridas e semiáridas.

Palavras-chave: Salinidade. Sodicidade. Culturas agrícolas. Produção de alimentos. Insegurança alimentar.

5.1. Introduction

Abiotic stresses such as drought, salinity, low and high temperatures, among other climatic adversities, have been limiting factors for human adaptation and food production since the formation of the first prehistoric populations (SHORT, 2019). Soil salinization and sodification were present in several civilizations, with Mesopotamia as one of the main examples, where its extinction is strongly related to soil degradation due to high salt concentrations and consequently decline in food production (SHAHID; ZAMAN; HENG, 2018).

One of the main techniques for human coexistence and food production in environments undergoing salinization is the cultivation of salt-tolerant plants. Currently, according to the global salt-affected soils map (FAO, 2021), approximately 833 million hectares are salt affected worldwide, which corresponds to 10% of arable lands. The Amaranthaceae family has a range of species considered halophytes (CHEESEMAN, 2015), the best known of which are quinoa (*Chenopodium quinoa* Willd.) and *Atriplex nummularia* L. Another species in this family, spinach (*Spinacia oleracea* L.), is not classified as a halophyte but has been found to be resistant to soil salt accumulation according to some studies (FERREIRA et al., 2018).

Quinoa is a facultative halophyte species from the Andes region in South America and is considered as a pseudocereal with high nutritional value and balanced composition of carbohydrates, fats, and proteins (BHARGAVA; OHRI, 2016). In Brazil, EMBRAPA (Brazilian Agricultural Research Company) has been developing and selecting quinoa genotypes capable of adapting to harsh environments, mainly for the prolonged drought that affects part of the Brazilian territory during the year (SILVA et al., 2021).

Little is known about quinoa and spinach potential to thrive under edaphoclimatic conditions present in the Brazilian semiarid. The selection of genotypes that tolerate drought, salinity, and high temperatures has become the key technique for introducing quinoa and spinach in this region of Brazil, where more than 50.3% of the population faces some degree of food insecurity, with 7.1% experiencing severe food insecurity (IBGE, 2020; SALLES-COSTA et al., 2022),

Quinoa was domesticated in regions with highly diverse soil and climate characteristics across countries like Bolivia, Colombia, Chile, Peru, and Argentina, resulting in significant genetic variability. Depending on quinoa genotype, quinoa can respond to salinity with greater or lesser tolerance, mainly in relation to the accumulation of sodium in the leaves and the control of Na^+ in plant xylem (FAO, 2011; SHABALA; HARIADI; JACOBSEN, 2013).

Spinach is also a plant species capable of tolerating irrigation with saline water. A diverse genetic material from all over that world have shown tremendous genetic variability in spinach for salinity tolerance (SANDHU et al., 2023). Depending on the variety, spinach plants can tolerate EC_w from 1.5 to 9.0 dS m^{-1} without significant losses in productivity (ORS; SUAREZ, 2016; FERREIRA et al., 2018; YAVUZ et al., 2022)

Despite the common lineage of quinoa and spinach (ZOU et al., 2017), few studies show the nutritional and physiological relationship between them in saline environments. Therefore, this work aims to evaluate the tolerance of two quinoa genotypes (CPAC 09 and CPAC 11) developed by EMBRAPA Cerrados in Brazil and spinach (cultivar Gazelle) under irrigation waters with increasing levels of salts and sodium.

5.2. Material and methods

5.2.1. Plant material

The experiment involved the evaluation of quinoa (*Chenopodium quinoa* Willd.), genotypes CPAC 09 and CPAC 11 developed by EMBRAPA Cerrados and spinach (*Spinacia Oleraceae* L. cv. Gazelle). The germination of both species began on March 19, 2023. Quinoa and spinach seeds were sown on March 15, 2023, in the greenhouse of US Salinity Laboratory, Riverside, California – USA. Ten seeds were sown per pot for each species at a depth of 1.5 mm for spinach and 2 mm for quinoa. After the germination, only two plants per pot were maintained until the end of the quinoa cycle and only three plants for spinach.

The CPAC 09 and CPAC 11 quinoa genotypes are tolerant to drought and are capable of producing 2.11 and 2.38 t ha^{-1}, respectively, with a water regime of 150 mm during the cycle. In particular, the CPAC 09 genotype has previously demonstrated a high capability for producing flavonoid and anthocyanin, suggesting a strong potential for adaptation in environments under abiotic stress (SILVA et al., 2021).

5.2.2. Water salinity

Salinity levels were based on seawater with a proportion of Cl^-:Na^+:Mg^{2+}:Ca^{2+}:SO_4^{2-} = 25.5:22.73:5.15:1:2.6. For the plant nutrition, half-strength modified Hoagland's solution was used and the potassium concentration was fixed at 5 $mmol_c$ L^{-1}. Water salinities for quinoa were 2, 25, 40, and 55 dS m^{-1} and for spinach the irrigation water salinities were 2 and 25 dS m^{-1}. The description and ionic composition of the saline waters is presented in Table 22.

Table 22 – Description and ionic composition of the saline waters

Targeted EC_w	Calculated EC_w	Na^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	K^+	NO_3^-	PO_4^{3-}
——dS m^{-1}——		——————————————————$mmol_c$ L^{-1}——————————————————							
2	1.89	2.1	1.7	3.3	0.6	3.7	4.9	7.61	1.5
25	24.80	196.1	5.4	44.2	21.5	221.4	4.9	7.61	1.5
40	39.96	343.1	11.9	76.7	39.0	389.9	4.9	7.61	1.5
55	54.84	489.1	18.3	109.6	56.5	557.7	4.9	7.61	1.5

5.2.3. Experimental design

The experiment was carried out with two quinoa (*Chenopodium quinoa* Willd.) genotypes, CPAC 09 and CPAC 11, developed by EMBRAPA Cerrados, and spinach (*Spinacia oleracea* L., cv. Gazelle) under different salinity levels and irrigation water qualities. Specifically, the quinoa genotypes were subjected to four levels of salinity (2, 25, 40, and 55 dS m^{-1}) in a randomized block design, with four replications. On the other hand, spinach was irrigated with water of two salinity levels (2 and 25 dS m^{-1}) in a completely randomized design with four replications. These two species were chosen to compare the genetic mechanisms in relation to salinity between a glycophyte species (spinach) and a halophyte species (quinoa) of the Amaranthaceae family.

The experiment was repeated twice, using pots filled with a mix of local soil and sand (1:1). The first experiment was carried out for six weeks with two plants per pot with 9 kg of mix soil. The second experiment was carried out until the harvest (approximately 150 days after sowing), with also two plants per pot with 15 kg of mix soil. The irrigation waters were applied with a 40% leaching fraction. The saline waters application started when leaf six was completely open approximately 25 days after sowing.

To determine the field capacity, three pots with 3 kg of mixed soil were saturated with water and allowed draining until leaching ceased. After the draining, all the pots were weighed. When the soil reached field capacity, three soil samples of each pot were collected and dried in an oven at 105 °C for 24 hours for analysis of the amount of water at field capacity. Then, the irrigation of all pots with a leaching fraction of 40% were calculated.

For destructive analysis such as nutritional assessments, plants from the first experiment were harvested, and the soils were also analyzed. The second experiment was carried out until

the grain maturation. The spinach plants were harvested after 6 weeks of saline water treatments for the nutritional evaluation.

5.2.4. Plant analyses

5.2.4.1. Biometric analyses

To determine the growth of quinoa, the plants were measured during the experiment in intervals of 15 days until 75 days after the beginning of the treatment's application. At the end of the growth cycle, we analyzed plant height (cm), shoot and root weight (g), and seed weight (g $plant^{-1}$). For the biometric analyses we used the second experiment.

5.2.4.2. Nutritional analyses

To evaluate content of macronutrients, Na and Cl in quinoa and spinach, the plants were harvested in the flowering stage, a period in which the highest concentration of elements had already been accumulated by the plant, after 52 days of saline water application. In each pot, the two plants from the experimental unit (first experiment) were collected, washed in tap and deionized waters to remove impurities. The plants were separated into leaves, stems, and roots and oven dried at 65°C for 72h. Each part of the dried plant (leaves, stems, and roots) was ground separately to carry out nutritional analyses. The elements evaluated were N, P, S, Ca, Mg, Na, K, and Cl.

Chloride was determined from nitric-acetic acid extracts by amperometric titration. The concentration of P, Ca, Mg, Na, K, and S were determined from nitric acid digestions of the dried, ground plant material by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, 3300DV, Perkin-Elmer Corp., Waltham, MA, USA). Nitrogen was analyzed by combustion using an Elementar Rapid N Exceed®.

5.2.4.3. Leaf gas exchange

Leaf net photosynthetic rate (Pn), leaf stomatal conductance (gs), and leaf transpiration rate (Tr) were measured using a Li-Cor 6400 Photosynthesis System (LI-COR, Lincoln, NE, USA) for spinach and quinoa. Both plants were grown in pots in the greenhouse at U. S. Salinity Laboratory, USDA-ARS, California. The most recent fully expanded exposed leaves in top portion of plants were used for the measurements. Measurements were conducted under the condition of photosynthetic photon flux density, 1000 $\mu mol_{photon}\ m^{-2}\ s^{-1}$ provided by a red light-emitting diode source emitting at 670 nm (LI-COR, Lincoln, NE, USA); operational or chamber ambient CO_2 concentration, 400 $\mu mol_{CO2}\ mol_{air}^{-1}$. Leaf chamber temperature and leaf to air vapor pressure deficit for the measurement ranged from 22.6 to 28.3 (25.8±0.0.17) (mean±1SE)

ºC and from 0.84 to 2.37 (1.74±0.05) kPa, respectively, for quinoa; and from 23.9 to 28.1 (26.0±0.37) ºC and from 1.04 to 2.48 (1.70±0.11) kPa, respectively, for spinach. Leaf water use efficiency (WUE) was calculated using the formula of WUE=1000*Pn/Tr.

5.2.4.4. Leaf SPAD readings

Leaf soil-plant analyses development (SPAD) chlorophyll readings were taken four times across the whole leaf blade avoiding the main vein on each of the leaves used for the leaf gas exchange measurement using a SPAD chlorophyll meter (SPAD-502; Minolta, Osaka, Japan) and the data were averaged for a leaf as an estimate of its chlorophyll content.

5.2.5. Soil analysis

The soil properties evaluated were soil pH in water (1:2.5); pH, soil electrical conductivity (EC_e), soluble cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and Cl^- by the saturated paste extract; exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) by the ammonium acetate method and cation exchange capacity by the index cation method (USSL STAFF, 1954).

The ions Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, 3300DV, Perkin-Elmer Corp., Waltham, MA, USA). The methods adopted are described by EMBRAPA (2017) and USSL STAFF (1954). Exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) values of the soils were calculated.

5.2.6. Data analysis

The results were initially subjected to normality tests (Shapiro-Wilk, $p < 0.05$) and homoscedasticity (Levene, $p > 0.05$). After these procedures, analysis of variance (ANOVA, $p<0.05$) was performed and Tukey's HSD test of a single-step multiple comparison in SAS GLM procedure (SAS version 9.4, 2020) was used for analyzing the significance of difference among/between the salinities within a genotype and, of difference between the genotypes at a salinity at $P\leq0.05$.

5.3. Results

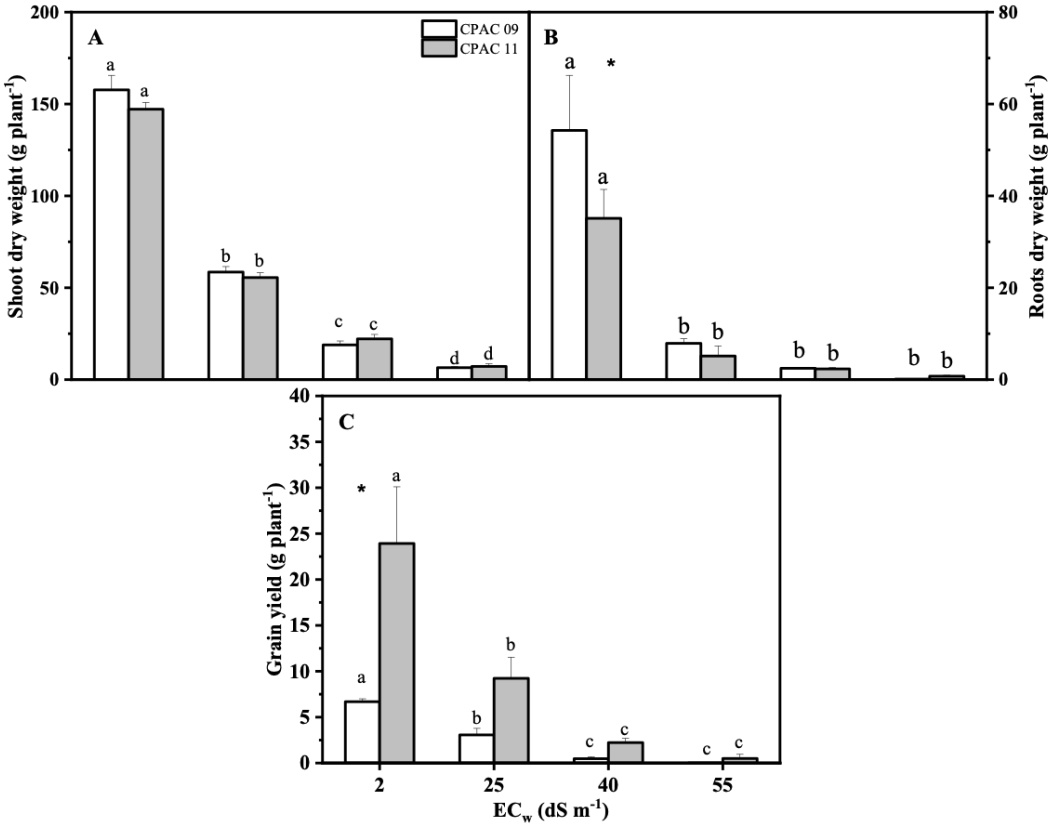
5.3.1. Biometric analyses

Through the biometric evaluation of the plants, it was possible to observe that in excessive salt concentrations, mainly from water with an EC_w of 40 dS m^{-1}, quinoa plants of both the CPAC 09 and CPAC 11 genotypes drastically reduced their biomass of the shoot and roots in 85.43% and 85.38%, respectively (Figure 13). With the increase in salinity from 2 to

55 dS m^{-1}, the CPAC 09 genotype experienced a reduction in shoot biomass of 95.87% and in root biomass of 99.78%. For CPAC 11, this reduction was 95.12% for the shoot biomass and 97.92% for the roots biomass (Figure 13).

Grain yield in the CPAC 09 genotype was reduced by 54.1% at EC_w 25 dS m^{-1} compared to the control. For the CPAC 11 genotype, the reduction in grain yield was 61.41%. Despite the survival of most plants under EC_w of 55 dS m^{-1}, in both genotypes, grain yield was greatly reduced, reaching 0.02 g $plant^{-1}$ in CPAC 09 and 0.5 g $plant^{-1}$ in CPAC 11. In general, the CPAC 09 genotype presented a lower grain production than the CPAC 11 genotype in all applied treatments, despite the high biomass of the shoot and roots (Figure 13).

Figure 13 – Dry biomass and grain yield of quinoa genotypes CPAC 09 and CPAC 11. (A) Shoot biomass, (B) roots biomass, and (C) grain yield

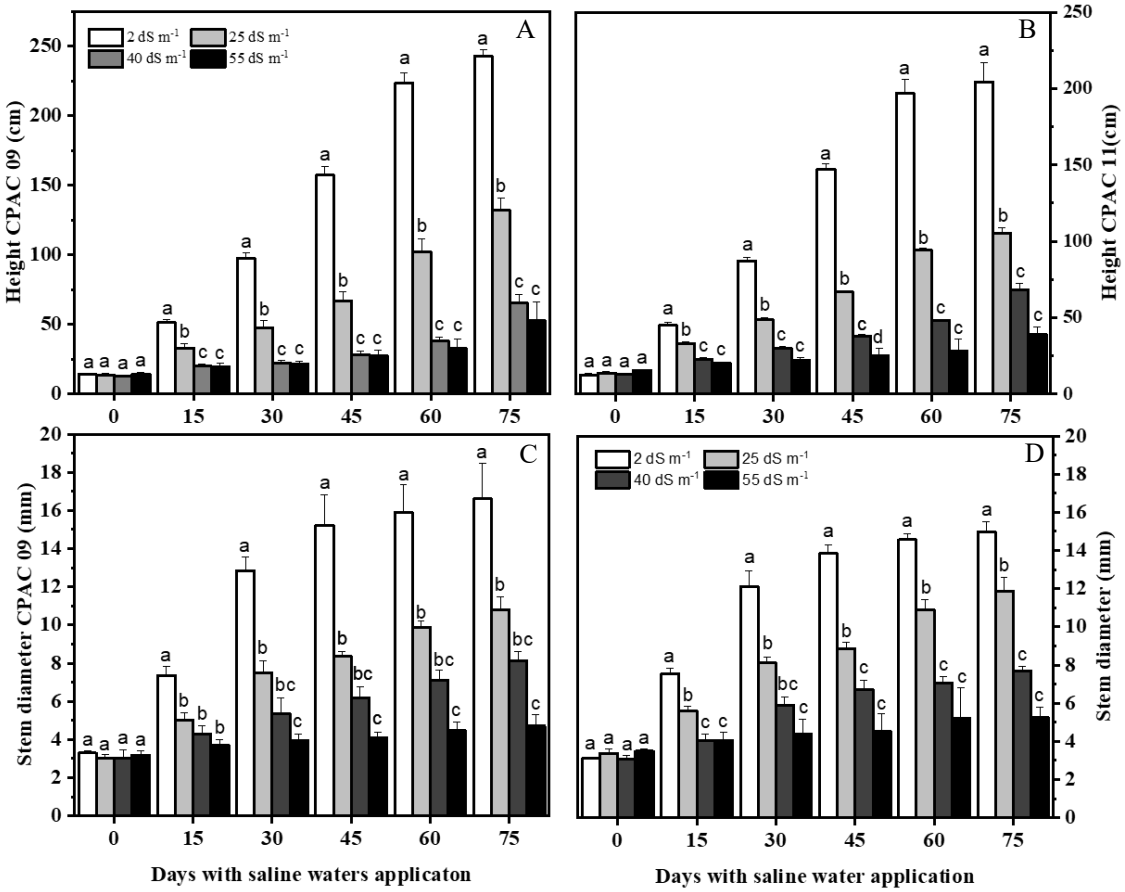


Different lower-case letters indicate the significant (P≤0.05) difference among the four EC_w treatments within a genotype. Significant (P≤0.05) difference between the two genotypes is marked with * at a salinity levels. No mark means that there is no corresponding significant difference between the two genotypes (P>0.05).

Figure 14 shows quinoa plant development from the beginning of the treatment application (after 25 days of sowing) until the grain maturation stage (after 150 days of sowing and 125 of the beginning of treatment application). With the irrigation with 40 and 55 dS m^{-1} waters, plants of the two genotypes (CPAC 09 and CPAC 11) had shown slow increases in both height and stem diameter (Figure 14). The tallest plants were those of the CPAC 09 genotype,

reaching heights of 242.75 cm and stem diameter of 16.61 mm after 75 days of treatment with EC_w of 2 dS m^{-1} (Figure 14A). CPAC 11 had an average height of 204.25 cm and stem diameter of 14.97 mm under the same conditions (Figure 14B). In treatments of 55 dS m^{-1}, plants of the genotypes CPAC 09 and CPAC 11 reached 52.33 and 39 cm in height, respectively, with a reduction of 78.45% at 55 dS m^{-1} compared to 2 dS m^{-1} in CPAC 09 and 80.91% in CPAC 11 (Figure 14A and 14B).

Figure 14 – Growth parameters for quinoa genotypes CPAC 09 and CPAC 11. (A) CPAC 09 height; (B) CPAC 11 height; (C) CPAC 09 stem diameter; (D) CPAC 11 stem diameter.



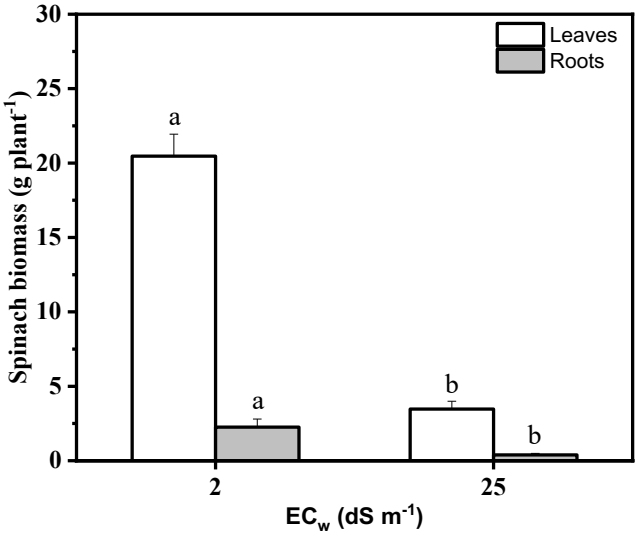
Different lower-case letters indicate the significant (P≤0.05) difference among the four EC_w treatments within a genotype. Significant (P≤0.05) difference between the two genotypes is marked with * at a salinity. No mark means that there is no corresponding significant difference between the two genotypes (P>0.05)

As the salinity increased from 2 to 25 dS m^{-1} there was a reduction of 45.62% in the height of plants of the CPAC 09 genotype and of 35.04% in the stem diameter. For CPAC 11, the decrease was 48.47% and 20.71% for height and stem diameter, respectively, between salinities of 2 and 25 dS m^{-1} (Figure 14A and 14B).

For spinach, there was a significant decrease ($p<0.001$) in the biomass of leaves and roots between salinities of 2 and 25 dS m^{-1} (Figure 15). Between the treatments applied, there

was a reduction of 83.04% in spinach leaf biomass and 82.74% in roots. Despite the reduction in growth and biomass, spinach plants were able to develop and reproduce with the application of irrigation water with an EC_w of 25 dS m^{-1}.

Figure 15 – Spinach leaves and roots biomass under ECw of 2 and 25 dS m^{-1}



Different lower-case letters indicate the significant (P≤0.05) difference among the two ECw treatments.

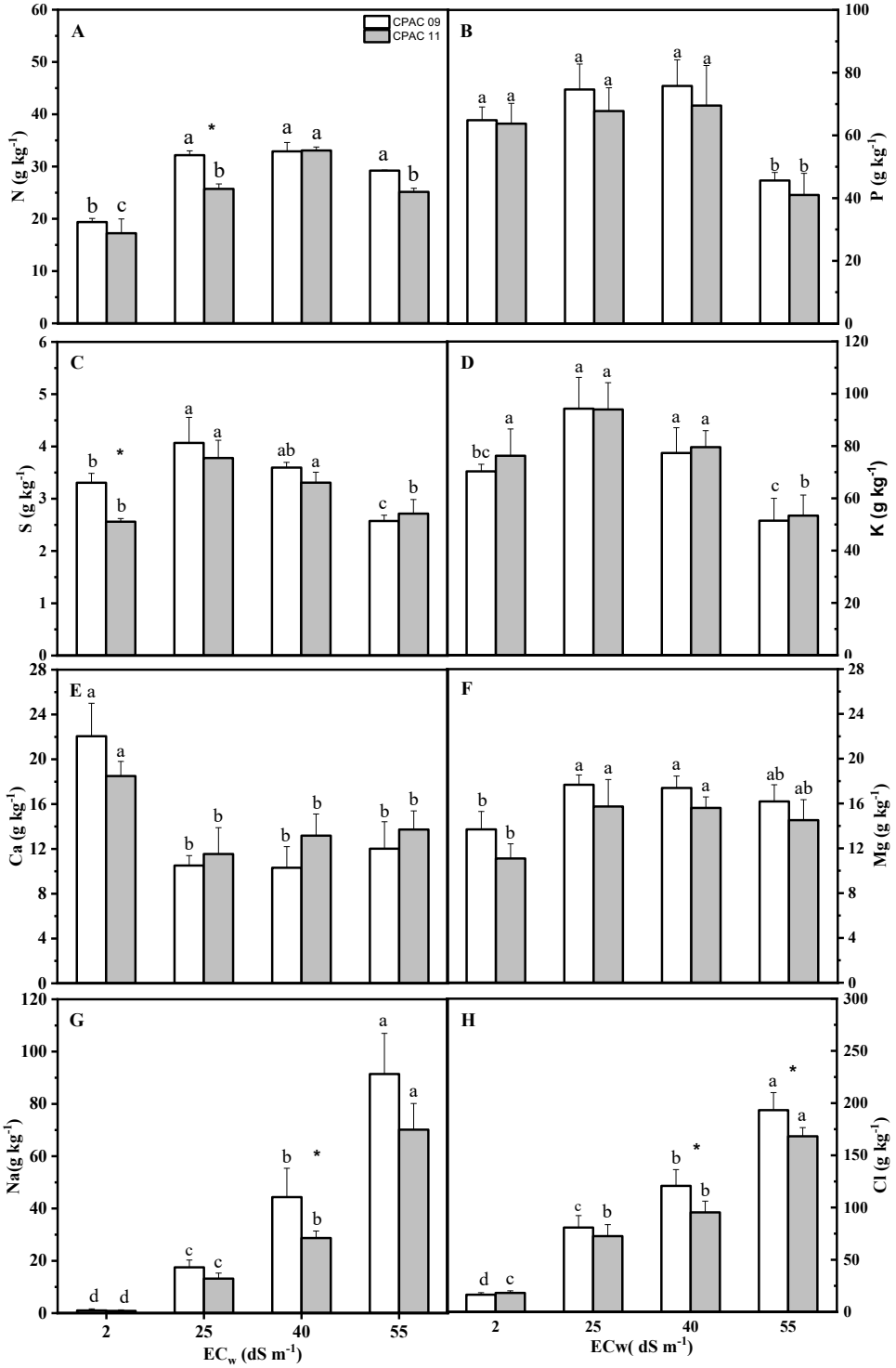
5.3.2. Nutritional analysis

5.3.2.1. Quinoa

The nutritional analysis of the leaves of genotypes CPAC 09 and CPAC 11 shows that, regardless of the genotype evaluated, there was an increase in the leaf concentration of Na, Cl, and Mg and a reduction in N, P, K, and Ca (Figure 16). The CPAC 09 genotype had higher concentrations of Na and Cl compared to CPAC 11, mainly when water with EC_w of 40 and 55 dS m^{-1} were applied (Figure 16). Among the elements evaluated, quinoa had the potential to accumulate salts in its leaves in the order of Cl > K > Na > Ca > Mg. In the 2 dS m^{-1} treatment, among different elements, K had the highest concentration in the quinoa leaf, in both genotypes (Figure 16).

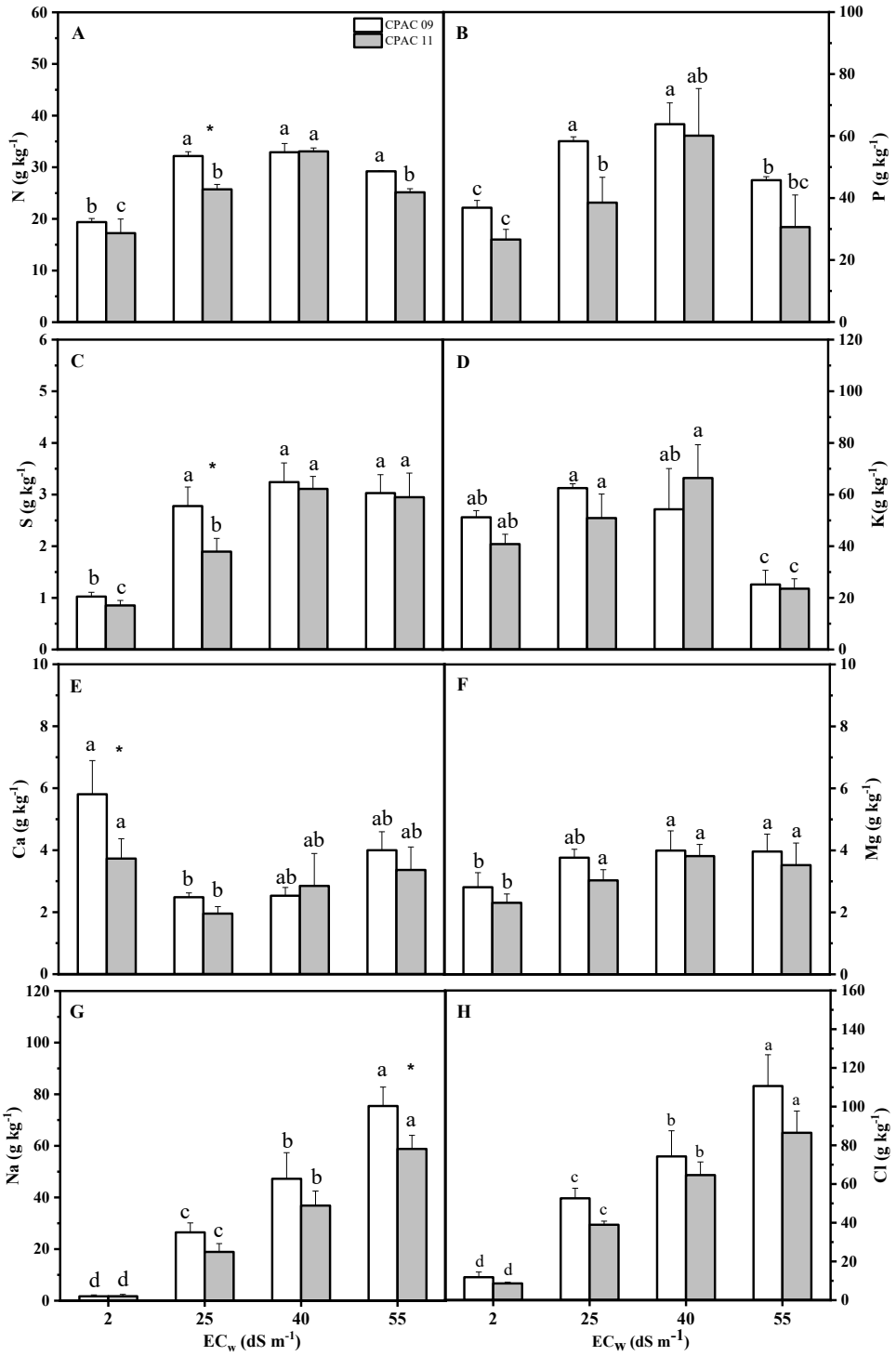
For nutritional analysis of the stems of the CPAC 09 and CPAC 11 genotypes (Figure 17), there was an increase in the concentration of S, Na, Cl, and Mg and a reduction in K and Ca. The CPAC 09 genotype had higher concentrations of Na and Cl compared to CPAC 11 under the salinity treatments. The concentration of ions in the quinoa stem follows the order Cl > Na > K > Ca > Mg with increasing salinity. In the 2 dS m^{-1} treatment, among the different elements, K was had the highest concentration in the quinoa stem, in both genotypes.

Figure 16 – Macronutrients, Na, and Cl concentration in quinoa leaves for the CPAC 09 and CPAC 11 genotypes. Different lower-case letters indicate the significant (P≤0.05) difference among the four EC_w treatments within a genotype



Significant (P≤0.05) difference between the two genotypes is marked with * at a salinity. No mark means that there is no corresponding significant difference between the two genotypes (P>0.05)

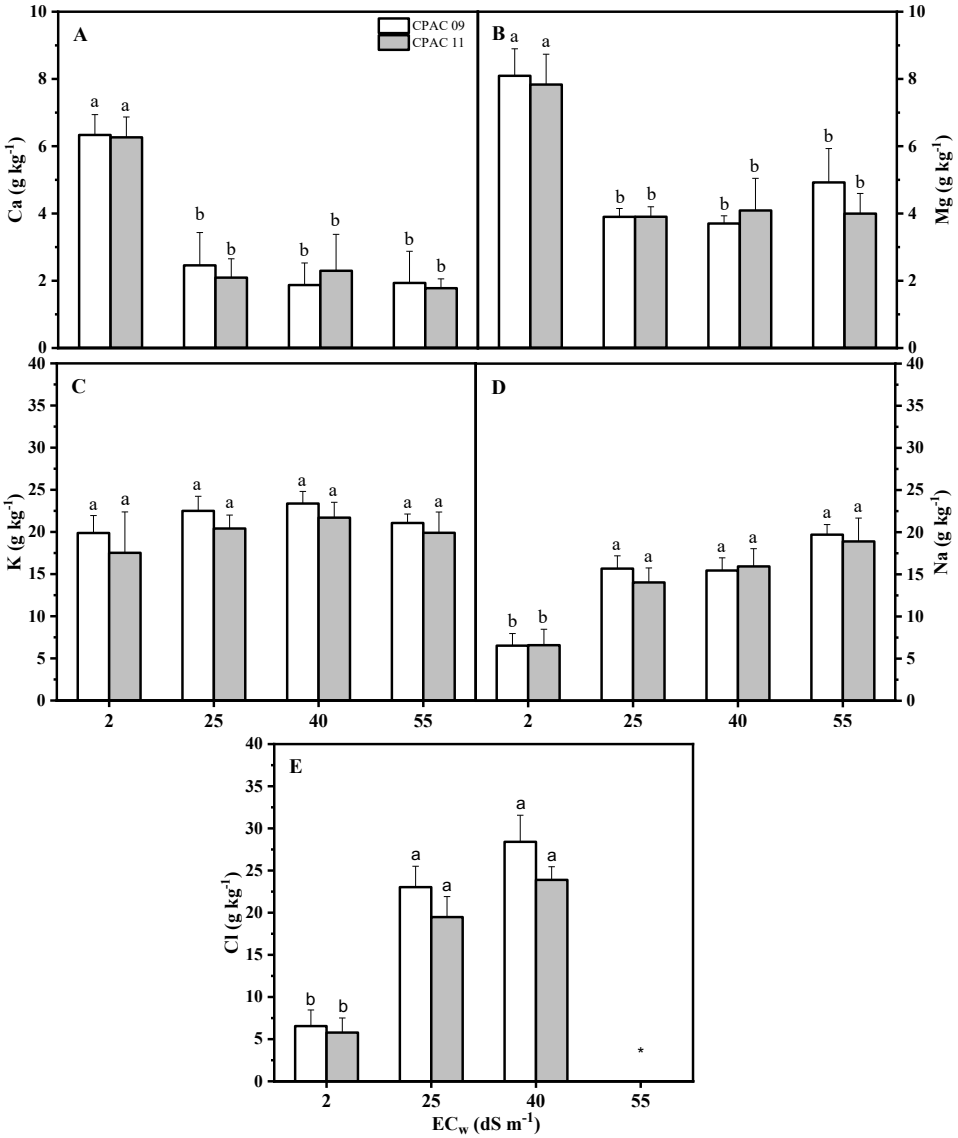
Figure 17 – Macronutrients, Na, and Cl concentration in quinoa stem for the CPAC 09 and CPAC 11 genotypes



Different lower-case letters indicate the significant (P≤0.05) difference among the four EC_w treatments within a genotype. Significant (P≤0.05) differences between the two genotypes is marked with * at a salinity. No mark means that there is no corresponding significant difference between the two genotypes (P>0.05)

For quinoa roots there was a reduction in Ca and Mg (figures 18A and 18B) concentrations with increasing salinity, whereas K did not change significantly (Figure 18C). On the other hand, there was an increase in Na and Cl concentrations. Due to the low biomass of roots in treatments of 55 dS m^{-1}, it was not possible to collect enough samples to perform the Cl^- analysis at this salinity.

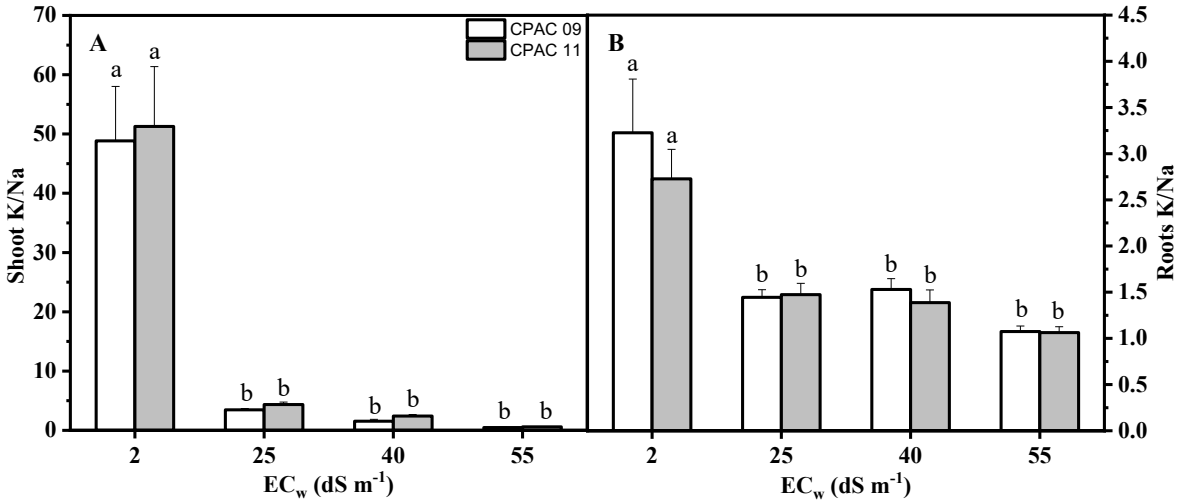
Figure 18 – Macronutrients, Na, and Cl concentration in quinoa roots for the CPAC 09 and CPAC 11 genotypes



Different lower-case letters indicate the significant (P≤0.05) differences among the four EC_w treatments within a genotype. * in figure 6E is related to a low roots biomass at ECw of 55 dS m^{-1} limiting Cl^- analysis.

For both genotypes, the K/Na ratio was reduced with the application of saline water. The greatest reductions were noticed in the quinoa shoot as shown in (Figure 19). For roots, the decrease in the K/Na ratio was less pronounced.

Figure 19 – K/Na ratio in quinoa shoot and roots for the CPAC 09 and CPAC 11 genotypes. (A) K/Na in shoot and (B) K/Na in roots

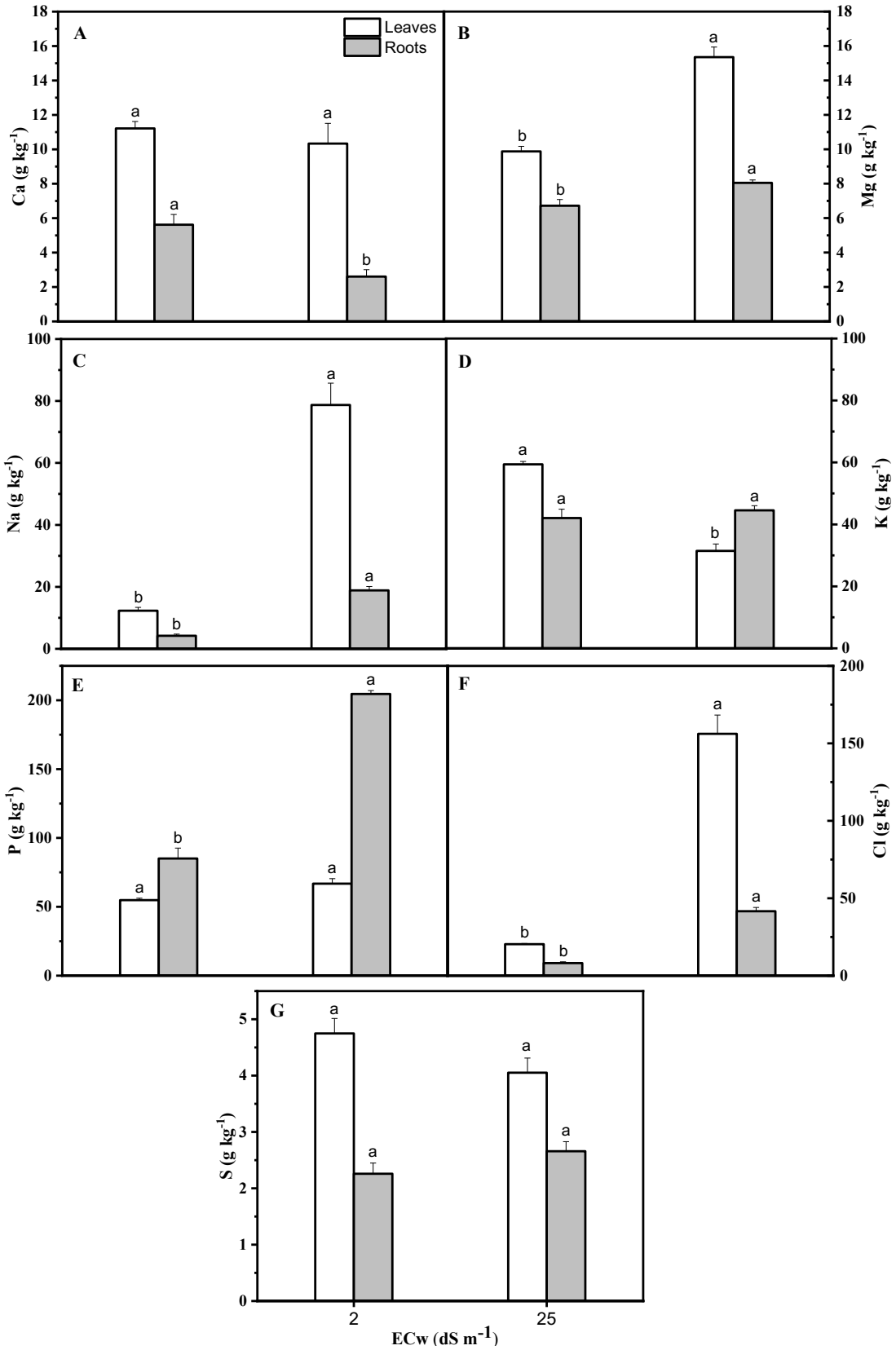


Different lower-case letters indicate the significant (P≤0.05) difference among the four EC_w treatments within a genotype.

5.3.2.2. Spinach

The concentrations of saline ions in spinach leaves and roots followed the trend seen in quinoa (Figure 20). For spinach leaves there was an increase in the concentrations of Mg, Na, and Cl in the 25 dS m^{-1} treatments and a reduction in K. For the roots, there was a reduction in Ca, an increase in Mg, Na, P, and Cl and K did not change significantly. Under non-saline conditions, spinach accumulated saline ions in the order of K>Cl>Na>Ca=Mg in the leaves, and K>Cl>Ca=Mg>Na in the roots (Figure 20). When saline water with an EC of 25 dS m^{-1} was applied, the ions most accumulated by spinach in the leaves were Cl>Na>K>Mg>Ca, and in the roots were K>Cl>Na>Mg>Ca. Thus, the ions most accumulated by spinach, whether in saline or non-saline conditions, were K, Na, and Cl, mainly in the leaves (Figure 20).

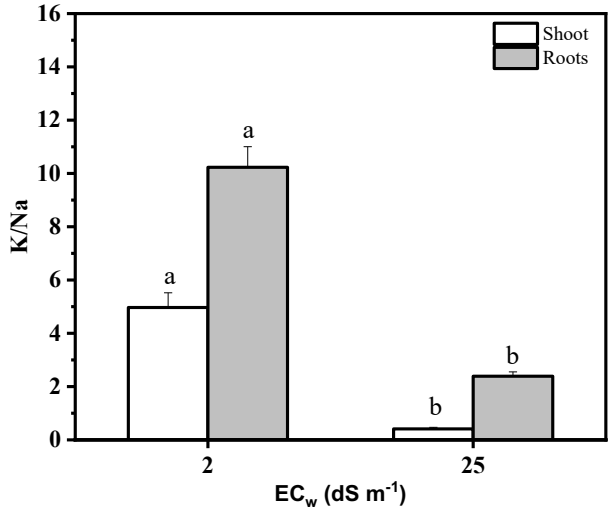
Figure 20 – Macronutrients, Na, and Cl concentration in spinach cv. Gazelle in leaves and roots



Different lower-case letters indicate the significant ($P \leq 0.05$) difference among the two EC_w treatments.

For spinach, the K/Na ratio was also reduced with the application of saline water. The greatest reductions were noticed in the spinach shoot as shown in (Figure 21). For roots, the decrease in the K/Na ratio was also less pronounced.

Figure 21 – K/Na ratio in spinach leaves and roots

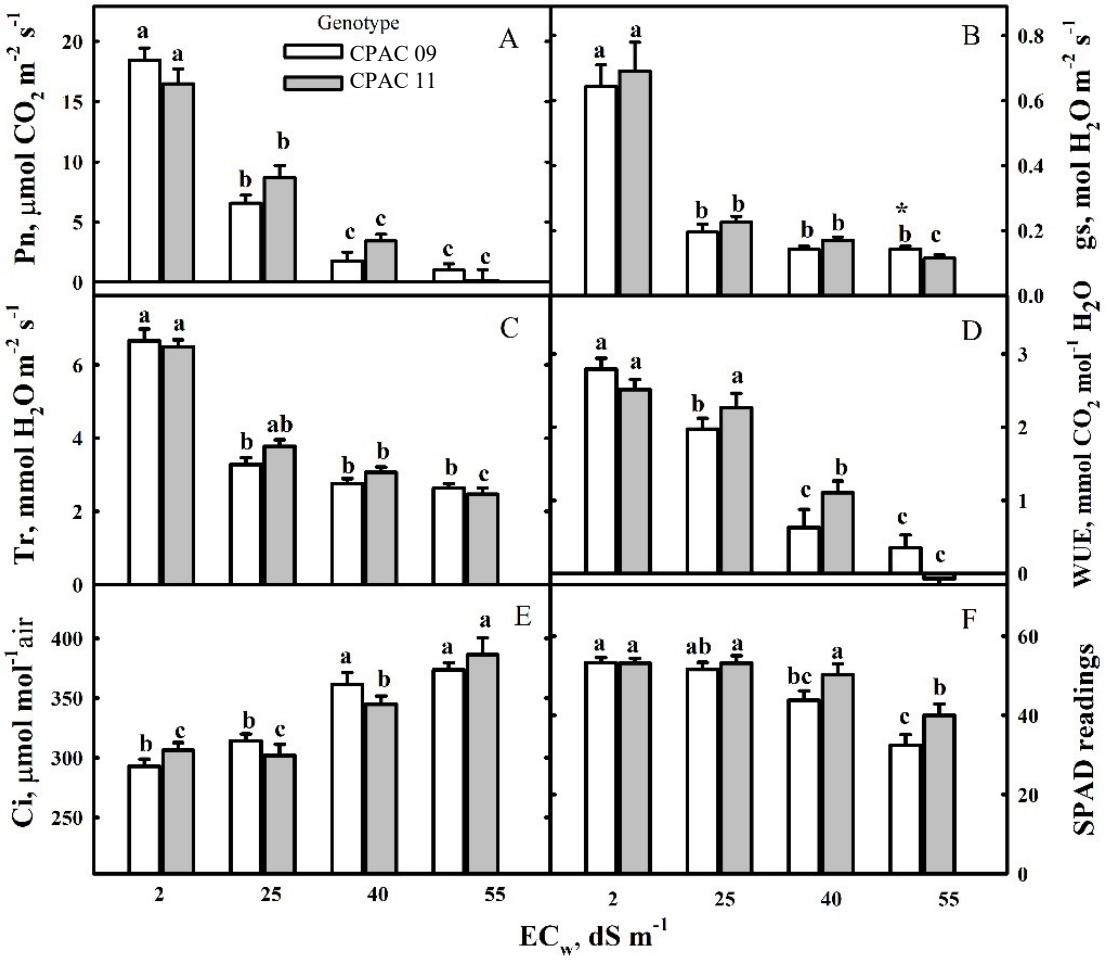


Different lower-case letters indicate the significant (P≤0.05) difference among the two EC_w treatments in leaves and roots.

5.3.3. Leaf gas exchange and SPAD readings

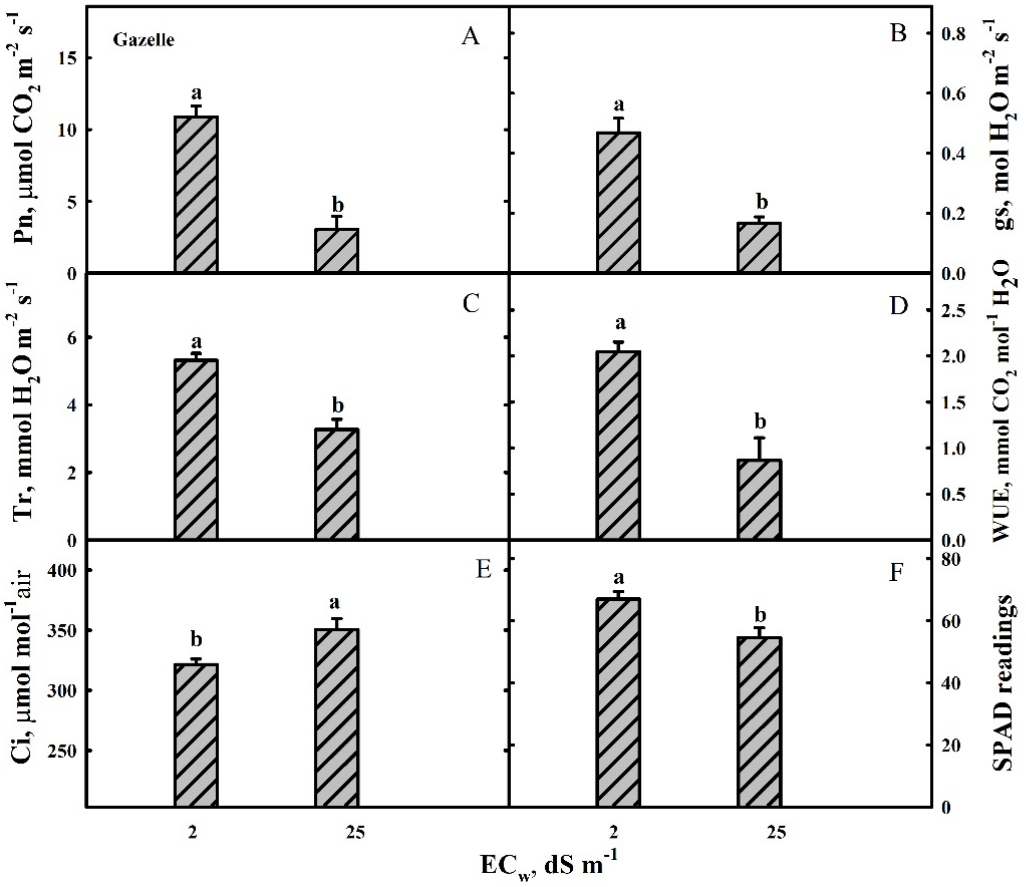
For quinoa, in the two genotypes evaluated, physiological parameters such as Pn, gs, Tr, WUE, and SPAD reading were significantly affected with increase in salt levels (Figure 22). Despite the reduction in stomata density (gs), CO_2 diffusion increased with increase in salinitiy. The same pattern was observed in spinach between salinities of 2 and 25 dS m^{-1} (Figure 23). In general, quinoa plants had their photosynthetic apparatus most affected in 40 and 55 dS m^{-1} treatments.

Figure 22 – Physiological responses of two quinoa genotypes, CPAC 9 and CPAC 11, under four irrigation water salinities. (A) leaf net photosynthetic rate (Pn); (B) stomatal conductance (gs); (C) transpiration rate (Tr); (D) water use efficiency (WUE); (E) intercellular CO_2 concentration (Ci); (F) SPAD readings



The salinities were measured as averaged electrical conductivity in the irrigation water (EC_w) during salt treatment. Data are represented as means±1SE bar with a sample size of n=8 (leaf, one leaf per plant, two plants per pot). Different lower-case letters indicate the significant ($P \leq 0.05$) difference among the four EC_w treatments within a genotype. Significant ($P \leq 0.05$) difference between the two genotypes is marked with * at a salinity. No mark means that there is no corresponding significant difference between the two genotypes ($P > 0.05$)

Figure 23 – Physiological responses of of spinach, cultivar: Gazelle, under two irrigation water salinities. (A) leaf net photosynthetic rate (Pn); (B) stomatal conductance (gs); (C) transpiration rate (Tr); (D) water use efficiency (WUE); (E) intercellular CO_2 concentration (Ci); (F) SPAD readings



The salinities were measured as averaged electrical conductivity in irrigation water (EC_w) during salt treatment. Data are represented as means±1SE bar with a sample size of n=8 (leaf, one leaf per plant, two plants per pot). Different lower-case letters indicate a significant ($P \leq 0.05$) difference between the two salinity treatments

5.3.4. Soil Analysis

There were significant changes in pH, EC_e, SAR, and ESP of the evaluated soil, at depths of 0-10 and 10-20cm with the application of water with high salinity levels in both quinoa genotypes (Tables 23 and 24). The highest concentrations of salts in the soil were observed in the 0-10 cm layer (layer with the greatest roots predominance). With the increase in the salinity, there was significant increase in EC_e, SAR, and ESP, reaching means (between both genotypes) of 65.59 dS m^{-1}, 72.35, and 30.28%, respectively, at the depth of 0 - 10cm. For the depth of 10-20 cm these values decreased to 49.29 dS m^{-1}, 64.46, and 40.26%, respectively.

The concentration of salts in the soil, expressed as EC_e, SAR, and ESP, was not significantly influenced by quinoa genotypes. Pots with CPAC 09 had a lower pH (mean of 8.03) than pots with CPAC 11 (mean of 8.17), in the 0-10 cm layer. In general, the pH in the 0-10 cm layer was reduced throughout the treatments for both genotypes, with means varying from 8.26 to 7.98 between salinities of 2 and 55 dS m^{-1} (Table 23). For the 10-20 cm soil layer, there was no significant difference between the genotypes; however, there was a variation in pH from 8.69 to 8.20 when salinity levels increased from 2 to 55 dS m^{-1} (Table 24).

Table 23 – Salinity and sodicity of soils after irrigation with saline waters in the 0 – 10 cm layer

ECw (dS m^{-1})	pH			pHes			ECe (dS m^{-1})		
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	8.23	8.29	8.26A	8.36	8.31	8.34A	2.19	2.37	2.28D
25	7.98	8.36	8.17AB	7.80	7.92	7.86B	34.95	34.68	34.82C
40	7.94	8.01	7.97B	7.59	7.63	7.61C	54.07	53.16	53.62B
55	7.96	8.01	7.98B	7.53	7.52	7.52C	63.62	67.56	65.59A
Mean	8.03b	8.17a		7.82	7.84		38.71	39.44	
ANOVA		F			F			F	
Variety		5.93*			0.90^{NS}			0.11^{NS}	
Salinity		6.02**			214.21***			149.88***	
Variety x Salinity		1.89^{NS}			2.16^{NS}			0.23^{NS}	
CV		2.03			0.90			16.32	
	SAR ($mmol_c$ $L^{-1})^{0.5}$			ESP (%)					
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean			
2	4.99	5.82	5.40D	11.07	11.24	11.16B			
25	46.64	47.83	47.24C	31.99	32.55	32.27A			
40	63.58	58.32	60.95B	31.10	30.21	30.66A			
55	72.93	71.77	72.35A	30.59	29.97	30.28A			
Mean	47.03	45.93		2,33	2,45				
ANOVA		F			F				
Variety		0.34^{NS}			0.02NS				
Salinity		243.36***			57.76***				
Variety x Salinity		0.62^{NS}			0.07^{NS}				
CV		11.41			14.26				

Uppercase letters compare means in the column and lowercase letters compare means in line. Means compared using the Tukey test at 5% probability. *** and ^{NS} are equal to 0.1% of probability and non-significant, respectively.

Table 24 – Salinity and sodicity of soils after irrigation with saline waters in the 10 – 20 cm layer

ECw (dS m^{-1})	pH			pHes			ECe (dS m^{-1})		
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	8,66	8,72	8,69A	8,37	7,08	7,72	2,42	2,35	2,39D
25	8,36	8,53	8,44B	7,97	8,00	7,99	26,10	29,15	27,62C
40	8,31	8,22	8,27C	7,85	7,80	7,83	41,31	39,81	40,56B
55	8,21	8,20	8,20C	7,76	7,73	7,74	48,32	50,27	49,29A
Mean	8,38	8,42		7,99	7,65		29,54	30,39	
ANOVA		F			F			F	
Variety		0.78NS			1.14NS			1.01NS	
Salinity		32.85***			0.14NS			578.11***	
Variety x Salinity		2.04NS			1.03NS			1.43NS	
CV		1.28			11.31			8.02	

	SAR ($mmol_c$ $L^{-1})^{0.5}$			ESP (%)		
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	7,89Ca	7,47Ca	7,68	13,72	12,70	13,21B
25	44,28Ba	49,74Ba	47,01	36,55	40,54	38,69A
40	59,91Aa	51,31Bb	55,61	38,73	36,35	37,54A
55	67,22Aa	61,69Aa	64,46	42,36	38,15	40,26A
Mean	44,83	42,55		32,88	31,97	
ANOVA		F			F	
Variety		1.97^{NS}			0.46^{NS}	
Salinity		239.12***			93.15***	
Variety x Salinity		3.62*			1.72^{NS}	
CV		10.48			11.62	

Uppercase letters compare means in the column. Means compared using the Tukey test at 5% probability. ***, *, and ^{NS} are equal to 0.1, 5% of probability, and non-significant, respectively.

Analyzing soluble ions, an increase in the general mean for Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and Cl^{-} was noted for both depths (0-10 and 10-20 cm) (Tables 25 and 26). There were no significant differences between genotypes and soluble ions in the soil. For Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and Cl^{-}, in the 0-10 cm layer, the general mean between the 2 and 55 dS m^{-1} treatments were 8.78 and 67.12 for Ca^{2+}, 3.37 and 112.88 for Mg^{2+}, 0.34 and 3.74 for K^{+}, 13.21 and 688.22 for Na^{+}, and 6.85 and 745.35 $mmol_c$ L^{-1} for Cl^{-}.

For the 10-20 cm layer, the mean values between salinities of 2 and 55 dS m^{-1} for Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and Cl^{-} were 7.03 and 42.49; 2.37 and 72.63; 0.15 and 1.25; 16.70 and 487.48 and 9.35 and 542.52, respectively. For all soluble ions evaluated in this work, there were no significant differences between the CPAC 09 and CPAC 11 genotypes, but with an increase in all ions evaluated with increasing salt levels.

Regarding exchangeable cations (Tables 27 and 28), there was a similar tendency for their accumulation with the soil among the treatments, except for Ca^{2+}, where there was a reduction in its concentration with the increase in salinity, but without significant changes between the genotypes. For the 0-10 cm layer, the concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} between salinities of 2 and 55 dS m^{-1} varied from 4.67 to 3.32; 1.16 to 2.44; 0.76 to 2.62, and 0.11 to 0.17 $cmol_c$ kg^{-1}, respectively. For the 10-20cm layer, the mean values of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} between the EC_w of 2 and 55 dS m^{-1} varied from 5.32 to 2.18; 1.16 to 2.21; 1.01 to 2.98, and 0.05 to 0.04 $cmol_c$ kg^{-1}, respectively.

Table 25 – Soluble cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) and Cl^{-} in soil after irrigation with saline waters in the 0 – 10 cm layer

ECw (dS m^{-1})	Ca^{2+}			Mg^{2+}			K^{+}		
	mmol$_c$ L^{-1}								
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	8.75	8.82	8.78C	3.38	3.36	3.37D	0.39	0.29	0.34C
25	37.40	35.02	36.21B	55.94	48.00	51.97C	2.17	1.64	1.90C
40	60.54	63.92	62.23A	93.16	84.84	89.00B	3.76	2.80	3.28B
55	63.30	70.94	67.12A	110.20	115.57	112.88A	4.11	3.38	3.74A
Mean	42.49	44.67		65.67	62.94		2.61a	2.02b	
ANOVA		F			F			F	
Variety		0.28			0.35NS			9.25**	
Salinity		40.60			105.92***			64.01***	
Variety x Salinity		0.26			0.51NS			0.90NS	
CV		27.37			20.40			23.35	

	Na^{+}			Cl^{-}		
	mmol$_c$ L^{-1}					
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	12.09	14.32	13.21D	6.34	7.37	6.85D
25	318.17	306.63	312.40C	312.24	337.25	324.75C
40	558.23	500.17	529.20B	561.96	583.72	572.84B
55	682.79	693.64	688.22A	695.88	794.82	745.35A
Mean	392.82	378.69		394.10	430.79	
ANOVA		F			F	
Variety		0.27^{NS}			1.52^{NS}	
Salinity		113.61***			116.64***	
Variety x Salinity		0.31^{NS}			0.52^{NS}	
CV		20.10			20.37	

Uppercase letters compare means in the column and lowercase letters compare means in line. Means compared using the Tukey test at 5% probability. *** and ^{NS} are equal to 0.1% of probability and non-significant, respectively.

Table 26 – Soluble cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) and Cl^{-} in soil after irrigation with saline waters in the 10 – 20 cm layer

EC_w (dS m^{-1})	Ca^{2+}			Mg^{2+}			K^{+}		
	mmol$_c$ L^{-1}								
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	6.99	7.07	7.03C	2.37	2.36	2.37D	0.15	0.14	0.15C
25	23.06	23.96	23.51B	30.33	31.69	31.01C	0.83	0.77	0.80B
40	33.54	42.66	38.10A	58.23	53.02	55.63B	1.14	1.04	1.09A
55	36.79	48.18	42.49A	72.59	72.68	72.63A	1.32	1.18	1.25A
Mean	25.10	30.47		40.88	39.94		0.86	0.78	
ANOVA		F			F			F	
Variety		3.74NS			0.34NS			2.66NS	
Salinity		33.34***			352.69***			117.14***	
Variety x Salinity		1.06NS			0.80NS			0.41NS	
CV		28.27			11.40			15.49	

	Na^{+}			Cl^{-}		
	mmol$_c$ L^{-1}					
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
2	17.18	16.22	16.70D	9.55	9.14	9.35D
25	228.80	258.57	243.68C	262.49	292.71	277.60C
40	404.78	352.97	378.88B	411.92	425.46	418.69B
55	496.43	478.52	487.48A	511.47	573.56	542.52A
Mean	286.80	276.57		298.86	325.22	
ANOVA		F			F	
Variety		1.11^{NS}			6.16*	
Salinity		437.00***			465.02***	
Variety x Salinity		3.07^{NS}			1.60^{NS}	
CV		9.74			9.63	

Uppercase letters compare means in the column. Means compared using the Tukey test at 5% probability. ***, *, and ^{NS} are equal to 0.1, 5% of probability, and non-significant, respectively.

Table 27 – Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) in soil after irrigation with saline waters in the 0 – 10 cm layer

ECw (dS m^{-1})	Ca^{2+}			Mg^{2+}			Na^{+}			K^{+}		
	cmol$_c$ kg^{-1}											
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
0	4.68	4.66	4.67A	1.17	1.16	1.16B	0.76	0.75	0.76B	0.15	0.07	0.11
10	2.61	3.52	3.07B	2.48	2.42	2.45A	2.44	2.73	2.59A	0.16	0.14	0.15
20	2.85	3.24	3.05B	2.71	2.19	2.45A	2.59	2.36	2.48A	0.20	0.10	0.15
40	3.27	3.37	3.32AB	2.50	2.38	2.44A	2.62	2.51	2.56A	0.22	0.12	0.17
Mean	3.35	3.70		2.21	2.04		2.10	2.09		0.18a	0.11b	
ANOVA		F			F			F			F	
Variety		0.73^{NS}			4.32^{NS}			0.06^{NS}			18.59***	
Salinity		3.60*			56.25***			138.02***			2.13^{NS}	
Variety x Salinity		0.26^{NS}			1.84NS			2.18^{NS}			1.37^{NS}	
CV		32.67			11.37			10.27			33.01	

Uppercase letters compare means in the column and lowercase letters compare means in line. Means compared using the Tukey test at 5% probability. ***, *, and ^{NS} are equal to 0.1, 5% of probability, and non-significant, respectively.

Table 28 – Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) in soil after irrigation with saline waters in the 10 – 20 cm layer

ECw (dS m^{-1})	Ca^{2+}			Mg^{2+}			Na^{+}			K^{+}		
	cmol$_c$ kg^{-1}											
	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean	CPAC 09	CPAC 11	Mean
0	5.30	5.35	5.32A	1.12Ba	1.20Ca	1.16	1.05	0.96	1.01B	0.07	0.02	0.05
10	2.63	2.24	2.43B	2.22Aa	2.05ABa	2.14	2.80	3.00	2.90A	0.04	0.05	0.05
20	2.18	2.44	2.31B	2.34Aa	1.83Bb	2.08	2.90	2.45	2.67A	0.07	0.02	0.04
40	1.93	2.43	2.18B	2.20Aa	2.23Aa	2.21	3.06	2.89	2.98A	0.04	0.05	0.04
Mean	3.01	3.11		1.97	1.80		2.45	2.33		0.03	0.05	
ANOVA		F			F			F			F	
Variety		0.40^{NS}			4.77*			1.32^{NS}			2.83^{NS}	
Salinity		82.85***			60.32***			72.38***			0.06^{NS}	
Variety x Salinity		1.28^{NS}			4.53*			1.53^{NS}			3.46^{NS}	
CV		15.34			9.48			12.95				

Uppercase letters compare means in the column and lowercase letters compare means in line. Means compared using the Tukey test at 5% probability. ***, *, and ^{NS} are equal to 0.1, 5% of probability, and non-significant, respectively.

5.4. Discussion

5.4.1. Quinoa and Spinach biometric, physiological, and nutritional responses under extremely saline waters

The use of saline waters with compositions proportional to seawater provides for quinoa (EC_w of 2, 25, 40, and 55 dS m^{-1}) and for spinach (EC_w of 2 and 25 dS m^{-1}) an understanding of the potential of these two crops under extreme saline/sodic stress.

With the increase in salts concentration, especially Cl^- and Na^+ ions, quinoa progressively reduced its shoot and roots biomass and grain yield (Figures 13A, 13B, and 13C). Despite being considered as a facultative halophyte, quinoa, when cultivated in extremely sodic environments, suffers deleterious effects on its growth and nutritional balance (ABBAS et al., 2021; TURCIOS; PAPENBROCK; TRÄNKNER, 2021).

With the increase in sodicity for both CPAC 09 and CPAC 11 quinoa genotypes, Na and Cl concentration in leaves, stems, and roots were elevated and K concentration reduced (Figures 16D, 16G, 16H, 17D, 17G, 17H, 18C, 18D, and 18E). This ionic imbalance directly affected the growth of quinoa plants, with a decrease of more than 45% in final growth in both genotypes between salinities of 2 and 25 dS m^{-1} (Figure 14). Between salinities of 2 and 55 dS m^{-1}, the deleterious effect of high salt concentrations reduced the final growth of quinoa plants in the two genotypes by more than 78%.

According to Abbas et al. (2021), sodicity has a greater deleterious effect on quinoa plants than salinity, as it increases the concentration of Na in plant tissues, favoring high oxidative damage and increment in enzymes activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), causing greater energy expenditure in the plant.

Despite the high concentrations of Na^+ and Cl^- in irrigation water (table 22), quinoa plants showed homeostasis in N and Mg concentration in shoot (Figures 16A and 16F), especially in treatments with extreme salinity (25, 40, and 55 dS m^{-1}) for the two genotypes.

In saline environments, quinoa has demonstrated a high capacity to produce grains and accumulate ions in its plant tissues to adjust its osmotic potential in relation to soil (JACOBSEN; MUJICA; JENSEN, 2003). For quinoa European varieties Atlas, Jessie, Pasto, and SelRiobamba, the reduction in quinoa grain yield was greater than 60% at salinity of 30 dS m^{-1}, similar to the results found in the present work. The accumulation of Na and Cl in leaves also had a significant increase for European varieties, where Jessie obtained the highest

concentrations when water with an EC of 30 dS m^{-1} was applied (approximately 200 mM for Na and 550 mM for Cl in quinoa leaves) (JARAMILLO ROMAN et al., 2020).

Spinach (cv Gazelle), despite classified as a glycophyte (species not tolerant to salinity), it was able to survive under salinity of 25 dS m^{-1}. This survival was accompanied by increased concentrations of Na and Cl in both leaves and roots (Figures 20C and 20F), similar to quinoa, although its biomass was reduced by more than 80% (Figure 13). Despite maintaining P homeostasis in the leaves (Figure 20E), when irrigated with salinity of 25 dS m^{-1}, the P concentration in the roots increased, possibly because the greater energy production in this part of the plant, as P is the element that participates in the ATP molecule responsible for the formation of free energy in the plant.

For spinach, the reduction in its biomass with the application of saline water was also described by Ors and Suarez (2017). The authors concluded that spinach initially increases its biomass under electrical conductivity of water (EC_w) up to 7 dS m^{-1}. However, compared to the control treatment (0 dS m^{-1}), biomass reduction only occurred under salinities of 15 dS m^{-1}. According to the authors, an increase in salinity also promoted greater uptake of Na^+ and Cl^-, primarily at the expense of K^+.

For Bhatti et al. (2021), using the English and Sindhi genotypes, spinach reduced its biomass by 30% when water with EC_w of 6 and 8 dS m^{-1} was applied. The authors also reported an increase of 3.4 and 2.7-folds more Na and Cl in the leaves compared to the 0.3 dS m^{-1} treatment, also with a reduction in K absorption. In this way, one of the mechanisms for surviving saline stress, used by spinach, is the reduction of its shoot and root biomass and accumulation of salts in the leaves for osmotic adjustment.

In halophyte plants such as quinoa, one of the tolerance mechanisms to excess of Na^+ in leaves is the rapid removal of this ion from the cell cytosol and a high concentration of K^+ in shoot and roots (high K/Na ratio), generating a high proton pump activity. This phenomenon is less common in glycophytic plants (SUN et al., 2017). Other quinoa strategies to tolerate high salts concentration are Na^+ sequestration in vacuoles, xylem Na^+ and K^+ loading, high tolerance to ROS, and reduction in stomatal density (HARIADI et al., 2011; ADOLF; JACOBSEN; SHABALA, 2013; SHABALA; BOSE; HEDRICH, 2014). In this work, high saline waters reduced the K/Na ratio in both quinoa and spinach, especially in shoot (Figures 21A and 23).

Ions such as Na^+ and Cl^- in high concentrations in cellular tissues can cause metabolic and osmotic problems in plants. These elements are transported via xylem in the plant, which leads to a higher concentration of Na^+ and Cl^- in the leaves rather than in roots (TESTER; DAVENPORT, 2003). This phenomenon was also observed in this work in both quinoa and

spinach. According to Tester and Davenport (2003), the phenomenon of greater salt accumulation in halophyte is not necessarily what differentiates a halophyte plant from a glycophyte, as a glycophyte can also accumulate large concentrations of salts. The main difference between the two classes of plants is how long each one can survive with these salts in their tissues, mainly in the shoot, without generating osmotic damage and ionic toxicity in the plant.

The reduction of approximately 80% in quinoa biomass is reached in salinity of 40dS m^{-1}, mainly in the genotype CPAC 09. For this reduction to occur in spinach, it was necessary to apply irrigation water with EC_w of 25 dS m^{-1}. Therefore, the quinoa genotypes evaluated in this work have greater tolerance to salinity than spinach. Despite this difference, irrigation with EC_w 25 dS m^{-1} already exceeds the critical salinity limit (4 dS m^{-1}) by more than six-fold, proving that there are spinach varieties that have a high tolerance to salinity in extreme environments.

As salinity EC_w increased from 2 (control) to 25 dS m^{-1}, for quinoa, leaf net photosynthetic rate, Pn, decreased significantly ($P \leq 0.05$) and dramatically by 65% and 47% for genotype CPAC 9 and CPAC 11, respectively (Figure 22A), quite similar to some reported results for other quinoa variety (HINOJOSA et al., 2018). As EC_w further increased, Pn of both genotypes continued declining significantly and the leaves of plants receiving the highest salt stress treatment at EC_w of 55 dS m^{-1} could only perform about 22% and 17% of net photosynthesis that the leaves of control plants performed for genotype CPAC 9 and CPAC 11, respectively (Figure 22A). This response of Pn to increasing EC_w could account for largely the reduction of quinoa growth biomass by the increasing salt stress.

The reduction in Pn might be due to the reduction in leaf stomatal conductance, gs, because significant ($P \leq 0.05$) and dramatical reduction in gs was also found: 70% and 67% reduction in gs when EC_w increased from 2 to 25 dS m^{-1}, and 78 % and 83% reduction in gs when EC_w increased from 2 to 55 dS m^{-1} for genotype CPAC 9 and CPAC 11, respectively (Figure 22B). The stomatal closuring could reduce CO_2 diffusion into leaves and thus cut down CO_2 uptake by leaves and supply to photosynthesis to cause a reduced Ci and a reduced Pn.

However, for both quinoa genotypes in this study, a reduced gs appears not resulting in a decreased Ci. Ci either remained unchanged as EC_w increased from 2 to 25 dS m^{-1} or increased significantly ($P \leq 0.05$) with further increase in EC_w (Figure 22E). This indicates that the supply of CO_2 was evidently not limited to be responsible for causing all the reduction in Pn. The unchanged Ci or higher Ci at a higher EC_w implies that the CO_2 fixation by photosynthetic enzymatic system might be slowed down, which caused the CO_2 diffused into leaves to accumulate more. This result strongly suggests that there was non-stomatal limitation on Pn

due to the salt stress and the function of some photosynthetic biochemical components were affected by the salt treatment.

Our findings agree with some recent reported result on leaf gas exchange of other quinoa cultivar under salt stress (KILLI; HAWORTH, 2017). They found that salt stress, induced by irrigating with saline water containing 300 mM of sodium chloride (equivalent to 60% of seawater salinity), reduced several biochemical components. These include the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase and the efficiency of electron transport for the regeneration of ribulose-1,5-bisphosphate.Because the non-stomatal limitation on Pn was observed on salt stressed and on drought + salt stressed plants but not on drought only stressed plants of quinoa, Killi and Haworth (2017) suggested that this non-stomatal or biochemical effects are largely due to ion toxicity in cells. Similarly, a possible non-stomatal limitation on Pn of spinach by the salt stress was also observed (Figures 23A, 23B, and 23E).

In general, for both quinoa and spinach, the application of extremely saline water increased the absorption of Na^+ and Cl^- and reduced K uptake, resulting in low plant development. This was also due to the reduction of physiological parameters such as photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (Tr), efficient water use (WUE), and SPAD reading (chlorophyll content in leaves). Despite the reduction in these parameters, CO_2 diffusion (Ci) was increased with increasing salinities, indicating a general similarity in the mechanisms of tolerance to saline stress between quinoa and spinach.

The similarities between quinoa and spinach can be explained due to their genetics. Both species belong to the same botanical family (Amaranthaceae) with a common ancestor that diverged approximately 16 million years ago (ZOU et al., 2017). In this present work, under the conditions established here, both quinoa (irrigated with water of 2, 25, 40, and 55 dS m^{-1}) and spinach (irrigated with water of 2 and 25 dS m^{-1}) managed to complete the whole plant cycle (even the seeds maturation) under greenhouse conditions in California – USA.

5.4.2. Soil chemical response to irrigation water similar to seawater under quinoa and spinach cultivation

One of the challenges for irrigation is the water quality in irrigated perimeters. As they are generally located in arid or semiarid areas, the water available is often saline and/or sodic, requiring the costly desalination technique for reuse these waters in agriculture (AMARAL; NAVONI, 2023). Even with the use of good water quality, whether from natural or desalinated sources, the high evaporation rate in these areas and soil conditions, naturally favor the

accumulation of soluble salts in the soil, generating pedogenetic processes called salinization and sodification (RENGASAMY, 2016; OLIVEIRA FILHO et al., 2020).

With the irrigation waters of 2, 25, 40, and 55 dS m^{-1} for pot cultivated quinoa, and a leaching fraction of 40%, there was a significant salt accumulation in the soil. This was particularly noticeable in treatments using saline waters, affecting both soil layers evaluated (0-10 and 10-20 cm, Tables 23 and 24). The general mean for EC_e among pots with both quinoa genotypes were 2.37, 34.68, 53.62, and 65.59 dS m^{-1} in the first layer (0-10 cm) and 2.39, 27.62, 40.56, and 49.29 dS m^{-1} in the second layer (10-20 cm) for each salinity tested in this work. This difference in salinity between soil layers is caused mainly by the effects of evapotranspiration, capillarity, and water absorption by the roots, favoring the ascendance of salts through the layers (RENGASAMY, 2016).

Among saline treatments, soil pH was reduced with increasing salinity (Tables 23 and 24). This behavior is related to the release of exchangeable H^+ from soil into solution, due to the high-pressure concentrations of cationic ions such as Ca^{2+}, Mg^{2+}, Na^+, and K^+ exert on soil colloids (VAN TAN; THANH, 2021). Between layers, the lowest pH was observed in the depth of 0-10 cm, mainly due to the acidifying effect that roots provide to soil. This occurs due to the ionic balance that exists in plants roots that, for a plant to absorb a cation, it is essential to release an H^+ proton into the soil (NYE, 1981). Therefore, depending on the anion or cation absorbed by the plant, the acidification phenomenon may occur in the rhizosphere. The roots, although present in the second layer, are found in smaller proportions, especially in saline treatments.

For parameters such as SAR and ESP, the increment in Na^+ in waters also favored the increase of these variables in the soil, reaching means above 60 for SAR and 30% for ESP in the 55 dS m^{-1} treatment. SAR and ESP values highly above the limit values for sodicity (13 and 15%, respectively) can already be seen in the 25 dS m^{-1} treatment. This would be lethal for most arable plants due to the osmotic effect (reduction in water absorption) and ionic effect (Na^+ and Cl^- toxicity) (TESTER; DAVENPORT, 2003). As described in the previous topic in this chapter, the increment in soil sodicity can causes deleterious effects on most agricultural crops. Despite being highly resistant to salinity and sodicity, quinoa plants greatly reduced their biomass and grain production when waters with EC_w greater than 25 dS m^{-1} are applied.

For all ions evaluated in this work, with the addition of saline water, there was a significant increase ($p<0.05$) in their concentrations in solution (Tables 25 and 26) and in exchangeable phase (table 6 and 7) of the soil. In the soluble phase, the ions in the highest concentration were Cl>Na>Mg>Ca>K. This behavior was already expected, as it reflects the

composition of the treatments, considering the proportion of ions present in seawater. In the colloidal phase, the soil adsorption of the ions was in the order of Ca>Mg=Na>K (Tables 27 and 28). This occurs due to the adsorption preference of elements, especially those with double valence, such as Ca^{2+} and Mg^{2+}. Despite the high concentration of Na^{+} in water, as it is a monovalent ion with a large, hydrated radius, Ca^{2+} has a greater adsorption force, mainly due to its bivalent charge (JALALI; ARIAN; RANJBAR, 2020).

In general, soils with a higher concentration of bivalent cations have a better physical structure than those with higher concentrations of monovalent ions such as Na^{+}, resulting in lower density, higher water infiltration rate, lower resistance to root penetration and greater distribution of pores in the soil (CHAUDHARI, 2001; QUIRK et al., 1997). Despite the high concentration of Ca^{2+} and Mg^{2+} in the exchangeable phase of the soil, the concentrations of Na^{+} are quite high. This also makes it difficult for plants to uptake K^{+}, which can be seen in figures 18D and 19D, where quinoa plants significantly reduced K^{+} absorption, especially in the 55 dS m^{-1} treatment.

As quinoa and spinach use K^{+} as a resistance mechanism to salts, the high concentrations of Na^{+} in the soil after the application of saline water indicates a reduction in the availability of K^{+} for the plants, favoring a reduction in growth and crop productivity.

Despite being a micronutrient, a high Cl^{-} concentration also causes harmful effects on agricultural crops. The concentration of Cl^{-} in soil solution, with values between 300 and 800 $mmol_c$ L^{-1}among saline treatments (Tables 25 and 26), may also have been one of the main factors limiting the development of quinoa under these conditions. High concentrations of Cl^{-} can promote a nutritional imbalance among anions in the plant, mainly inhibiting the absorption of $H_2PO_4^{-}$ and SO_4^{2-}. This is intensified in glycophytic plants, as they do not have mechanisms for excluding or using high concentrations of Cl^{-} in plant tissues (EL SABAGH et al., 2021).

Jaramillo Roman et al. (2020) obtained an EC_e of approximately 55 and 65 dS m^{-1} at the end of quinoa cycle with the water irrigation of 30 and 40 dS m^{-1}, respectively, similar concentration of those found in this work after using water of 40 and 55 dS m^{-1}. Despite Razzaghi et al. (2015), showed that the production of quinoa grain, cultivar Titicaca, one of the most tolerant to salinity, is reduced by approximately 50% at EC_e of 25 dS m^{-1}. The authors stated that at EC_e of 51.5 dS m^{-1} there was no grain production in quinoa. It was possible to observe, in this present work, that at soil salinity of 65 dS m^{-1}, quinoa managed to produce grains, despite the 99.70% reduction in grain yield (Figure 15C) between EC_e of 2.19 and 63.62 dS m^{-1} (Table 23) for CPAC 09 genotype and 97.9% reduction between EC_e of 2.37 e 67.57 dS m^{-1} for CPAC 11. Same pattern was also observed by Europian varieties such as Atlas, Jessie,

Pasto, and SelRiobamba (JARAMILLO ROMAN et al., 2020). This result proves the high potential of Brazilian genotypes to produce grain in extremely saline environments.

However, more studies must be carried out to evaluate waters with other saline compositions that can be used to irrigate quinoa and spinach under arid and semiarid climates. Thus, it will be possible to generate good quality food in these environments.

5.5. Conclusion

Due to the need to find strategies to reduce food insecurity in Brazil and worldwide, quinoa appears in the global context as one of the most promising crops to produce food in environments with abiotic stresses mainly related to salinity and drought. The CPAC 09 and CPAC 11 genotypes, developed by EMBRAPA Cerrados in Brazil, showed a high potential for grain production in saline soils commonly found in several arid and semiarid regions, mainly in the western of the United States of America and in the northeastern of Brazil. Both quinoa genotypes showed high resistance to extremely saline soils, being able to survive under EC_e of 65 dS m^{-1}.

After four months of saline water of 25 dS m^{-1}, the soils cultivated with quinoa reached a EC_e of 35 dS m^{-1}, which resulted in a grain yield loss of 54.1% for CPAC 09 and 61.41% for CPAC 11. In general, the CPAC 11 genotype has greater grain production capacity than the CPAC 11 genotype. Under salinity conditions similar to seawater (EC_w above 40 dS m^{-1}), the CPAC 11 genotype produced more grains than CPAC 09, despite the greater loss of production when compared to the 2 dS m^{-1} treatment.

Comparing quinoa genotypes with spinach, we can conclude that, despite spinach's lower resistance to salinity, this species has a high potential for survival in saline and sodic soils compared to other glycophytic crops, being better classified as a facultative halophyte, similar to quinoa, than a glycophyte. Although the reduction in growth, spinach was able to survive under EC_w of 25 dS m^{-1}, with a reduction of 83.04% in its leaf biomass. This suggests that spinach has salt tolerance mechanisms such as reduced growth rate and accumulation of ions such as Na^+ and Cl^- in the leaves for better osmotic adjustment.

In this way, both quinoa and spinach have great food production potential in areas considered unsuitable for agriculture due to the high concentrations of salts in both the USA and Brazil, reducing starvation among populations located in regions of food insecurity.

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6. CHAPTER V: SPINACH PLANTS HAVE SALT BLADDERS WITH SIMILAR SALT-RESPONSE MECHANISMS AS THOSE OF THE HALOPHYTE QUINOA

Abstract

Although spinach can accumulate sodium (Na) and chloride (Cl) in leaf tissues at levels similar to halophytes, and even tolerate NaCl at macronutrients levels, it is classified as a glycophyte. Quinoa, a member of the same family as spinach, possesses epidermal bladder cells (EBCs) capable of accumulating various ions. However, there are no existing reports on EBCs in spinach. In this study we provide the first detailed description of EBCs in spinach. We analyzed EBCs from two spinach cultivars irrigated with water having electrical conductivities (EC_{iw}) of 2.0 and 25.0 dS m^{-1}, and compared their performance to that of the Brazilian quinoa genotype CPAC09. EBCs from both spinach cultivars and quinoa were analyzed under optical and confocal microscopy. EBCs of both species were observed to consist of a stalk with a single cell in quinoa or multiple cells in spinach, each approximately 1.0 mm in diameter. Further, Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDS) of EBCs allowed for a comparison of the ionic signatures in plants irrigated with waters of low and high salinity. Under high salinity conditions, quinoa EBCs accumulated Cl and K but not Na, whereas spinach trichomes accumulated Na, Cl, and K. Potassium and Cl appeared to colocalize, while Na was found in a separate cluster. EBCs of spinach plants irrigated with 25 dS m^{-1} water had more Na, Cl, and K than EBCs of plants irrigated with 2.0 dS m^{-1} water. When comparing gene expression in spinach EBCs to leaf tissue, it was observed that certain genes were more actively expressed in the trichomes. These include genes associated with sodium transport such as *SOS3*, *NHX1*, *NHX2*, and *AKT1*; genes involved in chloride transport like *NPF2.5*, *SLAH1*, *NPF2.4*, and *ALMT12*; and some additional genes that play roles in regulatory mechanisms for managing salinity stress. These insights into gene regulation suggest potential strategies for enhancing spinach's resilience to salinity through targeted genetic interventions. Thus, our findings from SEM-EDS and gene expression analyses strongly indicate that spinach EBCs play a role in its salt-tolerance mechanisms. These observations support the notion that spinach, much like its halophytic relative quinoa, exhibits salinity tolerance and could potentially be classified as facultative halophyte.

Keywords: Salinity. Salt tolerance genes. SEM-EDS. Amaranthaceae Family.

CHAPTER V: PLANTAS DE ESPINAFRE TÊM GLÂNDULAS SALINAS COM MECANISMOS DE RESPOSTAS À SALINIDADE SEMELHANTES ÀQUELAS DA HALÓFITA QUINOA

Resumo

Apesar do espinafre acumular sódio (Na) e cloro (Cl) nos tecidos foliares em níveis semelhantes às plantas halófitas, e até tolerar NaCl em níveis de macronutrientes, ele é classificado como uma planta glicófita. A quinoa, um membro da mesma família do espinafre, possui células epidérmicas especializadas (glândulas) capazes de acumular vários íons. No entanto, não existem relatos sobre estas estruturas atualmente no espinafre. Neste estudo fornecemos a primeira descrição detalhada de glândulas de sais no espinafre. Analisamos as glândulas de duas cultivares de espinafre irrigadas com água com condutividade elétrica (CE_a) de 2,0 e 25,0 dS m^{-1} e comparamos seu desempenho ao do genótipo brasileiro de quinoa CPAC09. As glândulas de sais de cultivares de espinafre e quinoa foram analisados sob microscopia óptica e confocal. Observou-se que as glândulas de ambas as espécies consistiam em um pedúnculo com uma única célula na quinoa ou múltiplas células no espinafre, cada uma com aproximadamente 1,0 mm de diâmetro. Além disso, a Microscopia Eletrônica de Varredura acoplada à Espectroscopia de Raios X por Dispersão de Energia (SEM-EDS) permitiu uma comparação das assinaturas iônicas em plantas irrigadas com águas de baixa e alta salinidade. Sob condições de alta salinidade, as glândulas de quinoa acumularam Cl e K, mas não Na, enquanto os tricomas de espinafre acumularam Na, Cl e K. O K e o Cl pareciam colocalizar-se, enquanto o Na foi encontrado em estruturas separadas. As glândulas de plantas de espinafre irrigadas com água 25 dS m^{-1} apresentaram mais Na, Cl e K do que as de plantas irrigadas com água 2,0 dS m^{-1}. Ao comparar a expressão gênica nas glândulas de espinafre com o tecido foliar, observou-se que certos genes foram expressos mais ativamente nas glândulas. Estes incluem genes associados ao transporte de sódio, como SOS3, NHX1, NHX2 e AKT1; genes envolvidos no transporte de cloreto como NPF2.5, SLAH1, NPF2.4 e ALMT12; e alguns genes adicionais que desempenham papéis nos mecanismos regulatórios para gerenciar o estresse salino. Estas informações sobre a regulação genética sugerem estratégias potenciais para aumentar a resiliência do espinafre à salinidade através de intervenções genéticas direcionadas. Assim, nossos achados de SEM-EDS e análises de expressão gênica indicam fortemente que glândulas de espinafre desempenham um papel em seus mecanismos de tolerância ao sal. Estas observações apoiam a noção de que o espinafre, tal como a sua parente halófita quinoa, exibe tolerância à salinidade e poderia potencialmente ser classificado como halófita facultativa.

Palavras-chave: Salinidade. Genes de tolerância. SEM-EDS. Amaranthaceae.

6.1. Introduction

Soil salinization and sodification are rapidly increasing in agricultural lands worldwide, particularly in arid and semiarid regions. This trend is driven by factors such as inadequate drainage, excessive use of fertilizers, climate change, and other contributing factors (SINGH, 2020; HASSANI; AZAPAGIC; SHOKRI, 2021). The water/soil salinization is exacerbated by the over-taping of fresh groundwater and seawater intrusion in coastal areas. Currently, the main problem in saline/sodic areas is their abandonment, as they become unsuitable for the cultivation of most crops. Studies demonstrate that over 1 billion hectares are affected by salts in more than 100 countries and soil salinization expands by approximately three arable hectares per minute (SHABALA et al., 2014; IVUSHKIN et al., 2019). In the near future, soil degradation due to salinity is likely to cause important socioeconomic, environmental, and food-security issues.

One alternative for the use and/or mitigation of saline soils is the cultivation of salt-tolerant species (HANIN et al., 2016). The ability of plants to survive in saline soils largely depends on their mechanisms for tolerating saline stress, including tissue tolerance to high salt accumulation. Plants that are salt-tolerant are classified as halophytes, while those that are not as known as glycophytes (FLOWERS; COLMER, 2008; CHEESEMAN, 2015).

One of the strategies for salt tolerance in halophytes is salt accumulation and excretion through epidermal bladders. Epidermal bladder cells (EBCs) are specialized epidermal structures that can accumulate salts such as NaCl, improve K retention, and store other chemical elements (KIANI-POUYA et al. 2017; ZHANG, MUTAILIFU; LAN 2022). Approximately 50% of halophyte species, including *Chenopodium quinoa* Willd., *Chenopodium album* L., and *Atriplex spp*. have EBCs in their leaves as a component of their salinity tolerance mechanism. Studies on the ionic and genetic mechanisms related to salt-ion compartmentalization in EBCs are essential to advance the understanding of salt tolerance in plants and for further progress in the genetic modifications in glycophyte species that may allow them to maintain sustainable food production in saline soils (SHABALA et al., 2014; ZOU et al., 2017; NIKALJE et al., 2018; ZHANG et al., 2022).

Spinach (*Spinacia oleracea* L.), a member of the Amaranthaceae family, has traditionally been classified as a glycophyte, likely due earlier finding suggesting a low threshold of 2.0 dS m^{-1} (MAAS; GRATTAN, 1999; GRIEVE et al., 2017). However, a recent study has started to challenge this classification (SHABALA et al., 2014). Some additional

studies demonstrated that spinach exhibits tolerance to high salt concentrations, successfully surviving and yielding under saline waters with electrical conductivities (EC_w) ranging from EC_w 9.4 to 23 dS m^{-1} while maintaining its nutritional aspects and antioxidant capacity (FERREIRA et al., 2018; SANDHU et al., 2023). Furthermore, the spinach cultivars 'Raccoon' and 'Gazelle' exhibited only minimal leaf biomass loss, even under conditions of high salinity combined with potassium fertilization reduced to 2.5% of the normal level (UÇGUN et al., 2020). A recent study evaluating 16 regionally diverse spinach cultivars identified cultivars that exhibit even greater salt-tolerance than 'Raccoon' and 'Gazelle' (SANDHU et al., 2023).

EBCs have been reported to play an important role in the survival of halophytes in saline and sodic soils. However, the mechanisms involved in the storage of salts inside the vacuoles of EBCs remain incompletely understood. Additionally, the role of EBCs in quinoa has recently come under scrutiny (MOOG et al., 2022). Nonetheless, some authors have identified highly expressed genes in the EBCs of halophytes such as the anion transporters SLAH, NRT and CLC, and the cation transporters NHX1 and HKT1 (Zou et al., 2017; BÖHM et al., 2018)

While the involvement of salt bladders has been investigated in halophytes as specialized epidermal structures (ZHANG; MUTAILIFU; LAN, 2022), such glands in spinach have not been previously described, as the plant is typically classified as a glycophyte. On the contrary, spinach is reported as one of the Amaranthaceae members that lacks EBCs (SHABALA et al., 2014). Research connecting spinach leaf glandular trichomes (GTs) to the EBCs of halophytes and their role in salt tolerance mechanisms is currently insufficient. According to Zou et al. (2017), EBCs in quinoa resemble glandular trichomes, with certain genes related to salt tolerance exhibiting higher expression within these bladder cells compared to leaf cells. Notably, these genes are primarily involved in transferring salt from the leaf to the bladder cells (ZOU et al., 2017).

In this study, we report for the first time the presence of EBCs in the leaves of the 'Gazelle' and 'Seaside' spinach cultivars and compare them with the salt bladders of two quinoa cultivars. We utilized Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) and quantitative PCR (qPCR) to analyze ion accumulation in EBCs and explore the genetic mechanisms of salt tolerance in spinach. By examining both isolated spinach EBCs and spinach leaves without bladders, irrigated with waters of low (EC_w = 2.0 dS m^{-1}) and high salinity (EC_w = 25 dS m^{-1}), we linked the function of EBCs in spinach leaves to salt-tolerance mechanisms previously identified exclusively in halophytes.

6.2. Material and methods

For the comparison between quinoa and spinach epidermal bladder cells (EBCs), an experiment was carried out in a greenhouse at the United States Salinity Laboratory (USSL – Riverside, California). Two spinach (*Spinacia oleracea* L. cultivars Gazelle and Seaside) and two quinoa (*Chenopodium quinoa* Wild.) genotypes (CPAC 09 and CPAC 11) developed by EMBRAPA Cerrados in Planaltina, Brazil, were used in this experiment. In March 2023, spinach and quinoa were sown in pots containing 9 kg of soil, with two plants per pot for quinoa, in three plants per pot for spinach. After 25 days of seed germination, the plants were irrigated with waters with EC_w of 2 dS m^{-1} (control) and 25 dS m^{-1} (high-salinity water), balanced with mixed salts, in four replicates. The waters were built based on the salts ratio present in seawater with a proportion of Cl^-:Na^+:Mg^{2+}:Ca^{2+}:SO_4^{-2} = 25.5:22.73:5.15:1:2.6 (Table 29). Both treatments provided the basic plant nutrition through a half-strength modified Hoagland's, with potassium concentration fixed at 5 $mmol_c$ L^{-1}. The leaching fraction of the irrigated pots was 0.3.

Table 29 – Description and ionic composition of the saline waters

Targeted EC_w	Calculated EC_w	Na^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	K^+	NO_3^-	PO_4^{3-}
dS m^{-1}		mmolc L^{-1}							
2	1.89	2.1	1.7	3.3	0.6	3.7	4.9	7.61	1.5
25	24.8	196.1	5.4	44.2	21.5	221.4	4.9	7.61	1.5
40	39.96	343.1	11.9	76.7	39.0	389.9	4.9	7.61	1.5
55	54.84	489.1	18.3	109.6	56.5	557.7	4.9	7.61	1.5

6.2.1. Scanning Electron Microscopy with Energy Dispersive X-ray Spectrometry (SEM-EDS)

The EBCs were removed from the youngest leaves of quinoa and spinach with a small metal spatula after 60 days of saline water application to analyze the accumulation of salt ions using SEM-EDS. Spinach and quinoa bladders were carefully scraped onto the top of aluminum stubs without any tape to prevent contamination. The stubs were previously washed with molecular water and air-dried to eliminate the water interference. All the bladders were submitted to a layer of gold. The SEM-EDS analysis was carried out at the University of California in Riverside in a TESCAN Vega3 SBH microscope (TESCAN USA, Inc.,

Pleasanton, CA, USA). For both species, the energy of 15 kV on the bladders provided the best readings without rupturing the bladders.

6.2.2. Membrane Staining and Confocal Microscopy

Tissue samples from each plant were stained with Nile Red as describe previously (Li-Beisson et al., 2013) to visualize cell membranes of epidermal cell structures. Briefly, a stock solution of 1 mg mL^{-1} Nile Red or Nile Blue A Oxazone (Sigma-Aldrich Inc., St. Louis, MO, USA) prepared in 100% DMSO (Sigma-Aldrich Inc., St. Louis, MO, USA) was diluted to 1 µg mL^{-1} in sterile distilled water to create a working solution. Dissected leaf tissue samples were incubated in working solution for 5-10 minutes and then rinsed with sterile water for 1 minute before mounting.

Epidermal leaf structures were visualized using an inverted LSM 900 (Zeiss, Dublin, CA, USA) confocal microscope equipped with an EC Plan-NeoFluar 10x/0.3 NA objective. Nile Red (excitation wavelength 561 nm) and chlorophyll fluorescence (excitation wavelength 640 nm) were observed simultaneously by collected emission spectra from 400-650 nm and 650-700 nm, respectively. Z-series were collected in 1 µm intervals over varying distances to image the entire epidermal structure and converted into maximum-intensity projections using the Zen Blue Software (Zeiss).

6.2.3. Expression analysis

To compare gene expression in bladders and the other leaf cells, two spinach varieties (‘Gazelle’ and ‘Seaside’) were used. The bladders were manually removed from the selected leaves using a spatula/brush. RNA was extracted from both bladders and the remaining leaf tissues using the TRIzol® solution (Invitrogen, Carlsbad, CA, USA) and was subsequently treated with DNase I (Thermo Scientific, Waltham, MA, USA), adhering to the guidelines provided by the manufacturers. The quantitative Reverse Transcription-PCR (qRT-PCR) procedures were conducted using the iTaq™ Universal SYBR® Green One-Step Kit on a BioRad CFX96 System (Bio-Rad Laboratories, Hercules, CA, USA).

A set of 19 genes involved in Na, Cl, and K transport, ion compartmentalization, and tissue tolerance were examined for the expression analyses (Table S1). Each PCR reaction mixture, with a total volume of 10 µl, comprised 20 ng of RNA, 0.75 µM of specific primers (Table S1, supplementary material), 0.125 µL of iScript™ Reverse Transcriptase, and 5 µL of the 2x SYBR® Green Reaction mix. These experiments were repeated across three biological and two technical replicates. The PCR protocol involved an initial phase at 50 °C for 10

minutes, a 1-minute interval at 95 °C, followed by 40 cycles consisting of 10 seconds at 95 °C for denaturation, 30 seconds at 57 °C for annealing, and a 30-second extension at 68 °C. Relative expression levels were determined using the comparative $2^{-\Delta\Delta CT}$ method (LIVAK; SCHMITTGEN, 2001). For normalization of gene expression, reference genes used were spinach ACTIN (Spov3_chr2.02265), Actdf (Spov3_chr6.00169), and GAPDH (Sov3_C0001.00042).

Differences in gene expression were quantified by contrasting the cycle threshold values of the target gene against those of the reference genes. The formula employed for this calculation was:

$$\text{Normalized expression}_{\text{sample (GOI)}} = [RQ_{\text{sample (GOI)}}]/[RQ_{\text{sample (ref 1)}} \text{ x } RQ_{\text{sample (ref 2)}} \text{ x}\ldots\text{x } RQ_{\text{sample (ref n)}}\}\}^{1/n}$$

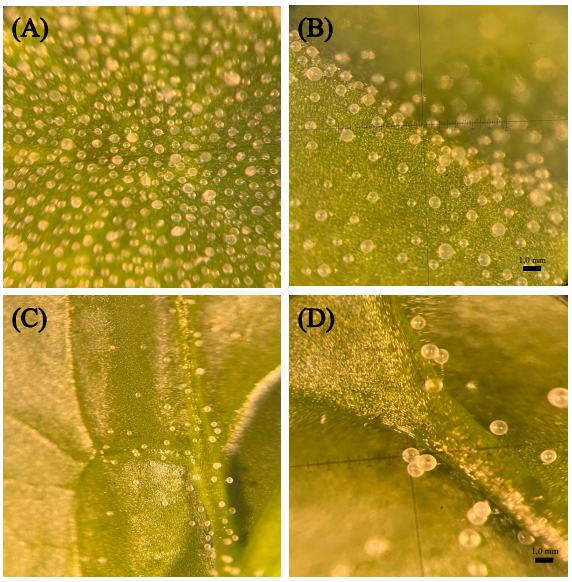
In the formula, RQ is the relative amount of a sample, the ref is the reference target gene in a run that includes one or more reference targets in each sample, and GOI is the gene of interest.

6.3. Results

6.3.1. EBCs Morphology

The results showed that the two spinach cultivars have modified trichomes similar to those salt bladders (EBCs) found in quinoa, with a diameter of less than 1.0 mm (Figure 24). It can be observed that the density of bladders in quinoa leaves (as Figures 24A and 24B) is higher than in spinach leaves. (Figures 24C and 24D). The similarity between the EBCs of spinach and quinoa, coupled with the fact that they both belong to the Amaranthaceae family, suggests that spinach EBCs may serve similar roles in salt storage as those observed in quinoa EBCs.

Figure 24 – Quinoa (A and B) and Spinach (C and D) bladders



The images from the optical, confocal and SEM microscopies illustrate the EBCs morphology. The stalk of quinoa EBCs shown in Figure 26A and 26B has only one cell, unlike the stalk of spinach trichomes, which have more than 4-5 cells (Figure 25B, 25C, and 25E). The top of the glandular structure is globular in spinach and quinoa, with the appearance of a large vacuole.

Figure 25 – Spinach bladders. (A) Spinach leaves with EBCs; (B) Spinach EBC - optical microscopy; (C) Spinach stalk with more than 8 cells- optical microscopy; (D) Spinach EBCin leaf - SEM; (E) Young spinach EBC- optical microscopy - (F) multiple EBCs in spinach leaves - optical microscopy; (G) Spinach EBC from confocal microscopy; (H) chlorophyll fluorescence in spinach EBCs, and (I) Spinach stalk – confocal microscopy

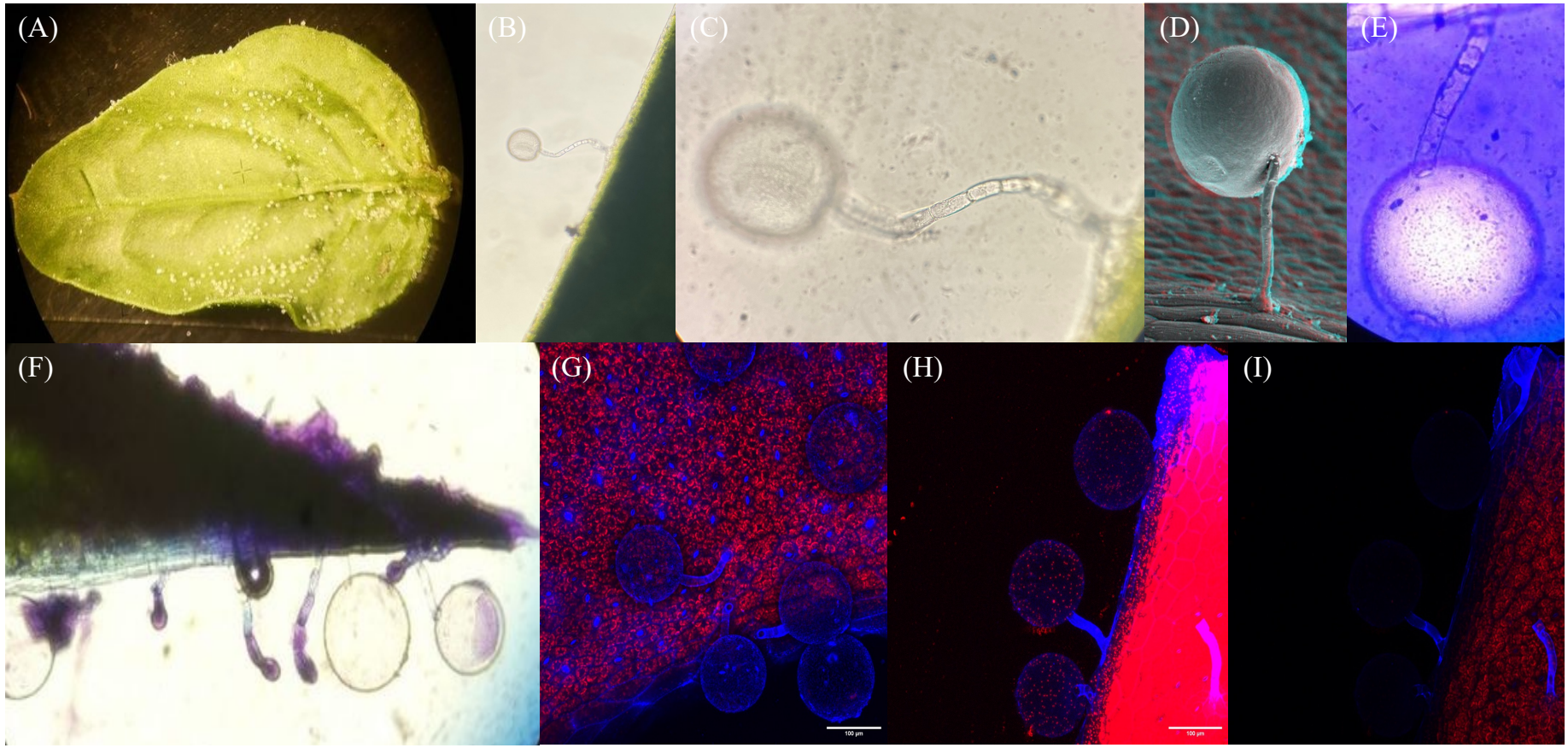
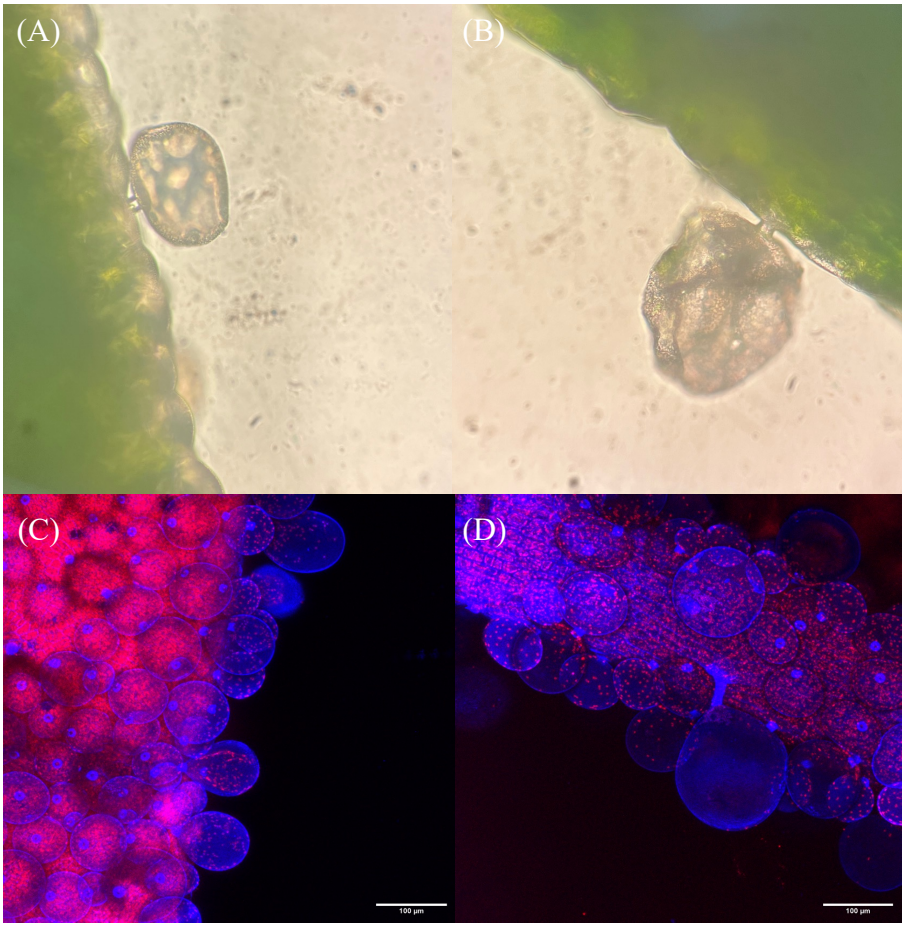


Figure 26 – Quinoa (CPAC 09) EBCs. (A and B) quinoa EBCs – optical microscopy; (C) quinoa EBCs in leaves – confocal microscopy, and (D) quinoa stalk and chlorophyll fluorescence in EBCs



The EBCs of both species exhibit chlorophyll fluorescence, indicating the presence of chlorophyll (Figure 25H and 26D). However, we were unable to confirm the presence of fully developed and functional chloroplasts. According to the trichome classification in *Solanum* species (WATTS; KARIYAT, 2021), the trichomes in quinoa can be characterized as EBCs with a large globular head and a single stalk cell. In spinach, the EBCs consist of a large globular head and a multicellular stalk, the length of which is similar to the diameter of the head.

6.3.2. SEM – EDS

SEM-EDS analysis revealed that both spinach and quinoa allocate salts inside EBCs (figures 27, 28, 29, 30, 31, 32, and 33). For spinach, EBCs were found to contain K, Cl, Na oxygen (O), and carbon (C) (Figures 27, 28, 29, 30 and 31). Under conditions with an EC_w of 2 dS m^{-1} (figures 27 and 30) the EBCs of the spinach plants exhibited higher concentrations of K, O, and C, compared to Na and Cl. However, when plants were submitted to EC_w of 25 dS

m^{-1} (Figures 28, 29, and 30) the concentration of Na and Cl increased, with K and Cl similar peak intensities, especially in ‘Gazelle’, suggesting that both ions were present in the EBCs. According to the distribution maps, Cl and K were colocalized in similar positions in the EBCs, while Na is associated with O and/or C (Figures 28, 29, and 30). Among the two spinach cultivars studied, 'Gazelle' demonstrated a higher capacity to secrete Na and Cl into the EBCs compared to 'Seaside' (Figures 28 and 31).

For quinoa, the presence of K, Cl, O, and C was observed in the EBCs (Figures 32 and 33). When low-salinity water was applied, the content of Cl was insignificant (Figure 32). However, the concentrations of Cl and K significantly increased when water with EC_w of 25 dS m^{-1} was applied (Figure 33). The EDS mapping for quinoa illustrates the colocalization of K and Cl in the EBCs. As the salinity of the irrigation water increased, there was a corresponding rise in K and Cl concentrations (Figure 32 and 33).

Spinach’s ability to sequester Na within EBCs differed markedly from that of quinoa. Quinoa CPAC 09 did not sequester Na into the EBCs under either low or high salinity conditions in this study (Figures 32 and 33).

After air drying the EBCs, a salt crust formed the spinach leaf (Figure 29). The mapping shows the salt crust, and the graphic exhibits the proportion of salts that are present in the crust (Figure 29). These results indicate that spinach employs a salt tolerance mechanism similar to other halophytes in the Amaranthaceae family.

Figure 27 – SEM-EDS of spinach bladders (cv Gazelle) under ECw of 2 dS m^{-1}

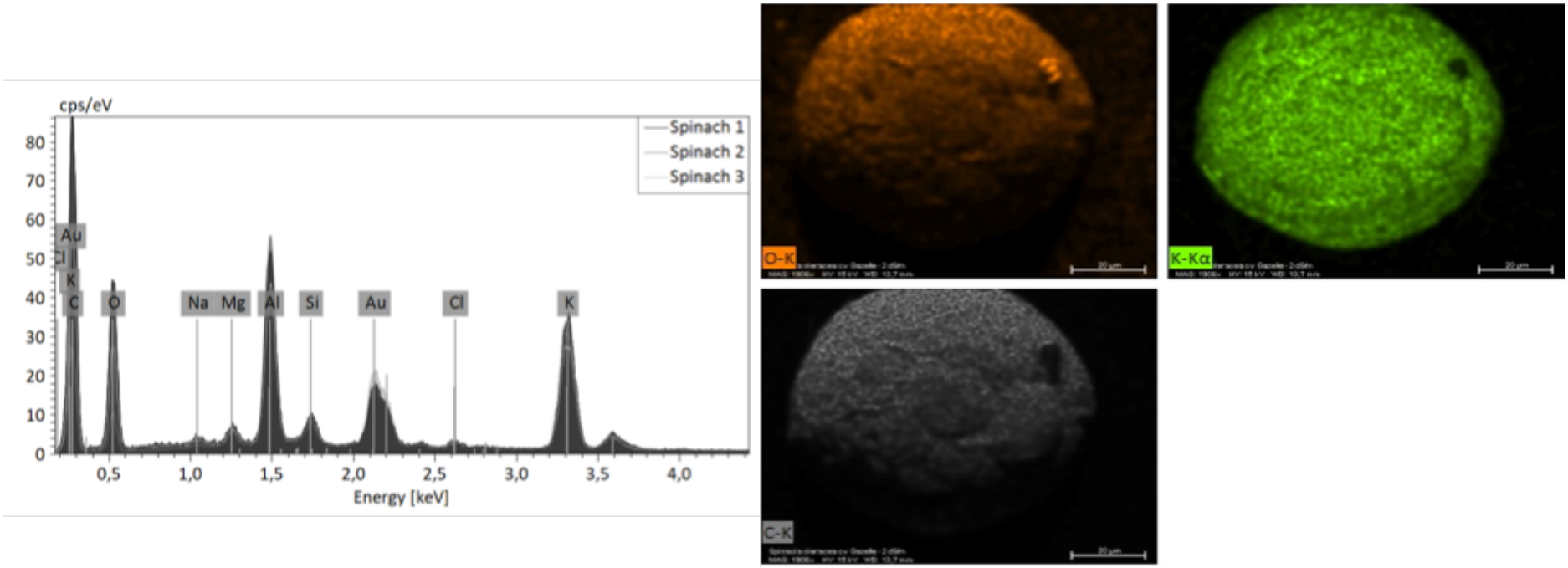


Figure 28 – SEM-EDS of spinach bladders (cv. Gazelle) under EC_w of 25 dS m^{-1}

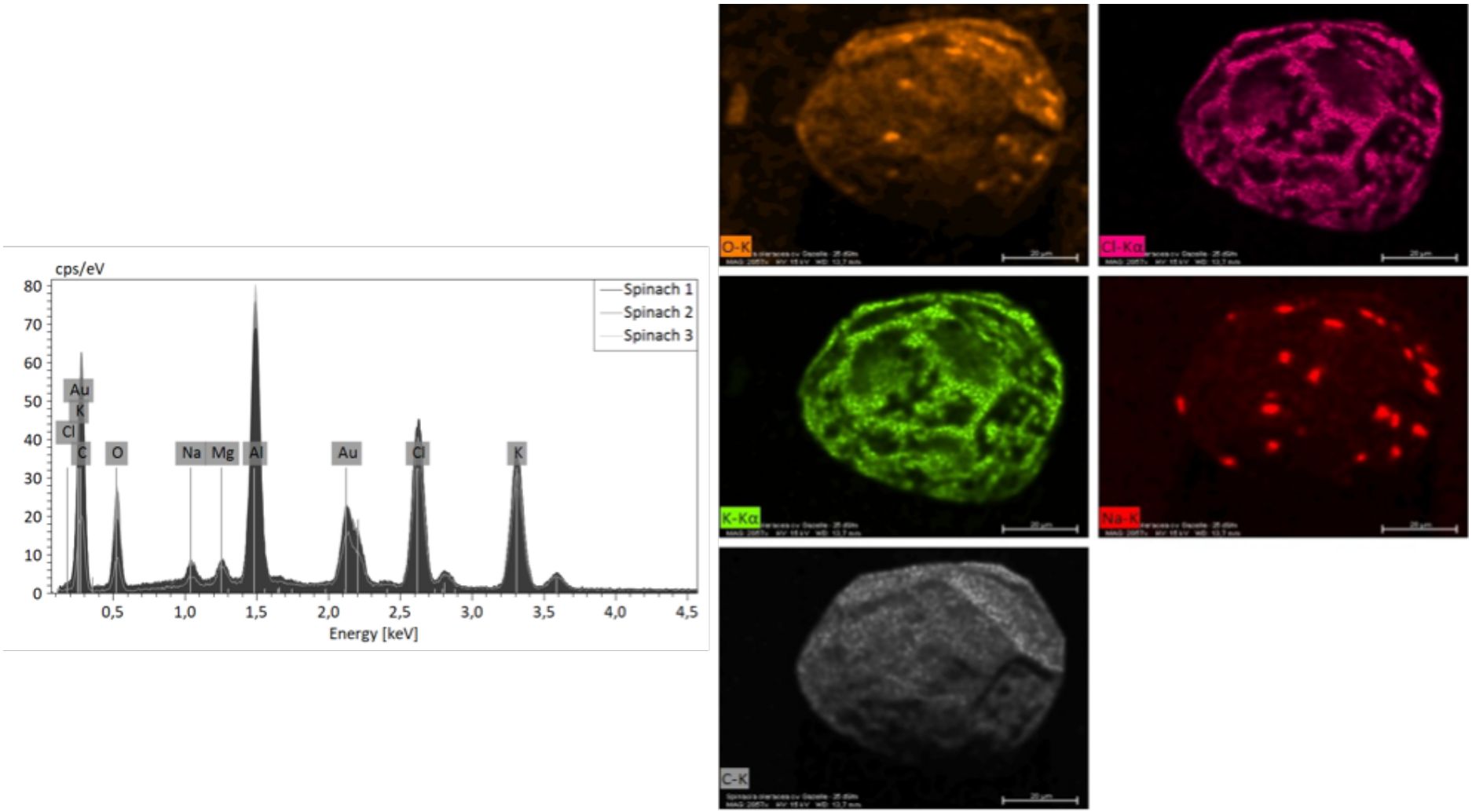


Figure 29 – SEM-EDS of salt crust in spinach EBCs (cv. Gazelle) under EC_w of 25 dS m^{-1}

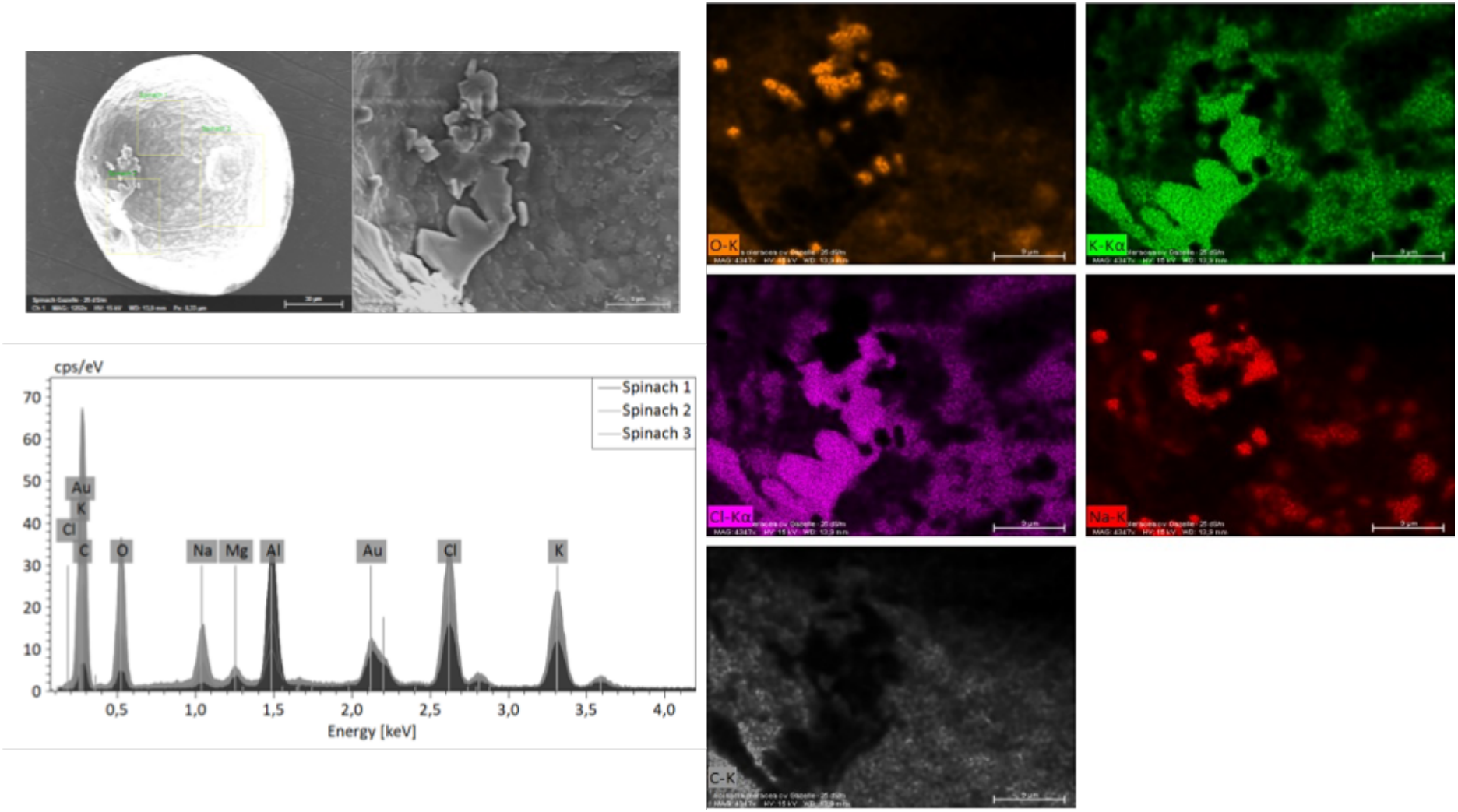


Figure 30 – SEM-EDS of spinach bladders (cv. Seaside) under EC_w of 2 dS m^{-1}

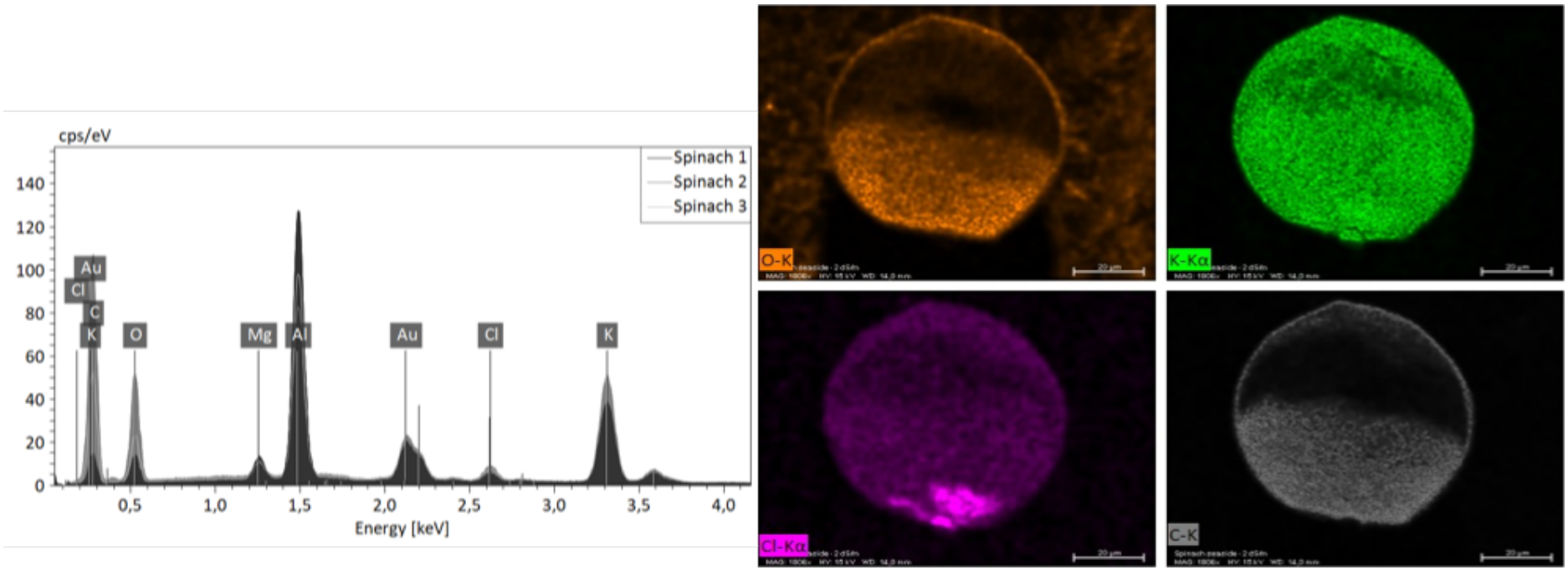


Figure 31 – SEM-EDS of spinach bladders (cv. Seaside) under EC_w of 25 dS m^{-1}

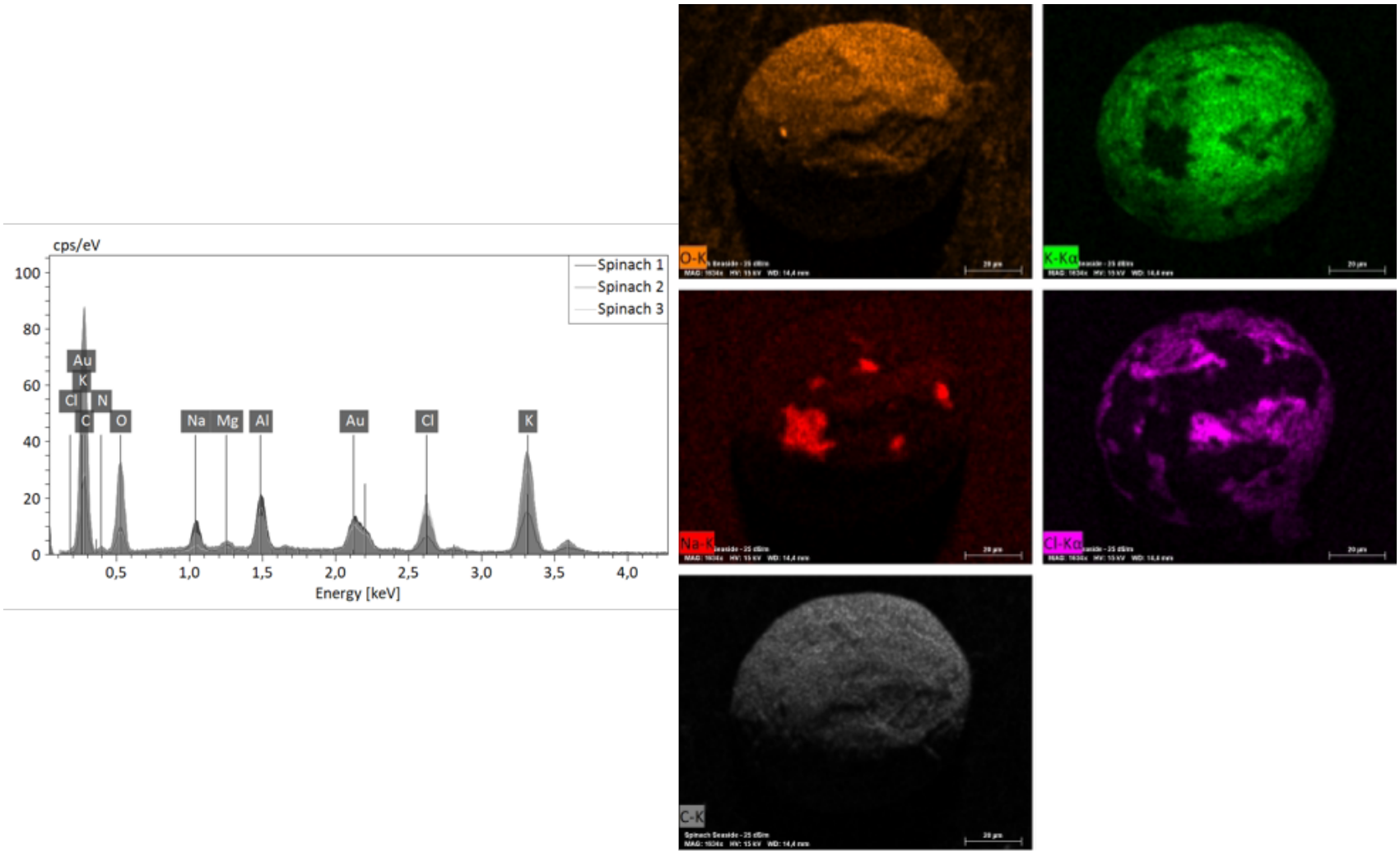


Figure 32 – SEM-EDS of quinoa bladders (CPAC 09) under EC_w of 2 dS m^{-1}

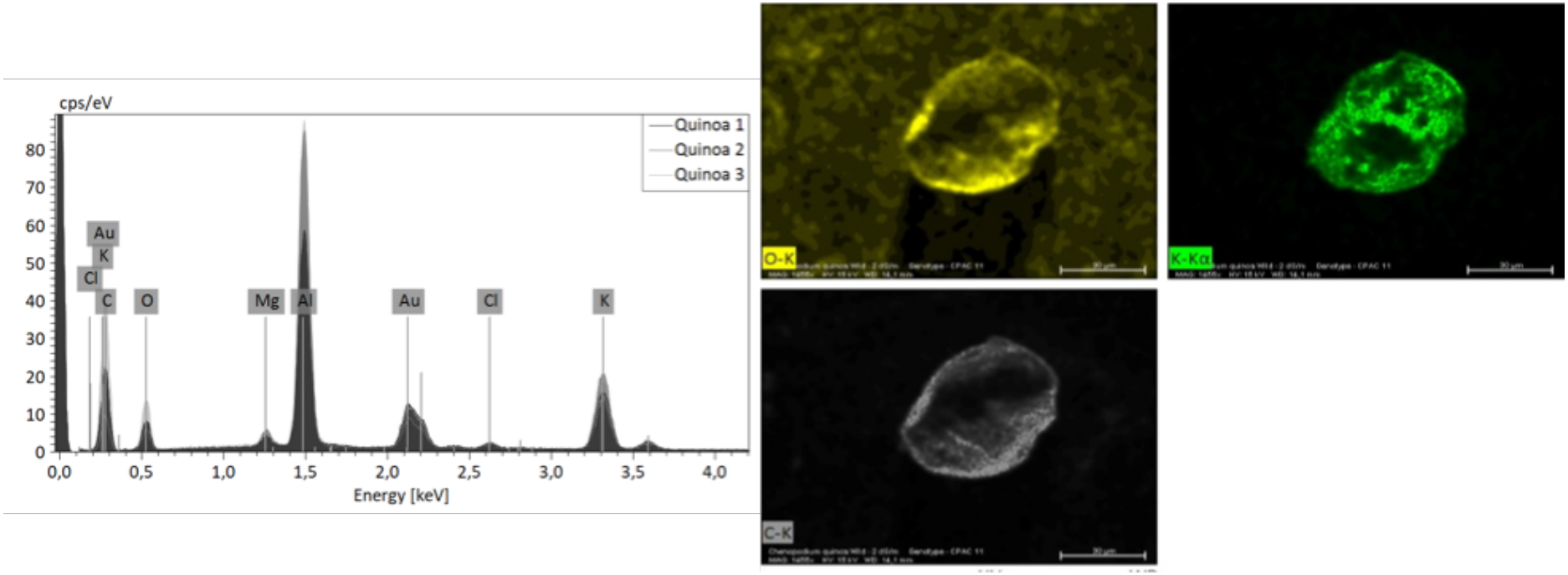
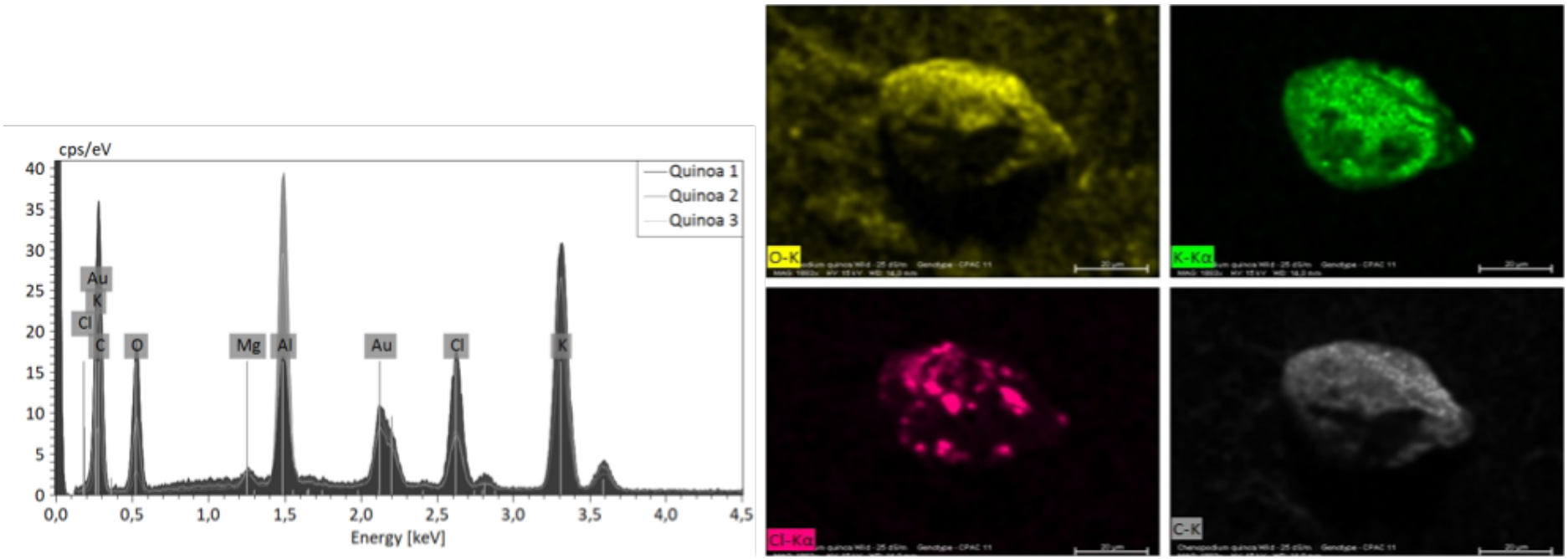


Figure 33 – SEM-EDS of quinoa bladders (CPAC 09) under EC_w of 25 dS m^{-1}

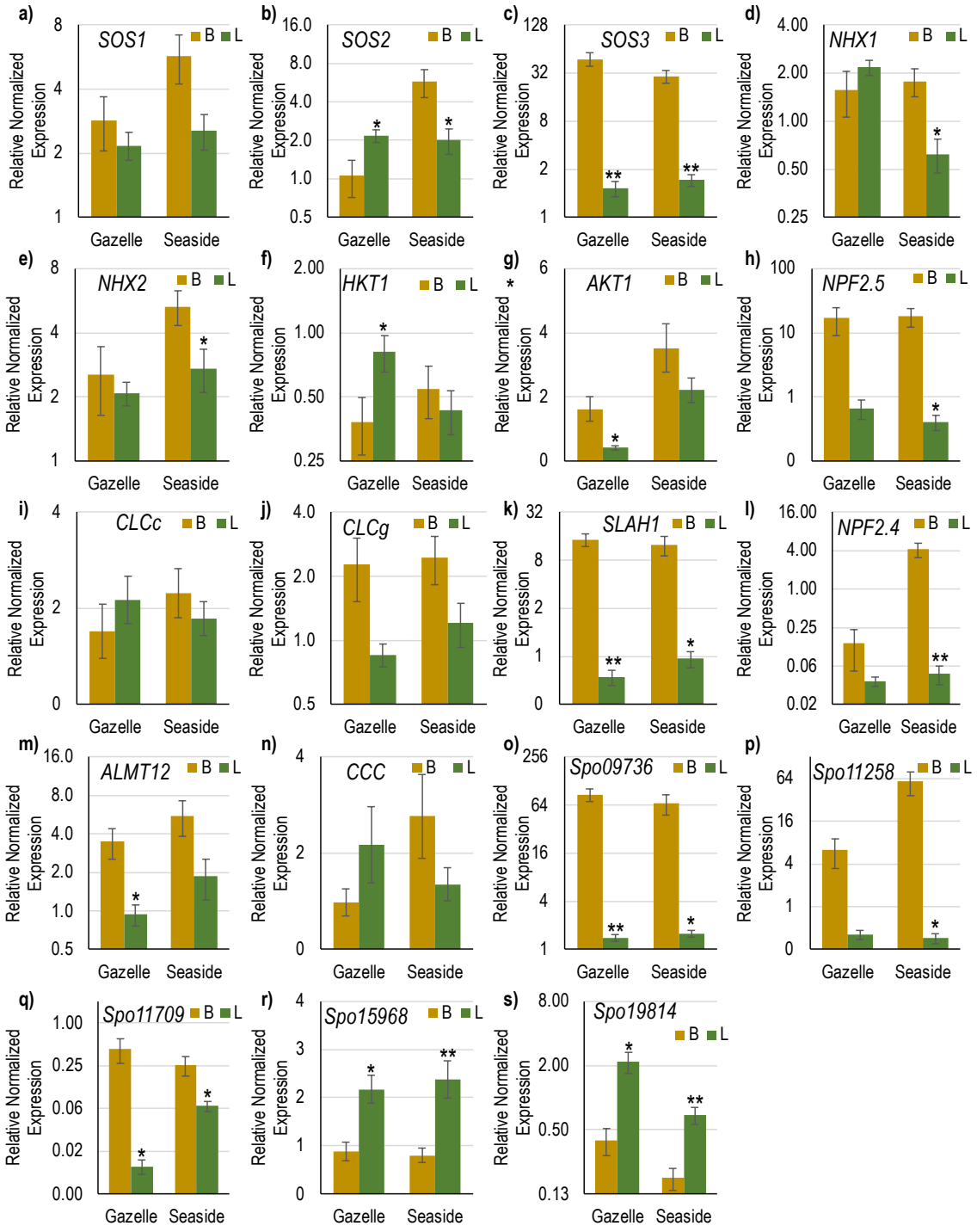


6.3.3. Expression analysis

Gene expression analysis was conducted on two distinct organs of spinach plants subjected to high salinity conditions: the bladders excised from the leaves and the residual leaf tissue devoid of bladders. This study utilized two spinach varieties, 'Gazelle' and 'Seaside', to assess the impact of high salinity on gene expression in these specific plant tissues. For the expression analysis, 19 genes associated with salinity tolerance in spinach were selected and categorized based on their roles and mechanisms in salt stress response (Figure 34).

This selection encompasses genes that regulate Na levels, including *SOS1*, *SOS2*, *SOS3*, *NHX1*, and *NHX2*. For potassium homeostasis, *HKT1* and *AKT1* were chosen. Chloride regulation is addressed through *NPF2.5*, *CLCc*, *CLCg*, *SLAH1*, *NPF2.4*, *ALMT12*, and *CCC*. Additionally, genes identified as differentially expressed in response to salinity stress in a previous RNA-seq study (*Spo09736*, *Spo11258*, *Spo11709*, *Spo15968*, and *Spo19814*) were also included in the analysis (ZHAO et al., 2021).

Figure 34 – Spinach gene expression in spinach EBCs and Leaves



In the analysis of genes regulating Na levels, *SOS3* exhibited notable upregulation in the bladders compared to the leaves lacking bladders across both spinach cultivars (Figure 34c). Furthermore, *NHX1* and *NHX2* showed enhanced expression in the bladders relative to the leaves without bladders in the 'Seaside' cultivar, whereas such induction was not observed in 'Gazelle'. Among the K homeostasis genes, *AKT1* experienced upregulation in the bladders of

'Gazelle' when compared to the leaves without bladders. This pattern of expression was not observed in 'Seaside' (Figure 34g) and of the genes responsible for Cl regulation, *NPF2.5*, *SLAH1*, *NPF2.4*, and *ALMT12* demonstrated significant upregulation in the bladders than bladderless leaves of at least one of the spinach cultivars studied (Figure 34h-n). From the genes identified for their differential expression under salinity conditions in a preceding RNA-seq analysis, *Spo09736*, *Spo11258*, and *Spo11709* were upregulated in the bladders relative to the leaves lacking bladders (Figure 33o-q). Conversely, *Spo15968* and *Spo19814* exhibited downregulation in the bladder tissues (Figure 34r and 34s). This comprehensive gene selection provided a focused insight into the genetic basis of salt accumulation in leaf bladders.

6.4. Discussion

6.4.1. Spinach contains EBCs similar quinoa

Although other members of the Amaranthaceae family, such as quinoa and atriplex, are known to contain EBCs, previous research has indicated that spinach lacks these specialized structures (SHABALA et al., 2014). The present study reveals that EBCs are extensively distributed across spinach plants. This finding expands our understanding of spinach's physiological adaptations, highlighting a significant presence of EBCs that was previously unrecognized in this species. The distribution of EBCs suggests that they may play a crucial role in the plant's ability to manage salt stress, similar to their function in other members of the Amaranthaceae family. This discovery not only challenges previous assumptions about the anatomical characteristics of spinach but also opens up new avenues for research into its salt tolerance mechanisms and potential agricultural applications in saline environments.

Spinach EBCs have a similar morphology to those of quinoa, as both are classified as glandular hairs with large globular heads. However, they differ in stalk structures: quinoa EBC feature a simple stalk, while spinach EBCs have a multicellular stalk (Figures 25C and 26A). We detected chloroplasts in the EBCs of spinach, consistent with previous discovery of chloroplasts in quinoa EBCs (BÖHM et al., 2018). Additionally, chloroplasts have been reported in the 10-celled glandular trichomes of *Artemisia annua* (DUKE; PAUL, 1993; FERREIRA; JANICK, 1995). These chloroplasts may provide energy for the biosynthesis of artemisinin precursors that are produced in glandular trichomes, as glandless Artemisia mutants do not possess EBCs and do not produce artemisinin (DUKE et al., 1994). While the exact role of chloroplasts in these EBCs remains unclear, it is hypothesized that the spinach glands require ATP to operate ionic pumps. These pumps are responsible for transporting H^+, Na^+, Cl^-, and K^+ into the EBCs and maintaining their separation from the cytoplasm (SHABALA et al.,

2020). The EBCs have been considered as an inverted external vacuole that allows halophytes to store excess Na^+ and Cl^- outside of leaf tissues to avoid salt toxicity while saving more energy than trying to extrude the excess N^+ and Cl^- outside the roots (SHABALA et al., 2020). These authors mentioned that this mechanism of salt tolerance allows halophytes, such as quinoa and Atriplex, to sequester large amounts of salt away from metabolically active cell compartments.

6.4.2. Ion storage in spinach and quinoa epidermal bladders

Our findings indicate that spinach EBCs are capable of storing salt ions, similar to the EBCs observed in quinoa. The sequestration of K^+, Na^+, and Cl^- is evident especially when spinach plants are submitted to high salt concentrations (Figures 28, 29, and 31). This ability to develop salt bladder structures is very common in halophyte species, as a way of protecting the plant against saline stress (KIANI-POUYA et al., 2017).

When comparing spinach and quinoa, the main difference between the EBCs of the two species is that quinoa EBCs did not accumulate Na^+. While some authors claim that quinoa can store Na^+ (BÖHM et al., 2018; KIANI-POUYA et al., 2017), other have concluded that the concentration of Na^+ in the EBCs is quite low, particularly when compared to K^+ (OTTERBACH et al., 2021). Our results revealed that quinoa's EBCs mainly accumulated K^+ and Na^+ sequestration is not a key salt-tolerance mechanism in quinoa (Figures 32 and 33). Under conditions of high salinity, these cells also show significant accumulation of Cl^-. These results are consistent with a previous study on mutant quinoa plants lacking EBCs, which reported that quinoa salt bladders accumulated K^+ but not Na^+ (MOOG et al., 2022). Nonetheless, the ability of these bladders to store Cl^- is also fundamental for tolerance to saline environments, considering that Cl^- is often the most concentrated anion in these environments, hindering plant development (KIANI-POUYA et al., 2017).

For quinoa, the EBCs of young leaves initially store excess salts, which are subsequently released into the environment as the leaves age and these structures rupture (Ding et al., 2010). The storage of salts within these structures is regulated by salt transporters based on the difference between the potential energy of the cytoplasm concerning the storage cells (EBCs), activated by specific genes related to the transport of cations such as K^+ and Na^+, and of anions such as Cl^- (SHABALA et al., 2014).

Although 'Seaside' has not been tested for salinity tolerance in our previous studies, 'Gazelle' has. In a study involving 'Raccoon' and 'Gazelle', both spinach cultivars could

accumulate large concentrations of Na and Cl in their leaf tissues when irrigated with saline water ranging from 1.2 to 16.7 dS m^{-1} (UÇGUN et al., 2020).

This is the first report to demonstrate that the spinach EBCs function as salt bladders and are crucial for its salt tolerance. As this is a characteristic observed and described in halophytic plants from the same botanical family (Amaranthaceae), as quinoa and Atriplex, we provide the possibility that spinach is a highly salt tolerant plant and contains features similar to halophytes. So, it coud he classified as a facultative halophyte as quinoa. According to Zou et al. (2017), some similarities between spinach and quinoa are explained genetically by the fact that they both belong to the Chenopodiaceae subfamily and share the same common ancestor. Also, our previous research with different spinach cultivars clearly showed that spinach can accumulate both Na and Cl in leaf tissues at the same levels, or higher than, of macronutrients like N without showing any visual symptoms of salt toxicity while maintaining tissue homeostasis of N, P, and K, even when the latter was provided at concentrations 20 to 40 times lower than ideal (FERREIRA et al., 2020; UÇGUN et al., 2020). ALSO, our recent work with cultivars from several origins determined that spinach can grow when irrigated with water salinity of 23 dS m^{-1} and still produce leaf biomass without any salt-toxicity symptoms (SANDHU et al., 2023)

6.4.3. Expression of genes related to salt tolerance in spinach bladders

This study has shed light on salinity tolerance in spinach by conducting gene expression analysis on both bladder-bearing and bladderless leaf tissues under high salinity conditions. It highlights how specific genes involved in the transport of ions into EBCs contribute to the plant's ability to cope with salt stress. Key findings include the upregulation of *SOS3*, a gene implicated in sodium regulation, in the bladder tissues of both spinach cultivars (Figure 33c). *SOS3* is a gene within the Salt Overly Sensitive (SOS) pathway, encoding a calcium sensor protein critical for detecting and responding to salt stress in plants (ZHU, 2003). Under salt stress, increased levels of Na^+ lead to changes in the intracellular Ca^{2+} concentration. The SOS3 protein detects salt stress by sensing elevated calcium levels, triggering a cascade where it activates SOS2, a kinase. This complex then phosphorylates and activates SOS1, an antiporter that expels excess sodium from cells, mitigating salt toxicity (QUINTERO et al., 2011). Beyond its established role in expelling sodium from roots back into the soil, our study highlights SOS3's vital function in directing sodium sequestration into leaf bladders, thereby diminishing sodium toxicity in photosynthetic tissues.

The differential expression of *NHX1* and *NHX2* observed exclusively in the bladders of the 'Seaside' variety suggests a genotype-specific strategy for managing sodium levels under salinity stress (Figure 34d and 34e). NHX1 and NHX2 proteins are key in sequestering excess sodium into the vacuoles, playing a crucial role in managing sodium toxicity within plant cells (YOKOI et al., 2002). This variety-specific response indicates that sodium compartmentalization into bladders, a mechanism critical for mitigating salt toxicity, may vary significantly between different spinach genotypes, highlighting the complex interplay between genetic makeup and physiological adaptation to environmental stresses.

The differential expression of *AKT1*, involved in K^+ homeostasis (RAGEL et al., 2019), further emphasizes the complex responses of spinach to salinity stress, potentially balancing ion homeostasis in leaf bladders to maintain cellular functions under salt stress.

The marked increase in expression levels of chloride regulation genes, including *NPF2.5*, *SLAH1*, *NPF2.4*, and *ALMT12* within the bladders points to a sophisticated mechanism for managing chloride ions (Figure 34h, k, l, and m). High concentrations of Cl, like Na, pose a threat to plant health, necessitating efficient regulation mechanisms. SLAH1, specifically, plays a pivotal role in facilitating the transport of chloride ions from the roots to the xylem (LI et al. 2016), indicating its crucial function in chloride movement within the plant. The consistent upregulation of *SLAH1* in the bladders, as opposed to leaves without bladders, across both studied genotypes, underscores its significant contribution to chloride accumulation within the bladders. This pattern of gene expression not only reinforces the critical role of these genes in Cl^- homeostasis but also suggests a targeted approach by the plant to mitigate the toxic effects of high Cl levels through compartmentalization, thereby maintaining overall plant health and function in saline environments.

The upregulation of genes identified from previous RNA-seq studies (*Spo09736*, *Spo11258*, *Spo11709*) in bladder tissues (Figures 34o, p, and q) reinforces the notion that these genes might play pivotal roles in salt tolerance, either through direct participation in salt transport or through regulatory mechanisms that enhance the plant's ability to cope with salinity stress. *Spo09736* codes for expansin-like B1 (EXLB1), a protein that mitigates salinity stress by loosening the cell wall (GEILFUS et al., 2015). This action enhances the cell wall's elasticity, facilitating improved uptake of nutrients and water. *Spo11258* encodes for an E3 ubiquitin ligase, which is known to modulate salinity tolerance via the abscisic acid signaling pathway (YANG et al., 2022). *Spo11709* encodes the cysteine-rich receptor-like protein kinase 10 (CRK10). While the precise mechanism of CRK10 remains unclear, mutants of *crk10* in Arabidopsis exhibit persistent activation of pathways associated with both biotic and abiotic

stress responses (PIOVESANA, et al., 2023). Further research is imperative to delineate the roles of expansin-like B1 (EXLB1), E3 ubiquitin ligase, and cysteine-rich receptor-like protein kinase 10 (CRK10) in facilitating ion sequestration in spinach leaf bladders.

The observed downregulation of *Spo15968* and *Spo19814* in spinach bladder tissues (Figure 34r and s) adds a layer of complexity to the plant's salt stress response. *Spo15968*, encoding the S-type slow anion channel-associated homologue 2-like (SLAH2-like) protein, plays a role in nitrate transport essential for maintaining ion balance under salinity stress (HEDRICH et al., 2017). On the other hand, *Spo19814*, which codes for the probable zinc metallopeptidase EGY3, contributes to salinity tolerance by regulating chloroplastic reactive oxygen species (ROS) homeostasis (ZHUANG et al., 2021). These findings underscore a sophisticated regulatory network where both the upregulation and downregulation of specific genes are critical for fine-tuning the plant's adaptation to salinity. This adaptive strategy, emphasizing the nuanced regulation of gene expression, highlights the potential for developing targeted genetic interventions to enhance spinach's resilience to salinity.

The highlights of these results offer significant insights into the mechanisms of salt tolerance in spinach, particularly through the movement of salt into leaf bladders. The differential expression of selected genes underscores the potential adaptive strategy of spinach to manage salt stress by compartmentalizing excess salts in leaf bladders, thereby mitigating the detrimental effects of salinity on vital plant tissues. The movement of salt into bladders, as evidenced by the upregulation of specific ion transport and regulation genes, likely contributes to higher salt tolerance in spinach by physically removing excess salts from the photosynthetically active leaf tissues. This sequestration reduces the osmotic and ionic stresses on the plant, allowing for continued growth and development even under conditions that would otherwise be harmful. The ability to compartmentalize salts into specialized structures such as bladders may represent an evolutionary advantage, enabling these plants to thrive in unfavorable environments.

6.5. Conclusion

This work provides the first detailed description of EBCs in spinach leaves and irrefutable evidence of their accumulation of Na^+, Cl^-, and K^+. In contrast, quinoa EBCs accumulate only Cl^- and K^+ when subjected to high-salinity irrigation. The evidence is supported by SEM-EDS, gene expression analysis in both bladderless leaf tissues and isolated EBCs, and tissue accumulation of Na and Cl in leaves of spinach and quinoa irrigated with saline water of 25 dS m^{-1}. Our data suggests that one of the functions of these modified

trichomes present in spinach leaves is the storage of Na^+, K^+, and Cl^-, particularly under high salinity. Similar functions are observed in EBCs of other *Amaranthaceae* family members, such as *Chenopodium album* L. and *Atriplex canescens*.

While further research is needed to explore additional salt tolerance mechanisms in spinach EBCs, preliminary evidence indicates these structures may also play a role in K^+ homeostasis, as previously observed in studies where spinach was grown with significantly reduced potassium levels. Given these findings, and previous observations that spinach can accumulate significant levels of foliar Na and chloride Cl while maintaining nutrient homeostasis—a hallmark of halophytes—we propose reclassifying spinach from a "salt-tolerant glycophyte" to a facultative halophyte. This reclassification is supported by spinach's ability to maintain effective biomass production under saline irrigation up to 25 dS m^{-1}, facilitated by its equipped salt bladders. This adaptation not only underscores its resilience but also its potential utility in sustainable agriculture in regions facing salinity challenges. This research advances our understanding of salt tolerance mechanisms in spinach and highlights its potential in sustainable agriculture in semiarid regions where water quality is a limiting factor.

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Supplementary material

Table S1. Primers used for the expression analysis

Gene Name	Primer Name	Primer Sequence (5' - 3')
NHX1	Spo20154_1F	GCACATTGCAGGTACTTAATCAG
	Spo20154_1R	CGAAGCTCTGGATAGCGTTAAA
NHX2	Spo09537_1F	CCCTTCTTCTTGGCATAGTGT
	Spo09537_1R	CTTAACCTGAAACCCAGCATTG
SOS1	Spo00867_1F	ACGGTATCCGCATTTGGG
	Spo00867_1R	CTGCACACCTCTTTATCTGGT
SOS2	Spo15557_1F	ATGGTTGAGCAGATTAGACGT
	Spo15557_1R	TCGGCTGGCTAAAACCTC
SOS3	Spo02501_1F	GCCTCAGAAACACCATTCAC
	Spo02501_1R	GTTGGAACTCTTCCCTGTGA
HKT1	Spo18318_2F	CATCACTGAGAGCACCAGTTTA
	Spo18318_2R	TCCCATATGCACTTACGACTTC
AKT1	Spo12230_2F	GCTTTTCCAGCTGGTTTCAG
	Spo12230_2R	GGATGATAAATTCCATTGCACCAG
NPF2.4	Spo16635_1F	CTCCTTACTGGGCGCAATAAT
	Spo16635_1R	CAAGGCTTGATGGAGGGTTAT
NPF2.5	Spo06264_1F	GTGGATTAATCAGTTTCTCTCCATTC
	Spo06264_1R	ACATAACAACTCCCAGTAATGAAAC
SLAH1	Spo20847_1F	TTCATGTCCTTGGTAAGTAGACC
	Spo20847_1R	TGCTAGTGCTAGTATTGTCATAGG
CCC	Spo03495_1F	TGCAATGAAGGGTGGTGG

	Spo03495_1R	AACACATAGAGAGATGCAGCA
ALMT12	Spo02965_2F	GACGAGGCCATGGCATTATAT
	Spo02965_2R	GACGAAGAACTGCTCCTACTTT
CLCc	Spo04588_2F	GCATCTCCATACACTGTGCT
	Spo04588_2R	AATTGGAGATCTCCCTTGACTC
CLCg	Spo08512_1F	AATGGCTTCTTGGTGGAGAA
	Spo08512_1R	CTTGCCACTGTAGCACAAATC
Spo09736 (EXLB1)	Spo09736_1F	TCCAACTGGAGCATGTGG
	Spo09736_1R	CCGTACCTGATAGCAACCAC
Spo15968 (SLAH2-like)	Spo15968_1F	GCAGATAACAGAGAAACTGAAGTAAC
	Spo15968_1R	CATGTGGAACAACTTTGGATGAA
Spo19814 (EGY3)	Spo19814_1F	AACAAGTTGAAGAATGGCTTTGG
	Spo19814_1R	GGCTTCCTCAAATTCCCTATGA
Spo11258 (E3-ubiquitin ligase)	Spo11258_1F	CCCTTAACTCATGCTCCTCAC
	Spo11258_1R	CGACGTGATCAATGGAGATGATA
Spo11709 (CRK10)	Spo11709_1F	GGTGTCTATAAGGGTACATTGTCA
	Spo11709_1R	TTGGCTACTAGTATGACCTCATTT
GAPDH	Spo21203_2F	GTGTCAACGAGGAAGGTTACA
	Spo21203_2R	CCCTTGATGATGCCAAATTTCT
ACTIN	Spo18993_2F	GGTCGTACTACTGGTATTGTATTGG
	Spo18993_2R	AGATCACGTCCAGCCAAATC
Actdf	Spo17116_1F	CAAAAGGTCAAACTCTCTTTCTGG
	Spo17116_1R	GCGATGATCCTTCTTCCTCTTTA

7. FINAL CONSIDERATIONS

Quinoa (*Chenopodium quinoa* Willd.) genotypes CPAC 09 and CPAC 11, developed by EMBRAPA Cerrados in Brazil, are extremely tolerant to soil salinity and sodicity. In naturally salinized soils, with the addition of rice husk biochar (RHB), quinoa (CPAC 09) developed adequately during the winter season in Brazilian Northeast, showing a drastic reduction in its survival during summer cultivation. Therefore, the indication for this genotype is winter cultivation, mainly in the Northeast of Brazil.

The addition of RHB, made from slow pyrolysis at 400 °C, in quinoa cultivation should be indicated for reducing soil pH, greater supply of K^+, reduction in Na^+ toxicity, and improvement in physical properties of degraded soils, mainly in sandy soils. In silty soils, low-grain RHB negatively affects the improvement of soil physical attributes such as porosity and saturated hydraulic conductivity (K_{sat}). In this study, in general, the RHB dose of 40 t ha^{-1} was the best agronomic dose.

The CPAC 09 genotype showed great potential for phytoextraction of salts, mainly K^+ and Cl^-. In its use for the reclamation of soils affected by salts, quinoa is not as efficient as other plants in the Amaranthaceae family, such as *Atriplex nummularia* L., due to its low potential for phytoextraction of Na^+. So, quinoa can be used to produce highly nutritious grains in food insecure regions of the country with salt affected soils.

Both CPAC 09 and CPAC 11 genotypes were able to survive in soils with electrical conductivity (ECe) of approximately 65 dS m^{-1}, but with drastic reductions in their phytomass and productivity. Despite these, it is possible to affirm the high potential of this crop to produce in saline-sodic soils considered unsuitable for the cultivation of other plants.

Spinach (*Spinacia oleracea* L.) was considered a plant that tolerates salinities of 25 dS m^{-1}, being able to develop and reproduce in saline soils. Based on the genetic similarity with quinoa, this work proposes that studies on this species be intensified, for its reclassification from a glycophyte plant to a facultative halophyte.

The presence of epidermal bladder cells (EBCs) in spinach, capable of storing Na, Cl, and K ions, indicates that this plant has mechanisms of tolerance to salt stress, being proven through the presence of genes such as SOS3, NHX1, NHX2 and AKT1, NPF2 .5, SLAH1, NPF2.4, and ALMT12 in both leaves and isolated EBCs, which play roles in regulatory mechanisms for salt stress management in spinach.