UNIVERSIDADE FEDERAL DE PELOTAS

Programa de Pós-Graduação em Fisiologia Vegetal

Tese

ALTERAÇÕES FISIOLÓGICAS EM PLANTAS DE AMARANTO E QUINOA CULTIVADAS SOB ELEVADO CO_2 E ALAGAMENTO

Bruna Evelyn Paschoal Silva

Capão do Leão, 2022

Bruna Evelyn Paschoal Silva

ALTERAÇÕES FISIOLÓGICAS EM PLANTAS DE AMARANTO E QUINOA CULTIVADAS SOB ELEVADO CO_2 E ALAGAMENTO

Tese apresentada ao Programa de Pós-Graduação em Fisiologia Vegetal da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Fisiologia Vegetal.

Orientador: Dr. Sidnei Deuner
Coorientador: Ph.D. Luis Antonio de Avila

Capão do Leão, 2022

Banca Examinadora:

_____

Ph.D. Walter Quadros Ribeiro Júnior

PhD – Dundee University (Scotland)

_____

Dr. Luciano do Amarante

Plant Biology - UNICAMP

_____

Dr. Marcus Vinicius Fipke

Doctor in Crop Protection – Federal University of Pelotas

_____

Dr. Sidnei Deuner

Doctor in Agronomy – Federal University of Lavras (advisor)

I dedicate this thesis to José Paschoal. I miss you more than words can say. Thank you for believing in my dream. I look forward to the day we meet again.

DEDICATION

ACKNOWLEDGEMENT

First of all, I would like to thank God for life and for always being my strength away from my family.

My whole family, in special my parents, Ana and José, who have always encouraged me to pursue my dreams. Brian and Cristhian, my siblings, for being part of my life, for their companionship, conviviality and fundamental friendship during this journey.

Igor, who is my boyfriend, and his family, for encouraging me and being my second family.

Few, but good friends that I made along this journey for sharing the good and bad moments in this "universe" called post-graduation.

Sidnei and Luis, my advisors, not only for their friendship, but also for believing in my potential and for sharing their knowledge during these years.

Postgraduate Program in Plant Physiology professors, for all the learning and teachings. There, I was able to meet people who changed my conception of what teaching is.

Embrapa Clima Temperado for providing the genetic material for the development of this research.

Federal University of Pelotas, for the opportunity to continue and qualify my education.

CAPES, for granting the scholarship and financial support.

Abstract

SILVA, Bruna Evelyn Paschoal. **Metabolic and nutritional changes in pseudocereals submitted to high CO_2 and flooding.** 2022. Thesis defense (Doctorate in Plant Physiology) – Postgraduate Program in Plant Physiology, Department of Botany. Institute of Biology. Federal University of Pelotas, Capão do Leão.

Responsible for causing a series of extreme weather events, climate change can lead to droughts, floods, rising temperatures and increased emissions of greenhouse gases such as carbon dioxide (CO_2). Since there is growing evidence that environmental changes and its impacts are leading to an understanding of potential pressures on the ability to ensure adequate food supplies for the human population, it is extremely important to comprehend how plants and natural ecosystems will respond to these changes. Thus, the present work aimed to investigate the potential effects of two different agents of climate change: CO_2 and flooding. Therefore, amaranth plants cv. BRS Alegria and quinoa cv. BRS Piabiru were evaluated in the transitional stage between vegetative and reproductive, since this is considered the key event for the reproduction of cultivated plants. Three studies were carried out: in open top chambers (OTC's) with different levels of CO_2 (400 and 700 ppm) evaluating the primary and secondary metabolism; in growth chambers where the seeds obtained in the previous experiment were evaluated for parental effects; and the third experiment involved submitting the plants to the condition of soil flooding. As a result, it was possible to observe that both cultures are capable of altering their primary and secondary metabolism due to elevated concentrations of CO_2. Changes in gas exchange, saccharolytic enzymes and carbohydrate metabolism reflected in growth parameters and photosynthetic pigments when at elevated CO_2 levels. These changes altered the nutritional dynamics of several nutrients, harming the productive potential of crops. In addition, evaluations related to secondary metabolism showed us there was an increase in antioxidant capacity, betalains and phenolic compounds. These results suggest that there was a generation of reactive oxygen species during the photosynthetic process. When evaluating amaranth and quinoa seeds and seedlings from plants grown in a CO_2-enriched environment, a parental effect can be observed in seeds and seedlings. The first germination count and the germination speed index showed that although its germination potential was not altered, this initial process occurred in a more accelerated way. These results could be confirmed through the biochemical activity of α-amylase and acid phosphatase enzymes in different periods and CO_2 concentrations. In another experiment, the plants were submitted to different periods of flooding, both species showed severe metabolic alterations when submitted to flooding in the periods of 48 and 96 hours. However, during recovery, photosynthetic pigments showed values close to the control, helping to maintain gas exchange in the fight against ROS only by amaranth and plants. On the other hand, analyzes of the invertases showed that sucrose synthase was not able to complete the cleavage process in its entirety. This suggests that compounds essential for metabolic pathways such as fructose and glucose were absent, in addition to a possible imbalance of sucrose between shoots and roots. The results of the present research show significant advances for the management of both crops in the face of climate change. However, studies aimed at the nutritional quality of these pseudocereals in the proposed scenario are still necessary.

Keywords: *Chenopodium quinoa* Wild. *Amaranthus cruentus*. Climate Change. Photosynthesis. Nutrition. Progenies. Enzymatic activity. Flooding. Oxidative stress.

Resumo

SILVA, Bruna Evelyn Paschoal. **Alterações metabólicas e nutricionais de pseudocereais submetidos ao elevado CO_2 e alagamento.** 2022. Defesa de Tese (Doutorado em Fisiologia Vegetal) – Programa de Pós-Graduação em Fisiologia Vegetal, Departamento de Botânica. Instituto de Biologia. Universidade Federal de Pelotas, Capão do Leão.

Responsáveis por ocasionar uma série de eventos extremos no clima, as mudanças climáticas podem levar à eventos tais como secas, inundações, elevação da temperatura e aumento das emissões de gases de efeito estufa como CO_2. Tendo em vista que são crescentes as evidências de que as alterações e impactos ambientais estão levando à compreensão das potenciais pressões sobre a capacidade de garantir suprimento adequado de alimentos para a população humana, é imprescindível compreender como as plantas e os ecossistemas naturais responderão a essas mudanças. Assim, o presente trabalho teve como objetivo investigar os potenciais efeitos de dois diferentes agentes das mudanças climáticas: CO_2 e alagamento. Para tanto, plantas de amaranto cv. BRS Alegria e quinoa cv. BRS Piabiru foram avaliadas no estádio de transição entre o vegetativo e o reprodutivo, uma vez que este é considerado o evento chave para a reprodução de plantas cultivadas. Foram conduzidos três estudos, sendo eles: em câmaras de topo aberto (OTC's) com diferentes níveis de CO_2 (400 e 700 ppm) avaliando o metabolismo primário e secundário; em câmaras de crescimento onde as sementes obtidas no experimento anterior foram avaliadas quanto aos efeitos parentais e por fim, a submissão das plantas a condição de alagamento do solo. Como resultado, foi possível observar que ambas as culturas são capazes de alterar o seu metabolismo primário e secundário devido a elevadas concentrações de CO_2. Alterações nas mudanças nas trocas gasosas, nas enzimas sacarolíticas e no metabolismo de carboidratos refletiram nos parâmetros de crescimento e pigmentos fotossintéticos quando em elevados níveis de CO_2. Estas mudanças foram capazes de alterar a dinâmica de nutrientes, prejudicando o potencial produtivo das culturas. Além disso, as avaliações relacionadas ao metabolismo secundário, nos evidenciaram o incremento da capacidade antioxidante, betalaínas e de compostos fenólicos. Estes resultados sugerem que houve geração de espécies reativas de oxigênio durante o processo fotossintético. Quando avaliadas as sementes e plântulas de amaranto e quinoa provenientes de plantas cultivadas em ambiente enriquecido por CO_2, um efeito parental pode ser observado em sementes e plântulas. A primeira contagem de germinação e o índice de velocidade de germinação mostraram que apesar de o seu potencial germinativo não ter sido alterado, este processo inicial ocorreu de forma mais acelerada. Estes resultados foram confirmados por meio da atividade bioquímica das enzimas α-amilase e fosfatase ácida nos diferentes períodos e concentrações de CO_2. Já para o experimento onde as plantas foram submetidas ao alagamento, ambas as espécies apresentaram alterações metabólicas severas quando submetidas a inundações nos períodos de 48 e 96 horas. Porém, durante a recuperação, os pigmentos fotossintéticos apresentaram valores próximos ao controle, auxiliando na manutenção das trocas gasosas no combate das ROS pelas plantas de amaranto. Por outro lado, as análises das invertases demonstraram que possivelmente a sacarose sintase não foi capaz de realizar em sua totalidade o processo de clivagem. Isso sugere que compostos essenciais para as rotas metabólicas como frutose e glicose

estavam ausentes, além de um possível desbalanço de sacarose entre parte aérea e raízes. Os resultados da presente pesquisa mostram avanços significativos para o manejo das duas culturas frente as mudanças climáticas, entretanto, estudos que visem a qualidade nutricional destes pseudocereais frente ao cenário proposto ainda são necessários.

Palavras-chave: *Chenopodium quinoa* Wild. *Amaranthus cruentus*. Mudança Climática. Fotossíntese. Nutrição. Progênie. Atividade Enzimática. Alagamento. Estresse oxidativo.

List of figures

List of Tables

SUMMARY

1. GENERAL INTRODUCTION

Climate is a major force in Earth's environmental system, and even minor changes in climate can have complex and serious effects on the environment and nature (VARANASI, PRASAD, JUGULAM; 2016). However, climate change projections are associated with a range of limitations and uncertainties, driven mainly by the model and uncertainties, because climate change every moment (RAO et al., 2016), but what can we effectively conclude as climate change? That is a very important question and there have been many discussions about the implications of these phenomena, due to abiotic stresses caused in the environment (ESPELAND; KETTERING, 2018).

Although the variables which are commonly used in studying climate are concerned mainly with the atmosphere, we cannot look at the atmosphere alone because the process in the atmosphere is coupled to the cryosphere and biosphere. Therefore, it is convenient to consider three categories of events that may affect the climate: events that occur outside the Earth (variations in the Sun); natural events on the surface of or within the Earth (plate tectonics; cryosphere; lithosphere; ocean circulation; biosphere) and human activities (carbon dioxide; ozone) (CRACKNELL; VAROTSOS, 2021). Moreover, we can consider anthropogenic activities as the main cause of altered weather conditions and their effects predicted changes are presented by the changes in frequency and severity of extreme climatic events which directly affect agricultural food production (MATZRAFI et al., 2016).

Responsible for causing a series of extreme events in the climate, climate change can lead to events such as droughts, floods, temperature rise, among others. Modifications in the atmosphere can also be observed with increasing UV radiation, concentrations of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3) (HORWATH; KUZYAKOV, 2018).

Carbon emissions related to human activities have contributed mainly to an increase of CO_2 concentration in the atmosphere. In the climate projection scenarios for this century, considering the absence of mitigations to restrict the emission of greenhouse gases, it is estimated that, by the year of 2030, the concentration of equivalent CO_2 will exceed 450 ppm, and possibly the concentration will reach values between 750 and 1300 ppm at the end of the century (IPCC, 2022). Also, if the amount of greenhouse gas emissions continues to grow at the current rates throughout the

next years, the planet's temperature may increase up to 4.8°C this century, which could result in an up to 82-centimeter rise in sea level and cause important damage to most coastal regions (ASSAD; RIBEIRO; NAKAI, 2019).

The increase of CO_2 concentration has a direct effect on plants. The mechanism of C_3 plants response to elevated concentrations of carbon dioxide e[CO_2] (elevated carbon dioxide) has been well-demonstrated over the years, as well as their primary effects, such as increased photosynthesis, reduced stomatal conductance and decreasing photorespiration (LI et al., 2019). Nevertheless, Ziska and Bunce (1997), already called attention to the need for studies with species of C_4 metabolism. Growth of C_4 plants also increases at e[CO_2] but for some authors (LEAKEY et al., 2006; MARKELZ; STRELLNER; LEAKEY, 2011), that's not a direct effect in photosynthesis. The argument used by this author is that C_4 photosynthesis is saturated at the current concentration atmospheric and the changes are possible only when there is a combination of drought and e[CO_2] (SOUZA et al., 2013; RUNION et al., 2016; FARIA et al., 2018; LI et al., 2019).

Soil flooding is a major abiotic stress that severely constrains plants growth and reduces agricultural production in many regions. It was estimated that flooding stress might affect 12% of cropped areas globally (SHABALA, 2011). Rio Grande do Sul, one of the main agricultural states of Brazil, is located on a flood-prone (alluvial and hydromorphic), 28-million-hectares territory. This fact favors the cultivation of plants such as rice. However, flooding is harmful to some crops which were commonly introduced to enhance the efficiency of the rice cropping system, like soybean (GARCIA et al., 2020).

Currently, some farmland management measures are used to alleviate the damage of the flooding on crop growth environment. Higher plants require large amounts of free water. Nevertheless, the excess of water in the root zone can be harmful or even deadly. One of the first changes that occur in flooded soil is the decrease in oxygen concentration. Oxygen scarcity in water-saturated soils is due to an imbalance between the slow diffusion of the gas into the water and the consumption of O_2 by micro-organisms and plant roots (VISSER et al., 2003). Furthermore, the effects of flooding are complex and vary depending on genotype, environmental conditions, growth stage, and flooding duration (TIAN et al., 2019).

Regarding the magnitude, as we experience these changes, we will continue to face other challenges. The ever-increasing world population demand increasing

amounts of food and to feed the nine billion people who are expected to inhabit the planet by 2050, a significant increase in grain yields of approximately 44 million metric tons per year will be needed (MBOW et al., 2019).

Climate changes affect food security both directly (impacts on yields) and indirectly (impacts on water availability and quality, pests and diseases, and pollination services (FANZO et al., 2017; WATTS et al., 2018;). Another route of food safety are risks during transport and storage, which can also be exacerbated by changing climate (MBOW et al., 2019).

Concerns about how plants and natural ecosystems will respond to such changes are relevant to the current scientific agenda since climate change is already responsible for changes in species distribution (LENOIR et al., 2008). Thus, one of the alternatives, in order to guarantee the food supply, is the use of plants considered “unusual” for large crops such as rice, soybeans and corn.

In this context, pseudocereals, part of a group of non-grass species, are considered crops of the 21st century. These crops possess a high nutritional profile and have been placed under the category of important crops by UNESCO due to the dwindling cultivation and exploitation in the wild. Besides, these pseudo cereals have gained worldwide importance in the nutraceutical industry due to the rich nutritional profile compared to cereals. These plants are enriched with various active principles such as polyphenols, flavonoids, amino acids, dietary fiber, lignans, vitamins, minerals, antioxidants, unsaturated fatty acids and other essential components like fagopyritols. Despite pseudocereals have been widely recognized for many years, interest has increased enormously since the turn of the century and research about them has intensified (MIR; RIAR; SINGH, 2018; PIRZADAH; MALIK, 2020; SAEID; AHMED, 2021).

Predominant in pseudocereals, secondary metabolism demonstrated phytochemicals that are not universal and not essential for the survival of these plants (CORDERO-DE-LOS-SANTOS et al., 2005). It is well known that plants produce these chemicals to protect themselves against other plants, pests and pathogens and environmental stress (FERGUSON et al., 2003; LI et al., 2010), therefore showing a greater resistance to adverse conditions imposed by climate changes.

The family *Amaranthaceae* is generally considered the “Amaranth family.” The word Amaranthus is basically derived from the Greek word “Anthos” (Flower) which means everlasting (RASTOGI; SHUKLA, 2013). Amaranth (*Amaranthus* spp) is a C_4

plant and an indigenous pseudo-grain, originated in North American. Despite amaranth grains were consumed by ancient pre-Columbian cultures, and we can consider, domesticated in South America (REYES-FERNÁNDEZ; REYES-MORENO; CUEVAS-RODRÍGUEZ, 2019).

Amaranth (*Amaranthus cruentus*) is a fast-growing crop. Due to its low production cost, it is one of the cheapest dark green vegetables in the tropical market, often described as the poor man's vegetable. Unlike other green vegetables, it is cultivated during summer, when other green vegetables are not available in the market. Considering its extraordinary nutritional quality, with a higher amount of protein content (14–19%) than other types of traditional cereal crops, there is potential worldwide agronomic value for this plant. Amaranth is also a good source of flavonoids and tocopherols. The lipid content in the seeds included 6-7% of squalene compounds can reduce cancer risk, lipid metabolism control, anti-aging effects on the skin and positive implications on the human immune system. Finally, is a rich source of magnesium, potassium, phosphorous and zinc minerals and one of the important characteristics a larger genetic diversity, phenotypic plasticity, tolerance of drought, and other stress factors (SAEID; AHMED, 2020).

Quinoa (*Chenopodium quinoa* Willd) is also an *Amaranthaceae* but unlike Amaranth, is a C_3 plant. A native of the Andes, quinoa dates back to more than 5000 years. Considered sacred by the Incas, it was called ‘‘the mother grain’’ and sustained ancient communities. It's an annual plant found in the Andean region of South America, between sea level and the heights of the Bolivian Altiplano at around 4000 m above sea level. It produces flat, oval-shaped seeds that are usually pale yellow but can range in color to pink. The Food and Agriculture Organization of the United Nations (FAO) chose quinoa as one of the crops destined for food security during this century (FAO, 2002) and launched the “International Year of Quinoa 2013” in this context (FAO, 2013).

Rich in macronutrients, quinoa have special proteins which are analogous to the quality of the casein. It's also gluten-free like amaranth, because of the lack of prolamins. There are useful levels of lipids, such as monounsaturated fat (as oleic acid) and small quantities of omega-3 fatty-acids such as *alpha-linolenic acid*, which are good for people's health. It also contains higher fiber, minerals and carbohydrates such as polysaccharides that have a low glycemic index and we can consider this species a pioneer in phytochemicals, antioxidants such as tocopherols and flavonoids such as

quercetin and kaempferol (SPEHAR; SANTOS, 2002; ABUGOCH-JAMES, 2009 ABUGOCH et al., 2009; IQBA et al., 2019).

The attenuation of the response mechanisms of pseudo cereals in the face of climate change is still a challenge, since each species, genotype and cultivar have distinct behavior. It is also important to consider the scarcity of studies that make it possible to anticipate and adapt practices to maximize agricultural production in future climate scenarios, aiming at the transition phase from vegetative growth to flowering, since this stage is considered the key event of the cycle of life in annual plants (SILVA; HANSSON; JOHANSSON, 2021). Therefore, the general objective of this study was to elucidate the effects of atmospheric CO_2 elevation and flooding on amaranth and quinoa plants.

REFERENCES

ABUGOCH, L. et al. Stability of quinoa flour proteins (*Chenopodium quinoa* Willd.) during storage. **International journal of food science & technology**, v. 44, n. 10, p. 2013-2020, 2009.

ABUGOCH -JAMES, L. E. Quinoa (*Chenopodium quinoa* Willd.): composition, chemistry, nutritional, and functional properties. **Advances in food and nutrition research**, v. 58, p. 1-31, 2009.

ASSAD, E. D.; RIBEIRO, R. R. R.; NAKAI, A. M. Assessments and How an Increase in Temperature may have an Impact on Agriculture in Brazil and Mapping of the Current and Future Situation. In: NOBRE, C. A.; MARENGO, J. A.; SOARES, W. R. (Ed.). **Climate change risks in Brazil**. Springer International Publishing, 2019.

CORDERO-DE-LOS-SANTOS, MY et al. Caracterização físico-química e funcional de isolados proteicos de amaranto (*Amaranthus hypochondriacus*) obtidos por precipitação isoelétrica e micelização. **Ciência e tecnologia alimentar internacional** , v. 11, n. 4, pág. 269-280, 2005.

CRACKNELL, Arthur P.; VAROTSOS, Costas A. **Understanding Global Climate Change: Modelling the Climatic System and Human Impacts**. CRC Press, 2021.

ESPELAND, E. K.; KETTENRING, K. M.; Strategic plant choices can alleviate climate change impacts: A review. **Journal of Environmental Management**. v. 222, p. 316–324. 2018.

FAO. **Food and Agriculture Organization of the United Nations**. 2013. Disponível em http://www.fao.org/. Acesso em: 10 de julho de 2021.

FANZO, J. From big to small: the significance of smallholder farms in the global food system. **The Lancet Planetary Health**, v. 1, n. 1, p. e15-e16, 2017.

FARIA, A. P. et al. The increase of current atmospheric CO_2 and temperature can benefit leaf gas exchanges, carbohydrate content and growth in C_4 grass invaders of the Cerrado biome. **Plant Physiology and Biochemistry**, v. 127, p. 608-616, 2018.

FERGUSON, A. W. et al. Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. **Agriculture, ecosystems & environment**, v. 95, n. 2-3, p. 509-521, 2003.

GARCIA, N. et al. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. **Environmental and Experimental Botany**, v. 172, p. 103975, 2020.

HORWATH, W. R.; KUZYAKOV, YAKOV. The Potential for Soils to Mitigate Climate Change Through Carbon Sequestration. In**: Climate Change Impacts on Soil Processes and Ecosystem Properties**. Chapter 3. p. 61-92. 2018.

IQBAL, S. et al. Yield potential and salt tolerance of quinoa on salt-degraded soils of Pakistan. **Journal of Agronomy and Crop Science**, v. 205, n. 1, pág. 13-21, 2019.

LEAKEY, A. D. B. et al. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. **Plant physiology**, v. 140, n. 2, p. 779-790, 2006.

LENOIR, J. et al. A significant upward shift in plant species optimum elevation during the 20th century. **Science**, v. 320, n. 5884, p. 1768-1771, 2008.

LI, B. et al., Genetic variation in nitrogen use efficiency of loblolly pine seedlings. **Forest Science**, Amsterdam, v.37, n.2, p.613-626, 1991

LI, P. et al. Photosynthesis and yield response to elevated CO_2, C_4 plant foxtail millet behaves similarly to C3 species. **Plant Science**, v. 285, p. 239-247, 2019.

MARKELZ, R. J C.; STRELLNER, R. S.; LEAKEY, A. D. B. Impairment of C_4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO_2] in maize. **Journal of experimental botany**, v. 62, n. 9, p. 3235-3246, 2011.

MATZRAFI, M. et al. Climate change increases the risk of herbicide-resistant weeds due to enhanced detoxification. **Planta**, v. 244, n. 6, p. 1217-1227, 2016.

MBOW, C. et al. IPCC Special Report on Land and Climate Change. **Chapter 5: Food Security**. 2019.

MIR, N. A.; RIAR, C. S.; SINGH, S. Nutritional constituents of pseudo cereals and their potential use in food systems: A review. **Trends in Food Science & Technology**, v. 75, p. 170-180, 2018.

MIR, S. A.; MANICKAVASAGAN, A.; SHAH, M. A. (Ed.). **Whole Grains: Processing, Product Development, and Nutritional Aspects**. CRC press, 2019.

MOTA, C. et al. Protein content and amino acids profile of pseudocereals. **Food chemistry**, v. 193, p. 55-61, 2016.

PIRZADAH, T. B.; MALIK, B. Pseudocereals as super foods of 21st century: Recent technological interventions. **Journal of Agriculture and Food Research**, v. 2, p. 100052, 2020.

RAO, S. et al. A multi-model assessment of the co-benefits of climate mitigation for global air quality. **Environmental Research Letters**, v. 11, n. 12, p. 124013, 2016.

RASTOGI, A.; SHUKLA, S. Amaranth: a new millennium crop of nutraceutical values. **Critical reviews in food science and nutrition**, v. 53, n. 2, p. 109-125, 2013.

RUNION, G. Brett et al. Varied growth response of cogongrass ecotypes to elevated CO2. **Frontiers in plant science**, v. 6, p. 1182, 2016.

REYES-FERNÁNDEZ, P-C.; REYES-MORENO, C. Edith-Oliva Cuevas-Rodríguez, and. **Whole Grains: Processing, Product Development, and Nutritional Aspects**, 2019.

SAEID, A; AHMED, M. A Review on Effects of Pseudo Cereals Flour on Quality Properties of Biscuit, Cookies and Cake. 2021.

SILVA, W. T. A. F; HANSSON, M; JOHANSSON, J. Light competition and phenological adaptation of annual plants to a changing climate. **Climate Change Ecology**, p. 100007, 2021.

SPEHAR, C.; SANTOS, R. L. de B. Quinoa BRS Piabiru: alternativa para diversificar os sistemas de produção de grãos. **Pesquisa Agropecuária Brasileira**, v. 37, p. 809-893, 2002.

SOUZA, A. P. et al. Elevated CO_2 increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. **Plant, cell & environment**, v. 31, n. 8, p. 1116-1127, 2008.

TIAN, Lixin et al. Effects of waterlogging stress at different growth stages on the photosynthetic characteristics and grain yield of spring maize (*Zea mays* L.) under field conditions. **Agricultural Water Management**, v. 218, p. 250-258, 2019.

VARANASI, A.; PRASAD, P. V. V.; JUGULAM, M. Impact of climate change factors on weeds and herbicide efficacy. **Advances in agronomy**, v. 135, p. 107-146, 2016.

WATTS, Nick et al. The 2018 report of the Lancet Countdown on health and climate change: shaping the health of nations for centuries to come. **The Lancet**, v. 392, n. 10163, p. 2479-2514, 2018.

CHAPTER 1

Pseudo cereals cultivated under an CO_2-enriched atmosphere alter physiological and growth parameters, nutrient distribution and grain yield

1. INTRODUCTION

The appearance of groundbreaking mechanisms capable of detecting small changes in climate, we can observe that these alterations occur for 2 million years (HANNAH, 2015). However, in the past, gas releases may have occurred naturally from massive seabed deposits of methane hydrates, emissions from volcanic eruptions, or decay of vegetation associated with asteroid impacts. Nowadays, anthropogenic emissions of greenhouse gases (GHGs) may have similar effects. These emissions have massive effects on the global carbon cycle and are causing major changes in climate (SHINDELL, 2015; SHINDELL et al., 2019; IPCC, 2022).

Accepted as being the main cause due to the rise of CO_2 in the atmosphere, global warming is recognized widely. The United Nations has been summarizing and correlating its impacts. The concentration of GHGs in the Earth's atmosphere is directly linked to the average global temperature of the Earth which, at the same time, has been rising steadily since the time of the Industrial Revolution and the most abundant gas, accounting for two-thirds of GHGs, is CO_2, the most common byproduct of fossil fuel burning (LETCHER, 2021).

Results of large-scale experiments presented large variations and showed that an increase in CO_2 does not necessarily promote plant growth, varying from species to species (LEAKEY et al., 2009; KIMBAL, 2016). These changes, considered positive in a short time, mainly promote the improvement of water use efficiency (amount of organic matter produced by the amount of water used) in both C_4 and C_3 plants. This process occurs, because of a decrease in stomatal conductance when CO_2 concentrations are higher (ALMEIDA et al., 2016; LIU et al., 2019).

The impacts of climate change on plant physiology, have added to the event of population increase and raised serious concerns about food security around the world (HERRERO et al., 2017). Besides, Brazil is a special case in this context because agriculture is a key economic activity and the majority of people working in agriculture

in the country are family farmers. Smallholders help to maintain the diversity of agricultural products and nutrients worldwide, collaborating with food security in this scenario (CAVALI et al., 2020; ANTOLIN; HEINEMANN; MARIN, 2021).

The most direct and simplest adaptive measure would be to encourage these farmers to carry out research that supports the implementation of different cultures (OMAR; MOUSSA; HINKELMANN, 2021). In addition, smallholders should preferentially cultivate products with higher added value, such as Pseudocereals. Most likely replacement products derive from protein-rich crops, including pseudocereals. Although these crops have declined in both production and consumption during several decades, they are achieving great recognition in recent years due to their effective and sustainable way (MANNERS; VARELA-ORTEGA; ETEN, 2020).

The term pseudocereals according to the American Heritage Dictionary of English language, can be defined as any plant that does not belong to the grass family. On the other hand, these plants, produce fruits and seeds usually used as flour for bread and other staple foods (MIR; RIAR; SINGH, 2018). Besides that, like cereals, they have starchy, dry seeds and are much more protein-rich. Plants usually included in the non-systematic grouping of pseudocereals are dicotyledonous and belong to different families. Consequently, plants such as quinoa and amaranth are placed in the *Chenopodiaceae* and *Amaranthaceae* family, which belongs to the *Caryophyllales* order, a subclass of *Caryophyllidae* (SCHOENLECHNER, 2016; CHANDRA et al., 2021).

Given the growing evidence that the research about changes and impacts in climate is leading to a better understanding of the potential pressures on the ability to ensure an adequate food supply for the human population, comprehend how plants response it is essential to precede studies related to these effects on the plant physiology (HATFIELD; WALTHALL, 2014).

Evidence that e[CO_2] can cause rapid growth has been proved over the years, since the first researches in the 1970s and 1980s (HOFSTRA and HESKETHSIONIT, 1975; HELLMERS; STRAIN, 1982). These alterations happen due to the fact that photosynthetic carbon gain results in an enhanced carbohydrate metabolism. As a consequence, there are also alterations in assimilate partitioning and sink-strength in plants (DRAKE et al. 1997; LONG et al. 2006; ZONG; SHANGGUAN, 2016).

As an important component of source-sink, the model of Munch, postulates that unloading and loading of the conducting tissue are mainly driven by concentration

and/or osmotic gradients (MÜNCH, 1930; HERBERS; SONNEWALD, 1998). From another point of view, the source-sink can be considered as the competitive ability of an organ to import photoassimilates (HO, 1998). However, studies about how the carbohydrate metabolism of pseudocerals responses to e[CO2] and what may be the impacts of the alteration of the source-sink function resulting from the effect of the increase in the concentration of atmospheric carbon on the nutrition, metabolism, and productivity of quinoa and amaranth plants are incipient.

Therefore, the aim of this study was to verify the effects of elevated CO_2 e[CO2] in Quinoa and Amaranth plants, considering that these species have C_3 and C_4 metabolism respectively. Here, it was demonstrated in a 2-year experiment that the relationship of physiological parameters as growing and photosynthesis are intimately linked with source-sink, sucrose metabolism and nutrients content influencing the grains' productivity.

2. MATERIAL AND METHODS

Plant material and growth conditions

The experiments were conducted in the agricultural years of 2019/2020 and 2020/2021 in Open Top Chambers (OTC) belonging to the Herbology Center of the Federal University of Pelotas, located in the municipality of Capão do Leão - RS. These chambers are equipped with sensors, an automated CO_2 concentration control center, coolers responsible for homogenizing the air inside them, a system of gas injection and distribution valves in each chamber, maintaining concentrations of 400 (control) and 700 ppm (elevated CO_2) respectively. The OTC has a useful area of $4m^2$ and 2.15 m in height, coated with a 150-micron thick, transparent polyethylene plastic film and equipped with a top-opening reducer to deflect the air and prevent the dilution of the desired concentration of CO_2 inside the chamber. Carbon dioxide (Messer®) used was 99.9% pure and was supplied through a storage cylinder (capacity of 25 kg CO_2) coupled to the injection and distribution system of the chambers. The internal temperature of the OTC's was monitored daily using a data logger (HOBO Pro v2), installed at the height of the canopy of the plants.

Seeds of the cultivar BRS Alegria (amaranth) and BRS Piabiru (quinoa) were seeded in polystyrene trays on commercial substrate (Plantmax®). After the appearance of the second pair of true leaves, the seedlings were transplanted into 8-L polyethylene pots filled with soil, which was previously analyzed for its physical and chemical attributes, amended and fertilized according to technical recommendations (EMBRAPA 1999), keeping only one plant per pot after the complete establishment of the plants.

Table 1: Physical and chemical characteristics of the soil collected in the experimental area of Palma, Campus Capão do Leão, UFPel.

pH H_2O 01:01	Ca^{2+}	Mg^{2+}	Al^{3+}	Effective CEC	% SOC	% Clay	Clay Class	K^{+}	P^{*}
	$cmol_c\ dm^{-3}$				m/v			$mg\ dm^{-3}$	
4.8	1.5	0.6	1.4	3.78	1.38	16	4	30	6

* Phosphorus(P) was extracted using Mehlich solution

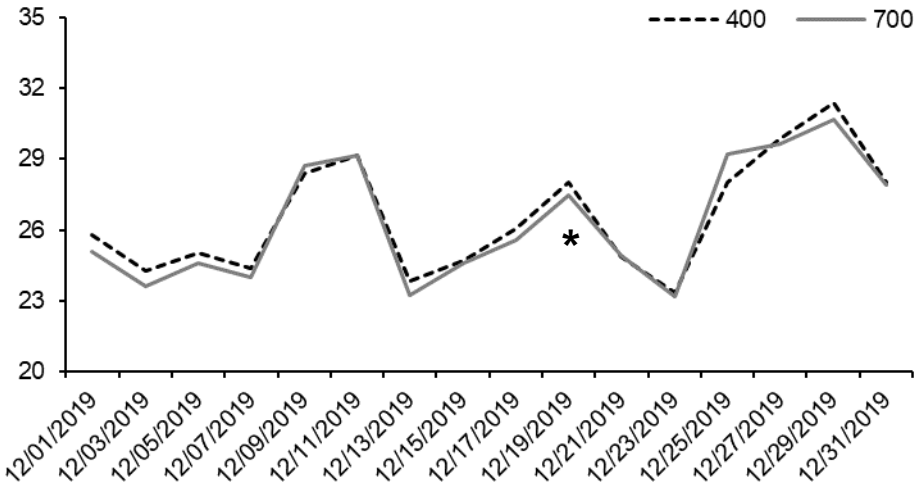

Figure 1: OTC's average indoor ambient temperature during the month of December 2019 – 400 ppm CO_2 and 700 ppm CO_2. *Transition period between vegetative and flowering.

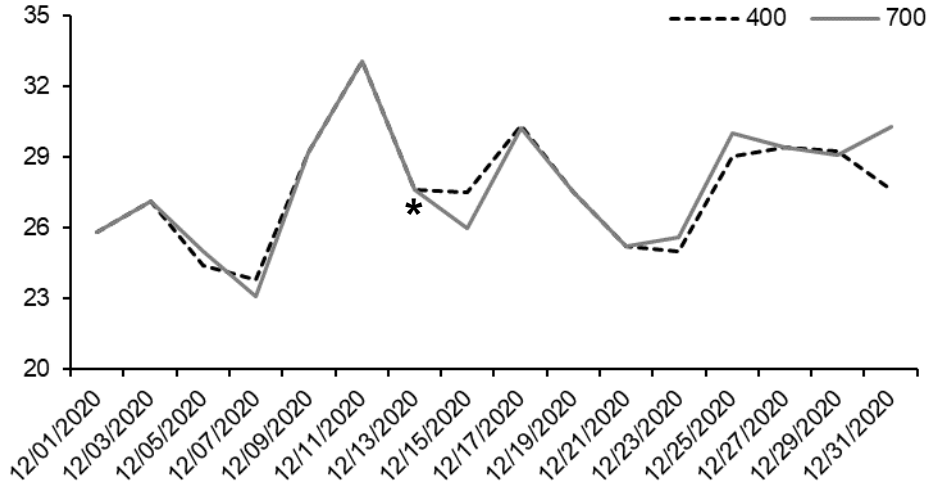

Figure 2: OTC's average indoor ambient temperature during the month of December 2020 – 400 ppm CO_2 and 700 ppm CO_2. *Transition period between vegetative and flowering

Growth parameters: Shoot (SL) and root length (RL) were evaluated using a ruler (cm). Shoot (SMD) and root (RDM) dry matter were obtained placing samples in an oven at 65ºC until constant weight (mg $plant^{-1}$). Stem diameter assessed using a caliper rule (mm). Panicle length (PL) evaluated using a ruler (cm). Branches per panicle (pot^{-1}). Leaf area (cm^2) was estimated using the following methodologies:

Amaranth Leaf area **(LA)** = 2HC/3 (MONTEIRO et al., 2005); **2/3** is the form factor determined for amaranth leaves, while **H** and **C** indicate the largest leaf dimensions in the longitudinal and transversal directions. The last pair of expanded leaves were used.

Quinoa Leaf Area **(LA)** = F(LxC), in which the correction factor of **F**, obtained by the Benincassa equation (2003) is 0.6079 (cultivar Piabiru); **L** and **C** indicate the largest leaf dimensions in the longitudinal and transversal directions. The last pair of expanded leaves were used.

Extraction and quantification of photosynthetic pigments:The photosynthetic pigments were quantified according to the methodology proposed by Wellburn (1994). For this purpose, leaf discs of two young, expanded leaves were used, in four repetitions per treatment. The leaves were cut into small segments, using 0.01 g of fresh sample inserted into test tubes containing 3.5 mL of dimethyl sulfoxide (DMSO) neutralized with 5% calcium carbonate. Then, the tubes were incubated in a water bath at a temperature of 65ºC for 1 hour, protected from light and then cooled in the dark until reaching room temperature. After, absorbance readings at 480 nm, 649

nm and 665 nm were taken in a spectrophotometer. Chlorophyll *a*, *b*, total and carotenoid contents were calculated based on the equations: Chlorophyll a = (12.47 x A665) - (3.62 x A649); Chlorophyll b = (25.06 x A649) - (6.5 x A665); Total chlorophyll = chlorophyll *a* + chlorophyll *b*; Carotenoids = (1000 x A480) - (1.29 x chlorophyll a) - (53.78 x chlorophyll b) / 220; and the results were expressed in mg g^{-1} FW.

Leaf gas exchange: The leaf gas exchange was performed using an IRGA infrared gas analyzer (LI6400, Licor). The evaluation was carried out between 8:30 and 10:00 AM. The concentration of CO_2 in the chamber was matched for each treatment (400 and 700 ppm) and the photon flux density was regulated to 1500 µmol of photons m^{-2} s^{-1} with a light source attached to the measuring chamber. Net CO_2 assimilation (*A*), Stomatal conductance (g_s), Internal concentration of CO_2 (*Ci*) and Transpiration rate (*E*) were measured in the youngest expanded leaf. Water use efficiency (*WUE*) was obtained through the *A/E* ratio.

Extraction and quantification of total soluble sugars (TSS), starch, sucrose (SUC) and total soluble amino acids (SAA) in leaves: The collected material was standardized, using approximately 250 mg of the middle third of two fully expanded leaves, with four repetitions per treatment. After weighed, the material was macerated in 8 mL of extracting solution M:C:W (methanol: chloroform: ultra-pure water in the proportion of 12:5:3) and stored in amber flasks for 24 hours in the dark. After this period, 2 mL of M:C:W solution were added and the extract was centrifuged at 2500 g for 30 minutes. After centrifugation, 8 mL of the supernatant was transferred to Falcon tubes, and 2 mL of chloroform and 3 mL of milli-Q water were added. The falcons were centrifuged again for 30 min at 2500 g for phase separation. The upper phase was collected and concentrated by evaporation to approximately 50% of the volume at 30ºC, in order to eliminate the excess methanol and chloroform residues present. The extract obtained at the end was later used for quantification of TSS (GRAHAM and SMYDZUK, 1965), SUC (HANDEL, 1968) and SAA (YEMM; COCKING, 1955).

Precipitate obtained from the first centrifugation, after drying at room temperature, was resuspended in 8 mL of 10% (w:v) trichloroacetic acid (TCA). In the above precipitate 10 mL of 30% perchloric acid were added. After stirring for 30 minutes, the tubes containing the reaction medium were centrifuged at 2500 rpm for 30 minutes. Starch was quantified from the collected supernatant (GRAHAM and SMYDZUK, 1965).

Quantification of TSS was done using test tubes with screw caps bathed in ice. After adding the extracts diluted in pure water, 1.5 mL of anthrone solution (0.15% in concentrated sulfuric acid) was added to each tube. After 15 minutes, the tubes were shaken and incubated at 90 °C for 20 minutes. Thereafter, the tubes were kept in the dark until reaching room temperature.

Starch determination was performed in the same way as TSS. At the end of the process, values obtained were multiplied by the correction factor 0.9, for conversion into starch contents. The determination of PSA was carried out accordingly to the same methodology used for AST. Readings were performed in a spectrophotometer, at wavelengths of 620 nm for total soluble sugars, starch, water soluble polysaccharides, sucrose and 570 nm for total soluble amino acids.

For the quantification of sucrose, test tubes with screw caps bathed in ice, extracts were used 100 µL of 30% KOH were transferred to tubes. The tubes were incubated in a water bath for 10 min at 100 °C. After reaching room temperature, 3 mL of anthrone (0.15% in 70% sulfuric acid) were incubated again in a water bath at 40 °C for 15 min.

SAA contents were determined from extracts plus 0.5 mL of 0.2 M citrate buffer pH 5.0, 0.2 mL of 5% ninhydrin reactive in ethylene glycol monomethyl ether and 1 mL of 2% (v/v) KCN in methyl cellusolve (prepared from the 0.01 M KCN solution in pure water). The capped test tubes were incubated in a water bath at 100 °C for 20 minutes. After 20 minutes at room temperature, 1.3 ml of 60% ethanol was added.

Determination of sucrose metabolism-related enzyme activity: Leaf samples from the apical portions of the plants (about 0.4 g) were ground until a fine powder in the presence of liquid N_2. The extraction of the neutral/alkaline invertase (CINV) and the acid invertase enzymes (CWINV and VINV) followed the methodology described by Zeng et al. (1999), with minor modifications. In each sample, 1.5 mL of extractor medium containing potassium phosphate buffer (200 mM, pH 7.5), PMSF (1 mM), $MgCl_2$ (5 mM), DTT (1 mM) and ascorbic acid (50 mM) was added and then centrifuged at 18,000 × g for 20 min at 4°C. The supernatant solution was collected to measure soluble invertase activity (VINV and CINV) and the precipitate was collected to measure insoluble invertase (CWINV). In addition to the reagents used for the soluble invertases, NaCl (1 M) and Tritonne-X-100 (1%) were also added for CWINV. The enzyme extract (500 µL) was added to 1000 µL assay medium containing 500 µL sodium acetate buffer (pH 4.5 for VINV and CWIN activity and pH 7.5 for CINV activity),

200 mM sucrose and 5 mM $MgCl_2$. The incubation temperature was 37°C, and 200 µL aliquots were collected after 10 and 40 min to determine enzymatic activity. Enzymatic activity was evaluated by quantifying reducing sugars produced according to the dinitrosalicylic acid (DNS) method described by Miller (1959). All enzyme activities were determined in triplicate and expressed in micromoles of glucose per gram of fresh weight per min (µmol glucose g^{-1} FW min^{-1}).

Susy activity was determined according to Lowell et al. (1989), with some modifications. The enzymatic extract of Susy was prepared using 0.5 g of homogenised samples, 0.05 M HEPES (pH 7.0), 1 mM EDTA (Ethylenediamine tetraacetic acid), 2 mM $MgCl_2$ and DTT (dithiothreitol), 0.1 M ascorbic acid and water. The homogenate was centrifuged at 13,000 × g for 20 min at 4°C. Then, 100 µL of extract (supernatant) was added to 1900 µL of the medium containing 0.1 M morpholino ethanesulgonic acid (MES) buffer (pH 6.0), 0.005 M $MgCl_2$, 0.3 M sucrose, 0.005 M uridine 5'diphosphoglucose disodium (UDP) and water. The determination of Susy enzyme activity was the same as that for the invertases.

Nutrient contents in leaves (TEDESCO, 1995): A mixture of leaves from different parts of the plants was collected and placed in a greenhouse at 65ºC until constant weight and then double ground in a mill. Approximately 200 mg were weighed on an analytical balance for subsequent sulfuric digestion of macronutrients and 500 mg for nitrous-perchloric digestion of micronutrients. From the digested material, the reading of Nitrogen (N) – Kjeldhal method (nitrogen distiller TE-0364); Phosphorus (P) - spectrophotometer at 660 nm; Potassium (K) - Flame Photometer (Micronal B462); Calcium (Ca); Magnesium (Mg); Zinc (Zn); Copper (Cu); Manganese (Mn) and Iron (Fe) - Flame Atomic Absorption Spectrophotometer (Model AA 990F - PG Instruments brand).

Yield components per pot: For the grain yield components, 10 replicates per treatment were used, where each pot with a plant was considered a replicate. The weight of one thousand grains pot^{-1}, number of panicle $grains^{-1}$ and weight of grains pot^{-1} were determined.

Experimental design: The experimental design was completely randomized, totaling 10 plants of each species per treatment. The data obtained were analyzed for homoscedasticity by the Bartlett test and for normality by the Shapiro Wilk test, and considering the assumptions, the analysis of variance (ANOVA) was carried out using

the statistical software R (ExpDes.pt / www.r-project. org/). Afterward, when F was significant, the means were compared to the control by the t-test (P≤0.05).

3. RESULTS

Growth parameters

Results presented in Figure 3 demonstrate that there was a significant difference among treatments (p≤0.05). When cultivated at 700 ppm of CO_2, plants showed greater growth, making the variables SL (3A), RL (3C), SDW (3B) and RDW (3D) higher than the concentration of 400 ppm of CO_2.

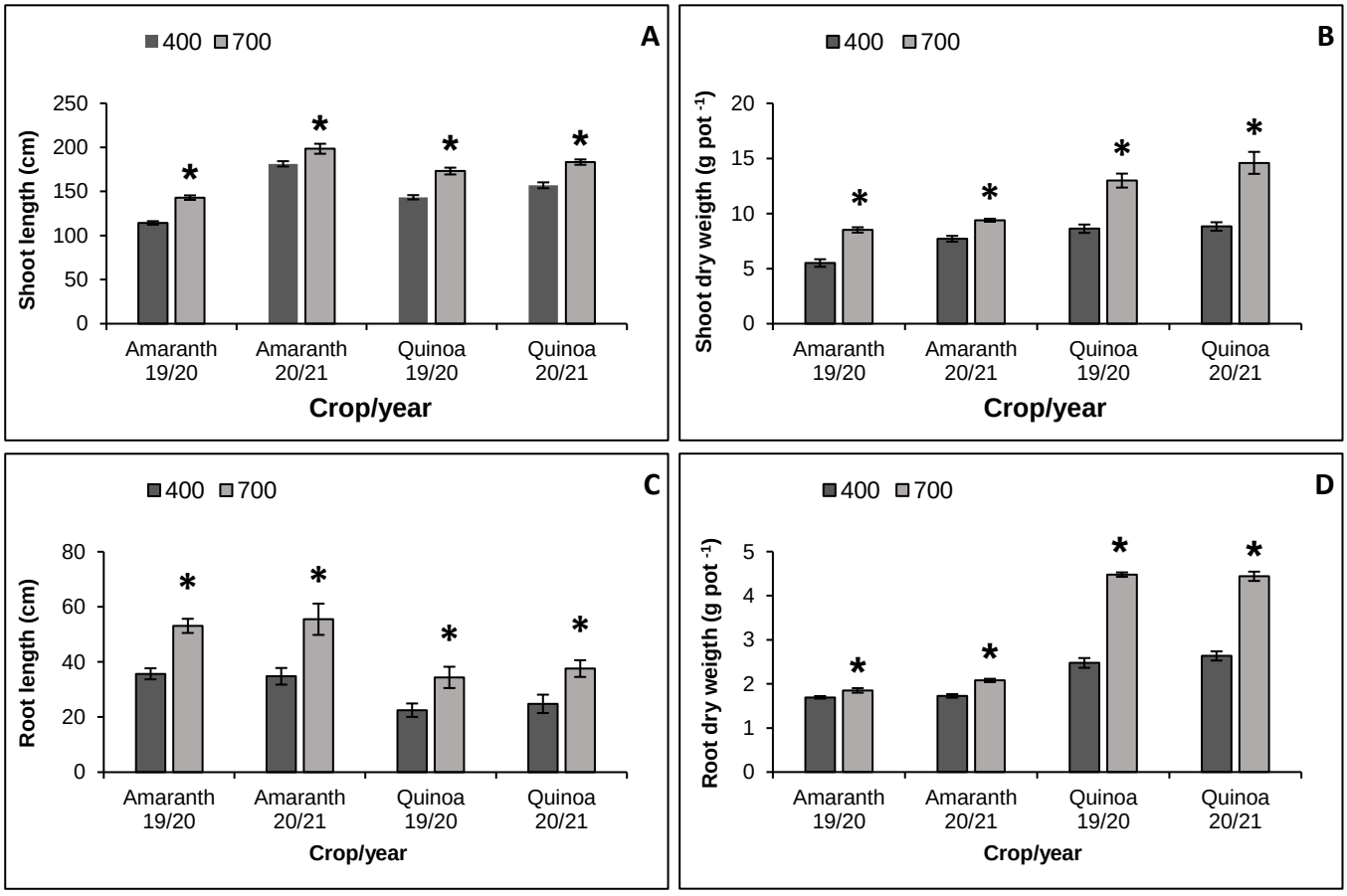

Figure 3: Effect of CO_2 on growth parameters. (A) Shoot length; (B) Shoot dry matter; (C) Root length; (D) Root system dry matter of amaranth and quinoa plants. 400 -Plants grown in OTC with 400 ppm CO_2 (control). 700-Plants grown in OTC with 700 ppm CO_2 e[CO_2]. The experiment was conducted during the crop years 2019/2020 and 2020/2021. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t test (P≤0.05, n=10).

When comparing values obtained for BPP, SD, LA (Figure 4B, 4C and 4D) between different levels of CO_2, data analysis showed significant differences. Amaranth and quinoa plants had higher growth in 700 ppm of CO_2. However, to PL (Figure 4A), quinoa plants had a significant and distinct behavior, with a higher panicle length in 400 ppm of CO_2.

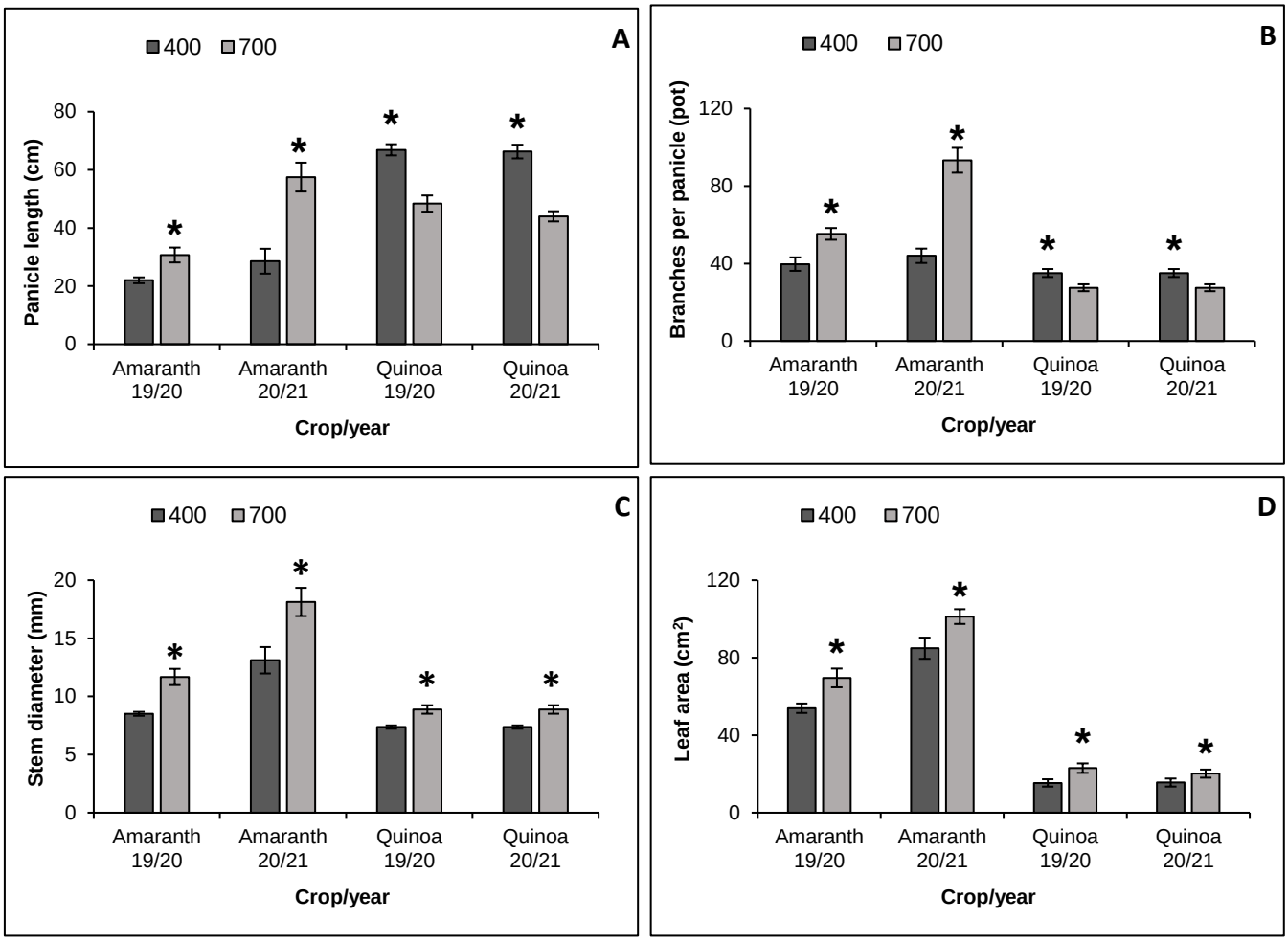

Figure 4: Effect of CO_2 on growth parameters. (A) Panicle length; (B) Branches per panicle; (C) Stem diameter; (D) Leaf area of amaranth and quinoa plants. 400 -Plants grown in OTC with 400 ppm CO_2 (control). 700- Plants grown in OTC with 700 ppm CO_2 e[CO_2]. The experiment was conducted during the crop years 2019/2020 and 2020/2021. Error bars correspond to the 95% confidence interval. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t test (P≤0.05, n=10).

Photosynthetic pigments

In general, photosynthetic pigments (Figure 5) did not present differences when comparing elevated CO_2 data to control. Relevant changes were observed only for CHL*a* (Figure 5A), CHL*b* (Figure 5B) and Carotenoids (Figure 5C) in amaranth plants in agricultural year 20/21

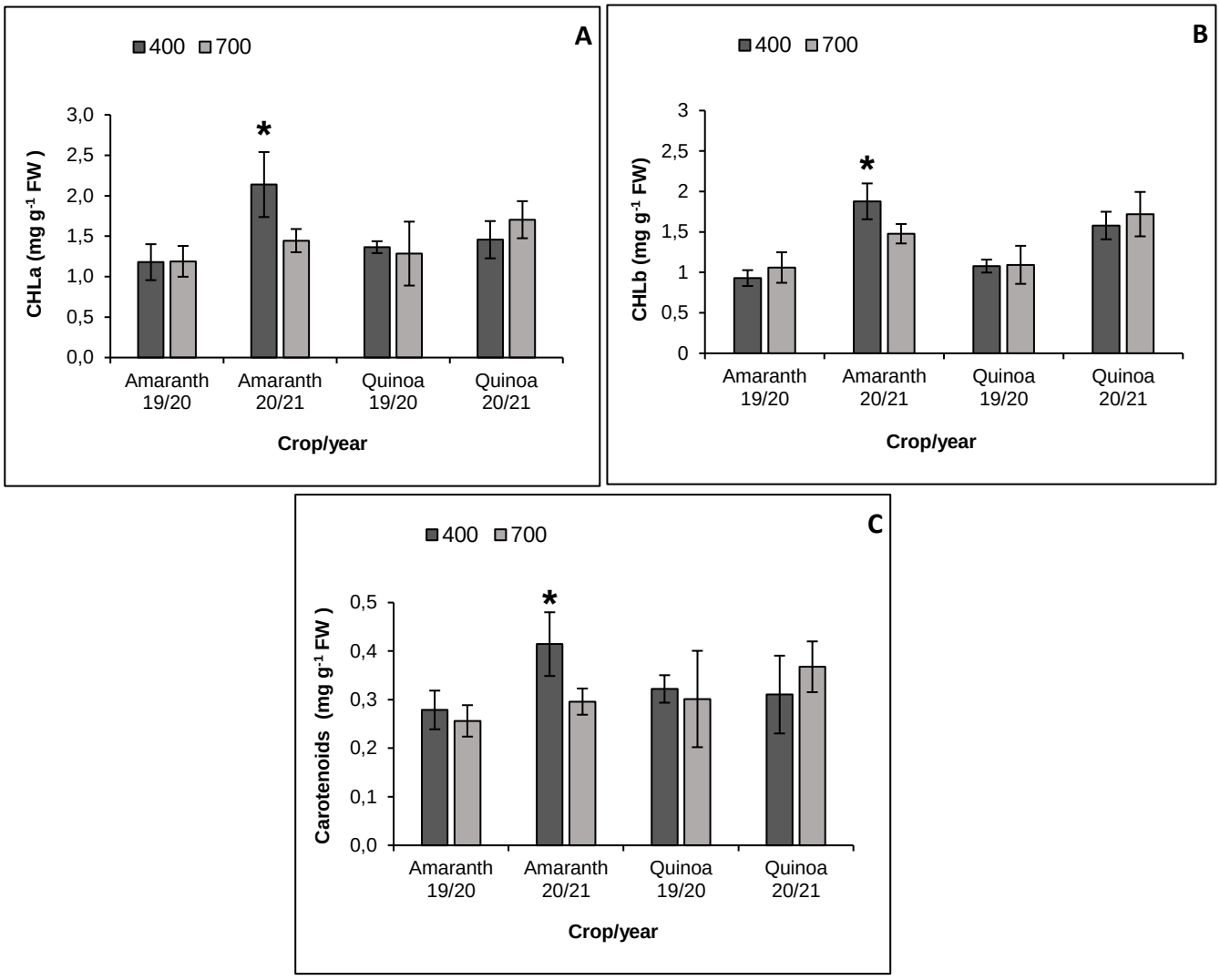

Figure 5: Effect of CO_2 on photosynthetic pigments. (A) CHLa; (B) CHLb; (C) Carotenoids of amaranth and quinoa plants in transition stadium between vegetative and flowering. 400 -Plants grown in OTC with 400 ppm CO_2 (control). 700- Plants grown in OTC with 700 ppm CO2 e[CO_2]. The experiment was conducted during the crop years 2019/2020 and 2020/2021.Error bars correspond to the 95% confidence interval. *Indicates significant difference by *t* test (P≤0.05, n=5).

Leaf gas exchange

Overall, e[CO_2] produced changes in the leaf gas exchange (Figure 6), demonstrating an expressive difference between the treatments ($p \leq 0.05$) to all measured parameters. Parameter A (Figure 6A) was higher for both amaranth and quinoa in 700 ppm. E[CO_2] promoted an increase of net CO_2 assimilation rate, resulting in higher Ci (Figure 6C) for both crops. Because of that, an decrease in stomatal conductance and transpiration rate were observed in e[CO_2] compared to control plants. Thus, the A/E ratio was higher in e[CO_2].

Sucrose metabolism-related enzyme activity

Analysis of sucrose metabolism-related enzyme activity (Figure 7) displayed a significant increase in the activity of the neutral invertase (CINV–7A) in quinoa and amaranth plants. Acid invertases, such as cell wall (CWINV–7B) and vacuole (VINV–7C), presented a similar trend, except for amaranth plants in 20/21. Amidst all agricultural years, sucrose synthase activity (SuSy–7D) was higher on e[CO_2].

Carbohydrate content

Significant changes were noted in most carbohydrate metabolism contents in leaves (Figure 8). Total content of soluble sugars (Figure 8A) and sucrose (Figure 8B) was higher for both crops in e[CO_2], whereas total content of starch (Figure 8C) presented a singular trend for each crop. Amaranth grow in e[CO_2], while quinoa plants showed an increase in this condition. Although total soluble amino (Figure 8D) acids content were higher in a[CO_2] for both crops, the agricultural year of 19/20 showed lower levels of the same component when compared with 20/21

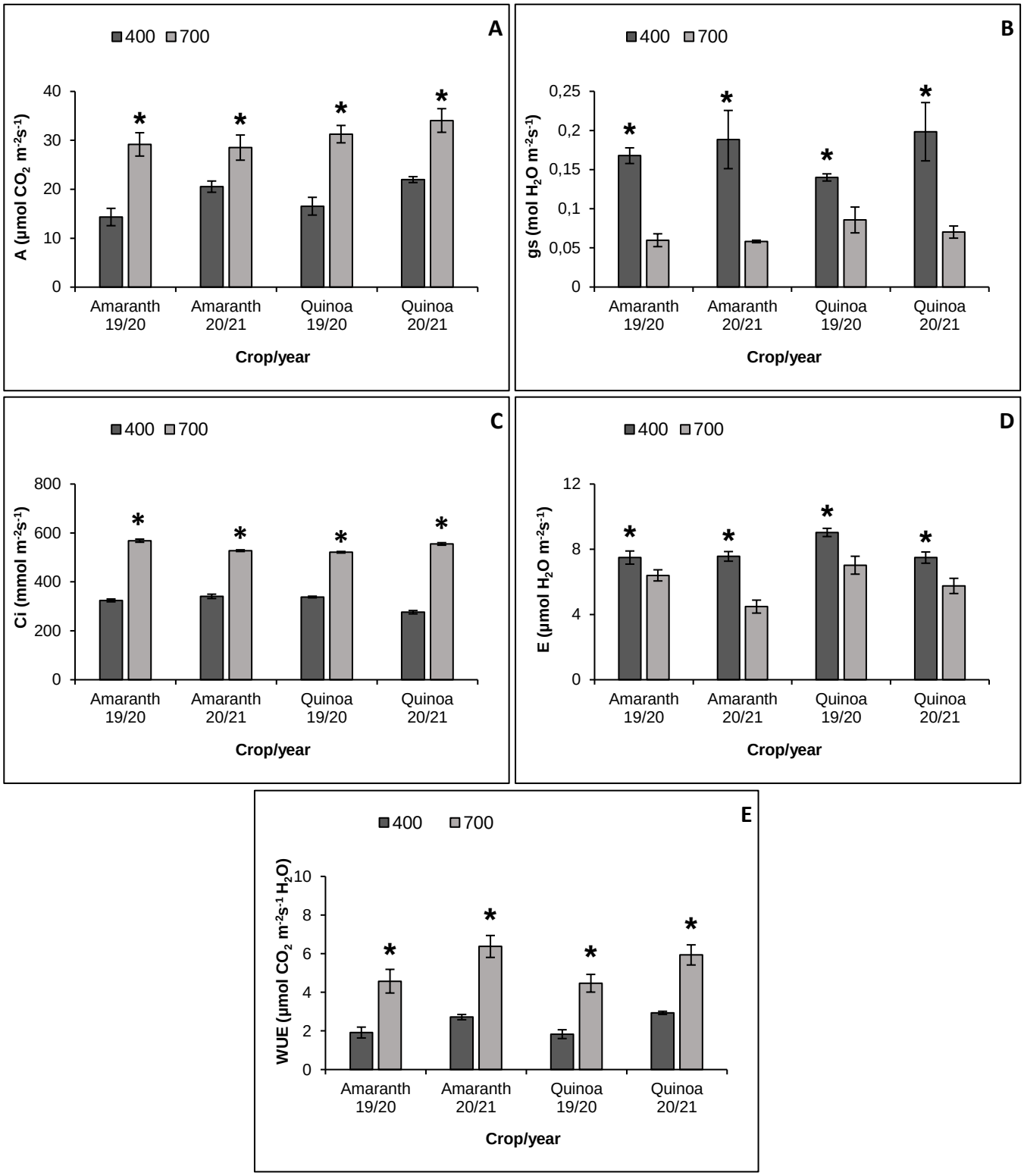

Figure 6: Effect of CO_2 on leaf gas exchange. (A) Net CO_2 assimilation; (B) Stomatal conductance; (C) Internal concentration of CO_2; (D)Transpiration rate; (E) Water use efficiency of amaranth and quinoa plants in transition stadium between vegetative and flowering. 400 - Plants grown in OTC with 400 ppm CO_2 (control). 700- Plants grown in OTC with 700 ppm CO_2 e[CO_2]. The experiment was conducted during the crop years 2019/2020 and 2020/2021. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

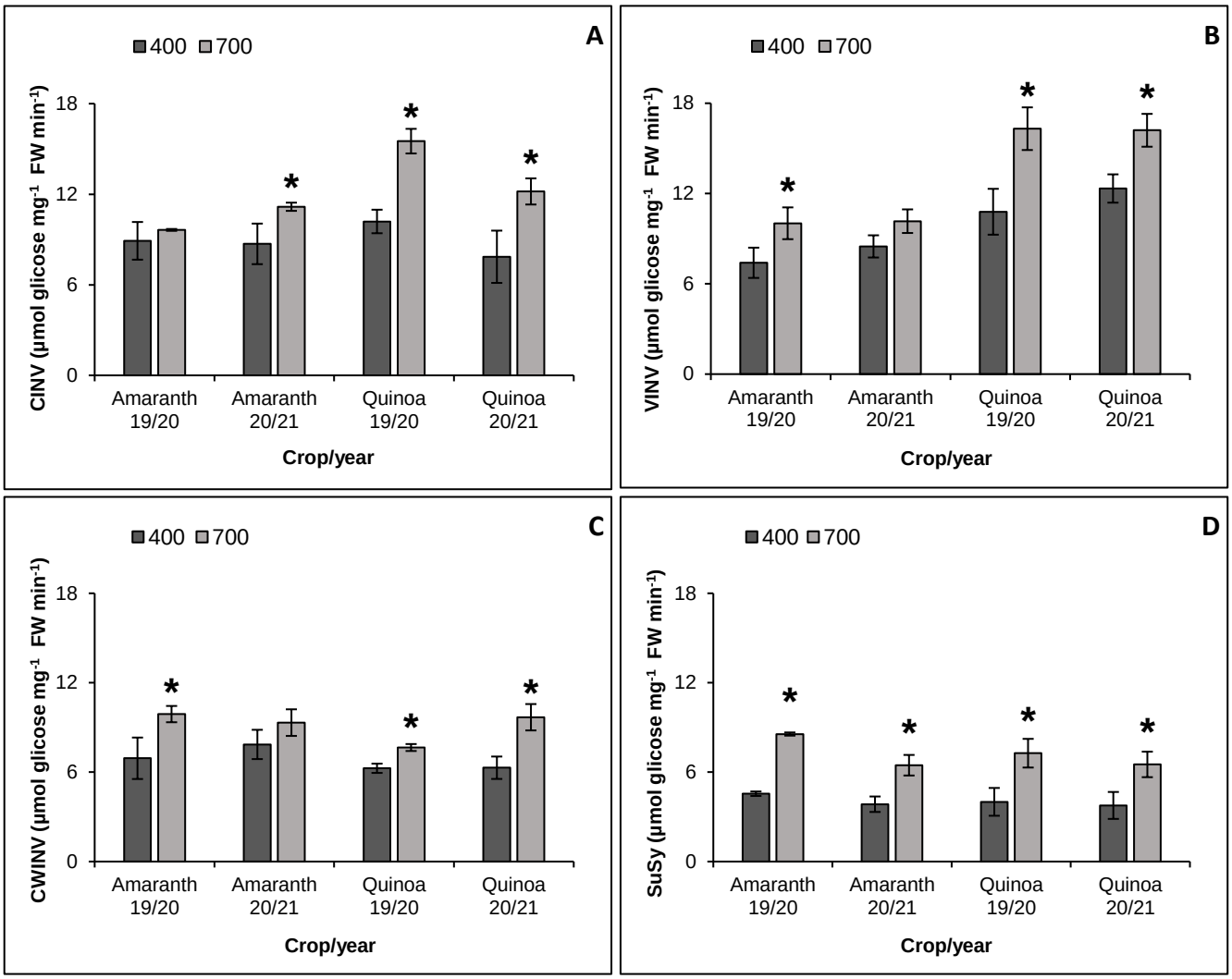

Figure 7: Effect of CO_2 on Sucrose metabolism-related enzyme activity in leaves. soluble acid invertases of cytosol (CINV–A); cell wall acid invertase (CWIV-B); soluble acid invertases of vacuole (VINV-C) and sucrose synthase (SuSy-D) activity. 400 - Plants grown in OTC with 400 ppm CO_2 (control). 700- Plants grown in OTC with 700 ppm CO_2 e[CO_2]. Experiment was conducted during the crop years 2019/2020 and 2020/2021. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

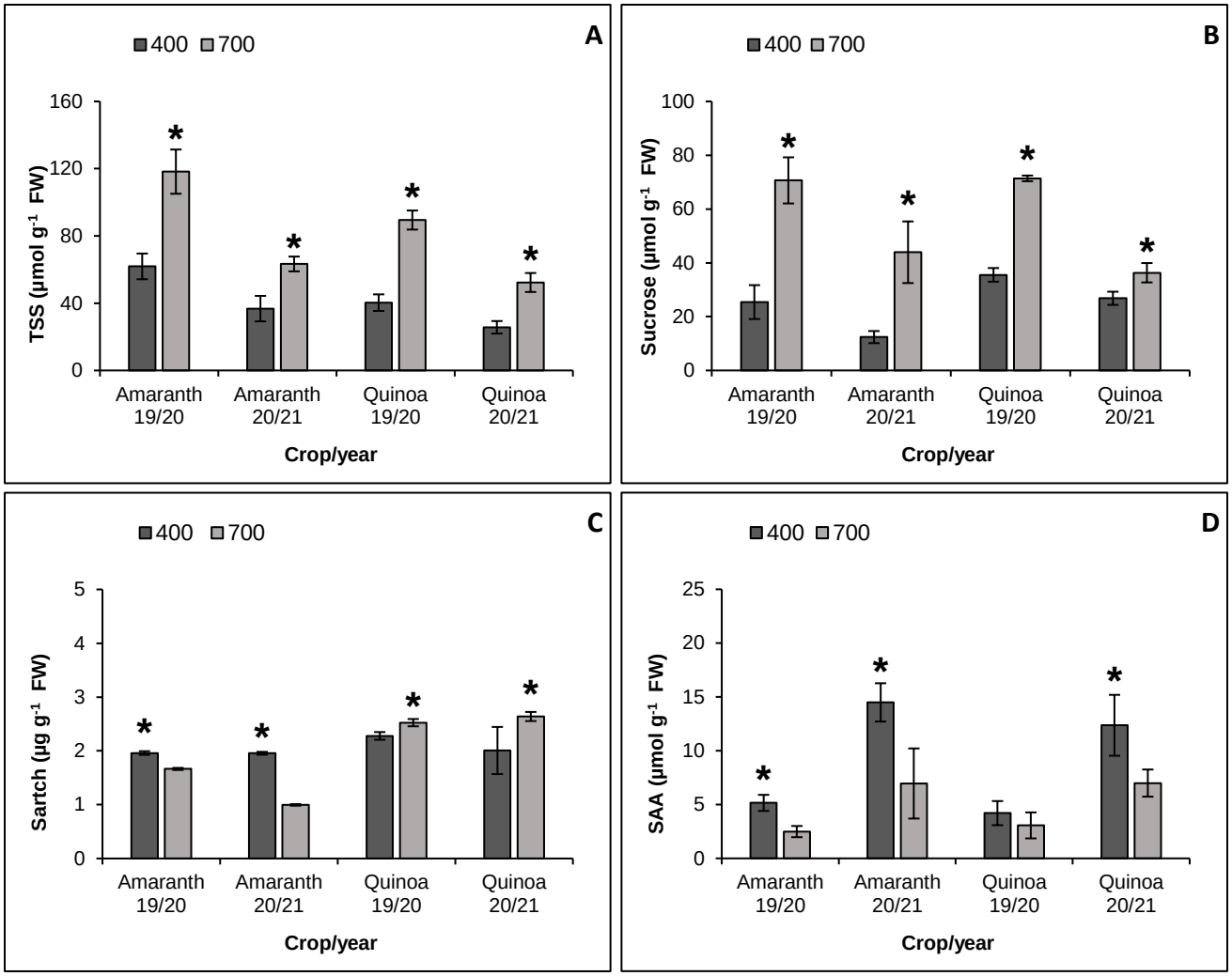

Figure 8: Effect of CO_2 on Carbohydrate content. (A) Soluble sugars; (B) Sucrose-SUC; (C) Starch and (D) total soluble amino acids-SAA of amaranth and quinoa plants in transition stadium between vegetative and flowering. 400 - Plants grown in OTC with 400 ppm CO_2 (control). 700- Plants grown in OTC with 700 ppm CO_2 e[CO_2]. The experiment was conducted during the crop years 2019/2020 and 2020/2021. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

Nutrient contents in leaves

Overall, e[CO_2] decreased some macro (Table 2) and micronutrients (Table 3) in amaranth and quinoa leaves. Here, we had different results for crops and agricultural year. Amaranth plants in e[CO_2] revealed a decline in leaf contents of N; K; Ca; Zn; Mn and Cu, whereas quinoa plants presented a decrease of N; P (quinoa 19/20); K; Ca (quinoa 20/21); Zn; and Mn in e[CO_2] (amaranth and quinoa 19/20-20/21). In general, magnesium leaf contents were non-significant. Fe and P levels were higher in e[CO_2] for both crops and agricultural years

Table 2: Macronutrients contents in leaves of amaranth and quinoa plants in transition stadium between vegetative and flowering.

	Crop / Agricultural year			
Treatment	Amaranth 19/20	Amaranth 20/21	Quinoa 19/20	Quinoa 20/21
CO_2 (ppm)	**N (g kg^{-1})**			
400	52.0 ±(1.79)*	53.0 ± (1.66)*	54.9 ± (4.57)*	53.2 ± (0.54)*
700	49.2 ±(0.98)	36.0 ± (1.79)	48.4 ± (0.27)	38.5 ± (1.71)
CV%	1.15	1.57	2.52	1.12
CO_2 (ppm)	**P (g kg^{-1})**			
400	3.4 ± (0.13)	3.1 ± (0.08)	5.1 ± (0.42)*	2.7 ± (0.08)
700	4.3 ± (0.17)*	3.7 ± (0.17)*	4.0 ± (0.37)	3.0 ± (0.18)*
CV%	1.65	1.56	3.54	2.03
CO_2 (ppm)	**K (g kg^{-1})**			
400	46.5 ± (2.33)*	46.5 ± (3.87)*	50.8 ± (2.07)*	50.9 ± (1.97)*
700	30.7 ± (4.20)	33.9 ± (1.62)	46.3 ± (1.86)	45.7 ± (0.64)
CV%	3.54	2.97	1.63	1.22
CO_2 (ppm)	**Ca (g kg^{-1})**			
400	16.9 ± (1.88)*	27.3 ± (2.75)*	11.3 ± (0.46)^{ns}	21.7 ± (1.63)*
700	12.9 ± (1.63)	16.9 ± (1.88)	11.2 ± (2.58)^{ns}	15.3 ± (2.97)
CV%	4.74	4.29	6.66	5.23
CO_2 (ppm)	**Mg (g kg^{-1})**			
400	8.1 ± (0.88)	8.8 ± (1.07)^{ns}	6.3 ± (1.13)^{ns}	8.9 ± (0.78)^{ns}
700	9.9 ± (0.21)*	7.8 ± (1.34)^{ns}	6.0 ± (1.03)^{ns}	8.8 ± (0.71)^{ns}
CV%	2.99	5.87	7.06	3.38

*Indicates significant difference by t-test (P≤0.05, n=4). ns indicates non-significant. CV: coefficient of variation. Values in parentheses correspond to the 95% confidence interval.

Table 3: Micronutrients contents in leaves of amaranth and quinoa plants in transition stadium between vegetative and flowering.

	Crop / Agricultural year			
Treatment	Amaranth 19/20	Amaranth 20/21	Quinoa 19/20	Quinoa 20/21
CO_2 (ppm)	**Fe (mg kg^{-1})**			
400	164.4±(4.5)	174.5±(1.33)	80.1±(7.83)	180.0±(3.63)
700	185.4±(2.37)*	207.8±(3.60)*	135.1±(8.64)*	235.5±(2.66)*
CV%	0.89	2.04	3.09	
CO_2 (ppm)	**Zn (mg kg^{-1})**			
400	22.2±(2.10)*	36.1±(2.99)*	11.3±(2.31)*	49.0±(1.03)*
700	15.5±(4.45)	26.0±(2.92)	6.4±(4.55)	20.8±(0.88)
CV%	7.41	3.82	16.37	1.11
CO_2 (ppm)	**Mn (mg kg^{-1})**			
400	554.7±(9.19)*	254.2±(4.18)*	348.4(5.42)*	755.0±(8.93)*
700	424.8±(2.60)	193.7±(8.29)	284.0±(3.86)	585.1±(9.43)
CV%	0.56	1.18	0.6	
CO_2 (ppm)	**Cu (mg kg^{-1})**			
400	17.6± (1.72)*	19.6±(1.72)*	12.4±(1.72)	14.0±(1.72)
700	15.2±(1.72)	17.6±(1.72)	31.4±(1.72)*	29.2±(1.72)*
CV%	4.21	3.73	3.15	3.2

*Indicates significant difference by t-test (P≤0.05, n=4); ns indicates non-significant. CV: coefficient of variation. Values in parentheses correspond to the 95% confidence interval. n=4

Yield components per pot

Table 4 shows yield components per pot at different agricultural years in two CO_2 concentrations for amaranth and quinoa plants. e[CO_2] contributes to a negative effect in weight of 1000 grains and grain weight per pot. However, regarding grains per panicle, there was a different performance. While the values for amaranth were non-significative, e[CO_2] provided a decrease in grains per panicle in quinoa plants.

Table 4: Yield components per pot of amaranth and quinoa plants in transition stadium between vegetative and flowering.

	Crop / Agricultural year			
Treatment	Amaranth 19/20	Amaranth 20/21	Quinoa 19/20	Quinoa 20/21
CO_2 (ppm)	**Weight of 1000 grains (g)**			
400	1.11±(0.01)*	1.10±(0.00)*	2.41±(0.07)*	2.48±(0.01)*
700	0.86±(0.03)	0.81±(0.01)	1.71±(0.04)	1.70±(0.00)
CV%	1.87	0.74	1.89	0.28
CO_2 (ppm)	**Grains per panicle**			
400	10.921±(11.28)^{ns}	9.879±(7.5)^{ns}	2.598±(2.66)*	2.772±(18.45)*
700	9.876±(10.52)^{ns}	10.922±(9.14)^{ns}	2.174±(3.48)	1.830±(14.0)
CV%	13.12	11.44	18.2	9.99
CO_2 (ppm)	**Grain weight per pot (g)**			
400	9.37±(0.96)^{ns}	8.40±(0.66)^{ns}	5.72±(0.69)*	5.67±(0.78)*
700	9.30±(0.73)^{ns}	8.41±(0.70)^{ns}	2.84±(0.62)	2.17±(0.47)
CV%	12.83	11.38	21.58	23.08

*Indicates significant difference by *t* test (P≤0.05, n=4); ns indicates non-significant. CV: coefficient of variation. Values in parentheses correspond to the 95% confidence interval. n=10

4. DISCUSSION

The hypothesis that C_4 plants can be responsive by ambient atmospheric CO_2 concentrations as much as C_3 plants has been proven useful, if not always entirely predictive, considering that due to genetic improvement (REICH et al., 2018), different behaviors can be observed according to genotype, variety and cultivar (ROCHA, 2019). Therefore, the idea which previously had been deeply embedded in models of past vegetation-climate interactions perhaps needs a new approach (XU et al., 2013).

Plant growth is considered a complex process, which can be influenced by numerous factors, such as the increase in CO_2. This is what makes growth parameters important, since they are related to greater light absorption (WESTOBY et al., 2002). Here, all of those results were increased by e[CO_2]. An increase in shoot and root length (consequently shoot and root dry matter) are major traits that can be attributed as the initial effects of elevated CO_2 in plants. Similar results were presented by Song et al (2012) for amaranth cultivated in 500 ppm and Bunce (2017) for quinoa in 600 ppm of CO_2, respectively. These experiments also were conducted into a OTC.

Regarding the root system, it not only it takes up soil nutrients and water for sustainable plant production but also pumps photosynthetically fixed C to soil organic matter (SOM) pools. It plays a crucial role in terrestrial C cycling. The e[CO_2] exerts a strong impact on these systems by influencing the morphology as root length and distribution. In addition, secondary roots control ecosystem C and N cycling as plants obtain water and nutrients and release exudates. In this study, it was possible to observe that the root system of amaranth and quinoa (Figure 8) plants collected after grain maturation, presented more secondary roots when in e[CO_2] , possibly as a strategy to maintain the nutritional and water status of the plants.

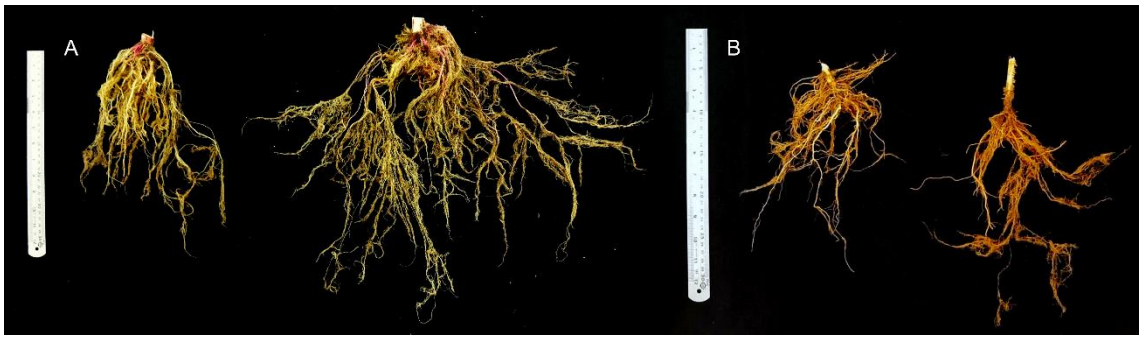

Figure 9: Effects of CO_2 increases on roots of amaranth and quinoa after grain maturation.

An increase in root elongation and branching was evidenced in *Sedum alfredii*, e[CO_2] (LI et al., 2012). However, other studies founded a variety of plant species show just an increased fine root production (PRITCHARD; ROGERS, 2000; TINGEY et al., 2000). Corroborating with the results presented here, a meta-analysis carried out by Nie et al (2013), showed fine root biomass of plants had a significantly stronger response to elevated CO_2 in OTC experiments (+35.8%).

According to Ferreira et al. (2014), the diameter of the stem is important because it is related to the falling of the plants. In order to maintain upright and support it, the e[CO_2] significantly increased stem diameter of amaranth and quinoa. Similar changes in plant morphology have been reported elsewhere, for example, e[CO_2] significantly increased the shoot of C_4 plants like maize (XIE et al., 2015), sugarcane (SOUZA et al., 2008), foxtail millet (LI et al., 2019) and C_3 plants like quinoa (OLIVEIRA-FILHO, 2017), coffee tree (TOZZI; GHINI, 2016), *Stylosanthes capitata* Vogel. (GONZALEZ-MELER, 2017). While to the panicle length, quinoa plants had a controversial result. The control treatment presented a higher PL than e[CO_2]. This phenomenon has been reported by some authors (AINSWORTH; LONG, 2005; DORNELES et al., 2019) as a down-regulation or acclimatization of species to the increase in CO_2 during the stadiums. Physiological processes often develop mechanisms of compensation that reduce or minimize the effects of CO_2 in the long term and sometimes, a limitation of the capacity of its regeneration can be observed (BUSCH; SAGE, 2017).

Chlorophylls and carotenoids are pigments capable of absorbing visible radiation and triggering photochemical reactions of photosynthesis (SEIFERMAN-HARMS, 1987). In general, plants grown in e[CO_2] show alteration in photosynthetic pigments. Some authors have mentioned the reduction of chlorophyll and carotenoids

in leaves due to the increase in biomass under e[CO_2], called the N dilution effect (CONROY and HOCKING, 1993; WANG et al., 2015). In some cases, changes in the C:N ratio cause an effect of nitrate (NO_3) assimilation inhibition by the roots (BLOOM et al., 2012, 2014; BHARGAVA; MITRA, 2021; KRÄMER et al., 2022). However, it was possible to observe two important factors: (1) change in pigments for amaranth plants (20/21), (2) non-significant change but mostly higher levels in the control treatment, evidencing the importance of chlorophyll breakdown. This a highly coordinated and integral process of the plant development stages that is programmed to facilitate the dynamic remobilization of nutrients from organs/tissues to parts of the plant that are still growing, in particular to reproductive/storage organs during the transition of plants to reproductive growth (WHITE et al., 2015; WHITE et al., 2016; KUAI; CHEN; HÖRTENSTEINER, 2018).

Growth, photosynthetic pigments and leaf gas exchange are closely linked. C_4 plant species possess a "distinct pathway" of photosynthesis from C_3 species, which in low atmospheric CO_2 concentration could be concentrated to enable more efficient carboxylation reaction, due to changes in the ratio of CO_2:O_2 (MAKINO; MAE, 1999). It does not mean that C_4 species do not change the Calvin-Benson cycle's fundamental machinery, but have functionalized structural and biochemical additions around C_3 photosynthesis to improve its efficiency. It is necessary to consider that most of C_4 plants fix CO_2 in mesophyll cells with phosphoenolpyruvate carboxylase (PEPC), an enzyme that, unlike Rubisco, is insensitive to O_2. Subsequently, CO_2 is released in the bundle sheath cells where Rubisco is localized and the Calvin-Benson cycle occurs. This additional step increases the availability of CO_2 around Rubisco and minimizes its chance of catalyzing the oxygenation reaction (TURKAN et al., 2018).

Concentrations of carbon dioxide are important regulators in the dynamics of opening and closing of stomata. Through these mechanisms, plants exchange gas with the external environment (TAUB, 2010). Opening the stomata allows the diffusion of CO_2 for photosynthesis, in addition to providing a path for water to diffuse from the leaves to the atmosphere (XU et al., 2016). Therefore, plants regulate the level of stomatal opening (stomatal conductance), seeking to maintain high rates of photosynthesis and reduce water loss. In the current study, e[CO_2] resulted in an increase on A (Figure 6A) and Ci (Figure 6-C) in quinoa plants. Partial closure of the stomata could have been responsible for reducing stomatal conductance and transpiration rate. Thus, it was possible to maintain high rates of photosynthesis

without compromising the internal concentration of CO_2, since the greater difference in CO_2 concentration between the atmosphere and the interior of the leaf compensates for the increase in stomatal resistance (WANG, et a., 2022).

This compensation is relatively less understood for C_4 species (ZHANG et al., 2021). Theoretically, when it comes to C_4 species in general, researchers consider C_4 plants saturated at a[CO_2] (CO_2 ambient/400 ppm). Also, they might not be stimulated by e[CO2] (AINSWORTH; LONG, 2005). We found significant stimulation rates, like Zhang et al. (2021) to Broomcorn millet; Li et al. (2019) to Foxtail millet; Davis and Ainsworth (2012) to *Amaranthus rudis*., even though Santos (2018) and Leakey et al. (2006) had opposite results for *Amaranthus viridis* and maize, respectively.

C_4 plants such as amaranth have increased vein densities during the evolution process, causing a reduction in intercellular air spaces and enhancement of bundle sheath organelles (MONSON, 1999; SAGE, 2004). In addition, the increasing vein densities may not only increase structural integrity or enhance leaf water status (SAGE, 2004), but may also allow xylem-transported CO_2 to be utilized for photosynthesis by reducing the distance between vascular bundles transporting xylem-transported CO_2 and photosynthetic cells (STUTZ; HANSON, 2019).

Results of changes in C_4 photosynthesis are not a consensus among researchers yet. Some authors mention the fact that increases observed in A at e[CO_2] such as our results are noticeable during the transition stadium between vegetative and flowering or apparent only during early stage in some crops because of the carbohydrate metabolism. At this stage, plants are preparing for exporting of macro e micronutrients for the produce of grains/seeds (WAND 1999; PRADO, 2020). In addition, studies related to gene regulation have increasingly reported the particularity of genotypes, showing different responses to e[CO2] for the same species mainly for C_4 plants (SILVA; ALVES; ZINGARETTI, 2020).

Carbon assimilated in photosynthesis is stored through carbohydrates, which are compounds generated in high quantities by plants and have high proportions of carbon (LEEGOOD et al., 2000).

In general, increases in carbohydrate production resulting from the increment in photosynthesis by e[CO_2] can result in alterations in the production and partition of carbohydrates, as it raises the activity of enzymes that hydrolyze sucrose into sink organs. (ROITSCH and GONZÁLEZ, 2004; LI et al., 2019).

Among the sugars synthesized in a plant, only a few of them are transported in the phloem over a long distance (LEMOINE et al., 2013). Upon arriving at sink tissues, sucrose can follow different pathways which can modulate sink strength and carbon flux (MA et al., 2018). Sucrose might be unloaded from the phloem to the apoplast by transporters or be hydrolyzed by invertases (CINV, CWINV and VINV) to yield glucose and fructose, which can enter the sink cells via hexose transporters (RUAN, 2014). Besides that, as a reversible cleavage, SuSy might catalyze sucrose using UDP to yield fructose and UDP-G (LAL et al., 2022) and also utilize other nucleotide phosphates for the cleavage, especially ADP, but usually with a lower affinity (STEIN; GRANOT, 2019).

In the present study, activity of sucrolytic enzymes was altered in leaves of amaranth and quinoa in transition stage between vegetative and flowering by e[CO2]. At this stage, considering that grain yield is dependent on the plant source/sink relationship, the top two leaves are the primary source, and the florets are the primary sink for photosynthesis. This might also change plant carbon and nitrogen metabolism (PELEG et al., 2011; WHITE et al., 2016). In addition to this direct effect on photosynthesis, many physiological processes are indirectly regulated, particularly through sugar detection and signaling pathways. Sugar signaling plays an important role in the plant's response to e[CO_2]; however, this is not well understood concerning the plant's nutritional quality (THOMPSON et al., 2017).

Accumulation of soluble sugars is a direct effect of e[CO_2] due to the growth of triose phosphate synthesis in leaves, which can be further transformed into other carbohydrates, e.g., glucose, fructose, and sucrose. A meta-analysis made with publications between 1990 and 2018 showed that e[CO_2] increased the concentrations of sucrose by 3.7% (at $p = 0.07$) and total soluble sugar by 17.5% in leaves. This data shows a non-integration of newly fixed carbohydrates to growth, accumulating them in the leaves (POORTER; PÉREZ-SOBA, 2002) and confirms results presented here. Dong et al (2018) still proposed that the synthesized carbohydrates in leaves cannot be fully translocated to fruits as well as to roots, although one needs to be cautious regarding the species variation.

Regarding sucrose, it is important to remember its relationship to plant growth and development. If photosynthetic capacity exceeds demand, excess photoassimilates remain in the chloroplast and can be stored in the form of starch. Thus, it is believed that the sink can control the activity of the source. Furthermore,

there is an intricate relationship between source and sink, as both activities are controlled by environmental factors such as CO_2 (TAIZ et al., 2017; MA et al., 2018).

As we have seen so far, e[CO_2] plays a crucial role in physiology of plants. The plant photosynthesis, stomatal aperture, biomass production, yield, and water use efficiency could be modulated by CO_2 environment (PAZZAGLI et al.,2016). As a result, more carbohydrates could be transferred into the grains due to the increased photosynthesis in plants grown under CO_2-enriched environment. Nevertheless, there is generally a reduction in mineral contents, particularly N concentrations (Table 3), in grown plants at e[CO_2], probably due to restricted root nutrient uptake, caused by reduced mass flow and dilution effect, which reflected directly on the total soluble amino acids content in leaves (LI et al., 2019).

Re-translocation of nutrients from leaves could be a strategy to efficiently retain P. However, this is more observed in senescence (ESCUDERO et al., 1992; AERTS, 1996; KILLINGBECK, 1996). Re-translocation by resorption serves to withdraw nutrients from leaves prior to abscission for later redeployment in developing tissues. The extent to which P is re-translocated and re-used depends on the plant nutrient status. Generally, with decreasing nutrient availability in an ecosystem, the amount of resorption of both N and P tends to increase (REED et al., 2012; VERGUTZ et al., 2012). This ratio could also be an indicator of which nutrient is most limiting in a given ecossystem (DE CAMPOS et al., 2013). Considering that both nitrogen and phosphorus are involved in the photosynthetic machinery, most studies have reported that these nutrients also decreases due to increased carbon assimilation in several crops (PANG et al., 2006). However, on this experiment, amaranth and quinoa (20/21) plants demonstrated an increase of P (Table 3).

Given that N and P availability control the global carbon-cycle response to environmental changes, aspects like stoichiometric balance are important to consider. Nevertheless, the importance of P dynamics in elevated CO_2 is less clear and the assumption of a homeostatic N:P ratio in elevated CO_2 due to similar proportional N and P responses needs to be more clarified (CROUS et al., 2019; ZHANG et al., 2014). Xu et al. (2019) also reinforces the idea that C_3 and C_4 plants also have different nutritional changes under e[CO_2]. In a study where nitrogen availability was analyzed, Sudderth et al. (2005) observed that N availability in the presence of e[CO_2] increased foliar N content in *Amaranthus viridis* (C_4 plant).

Several studies reported that e[CO_2] decreased the mineral concentration by a dilution effect, suggesting that the decrease in mineral concentration is not specifically regulated by certain metabolic processes but by a dilution effect due to the increased biomass, as it was demonstrated earlier. It is well elucidated that the higher growth rate under high CO_2 also increases the activity of anabolic processes that require nutrients, including osmoregulation (K), cell elongation and nucleic acid metabolism (B), metabolic pathways that requires nutrients as cofactors (Ca, Mg and Mn) and redox reactions (Fe, Zn and Cu). Furthermore, to maintain homeostasis, plants are able to alter the absorption of these nutrients (XU et al., 2019; AINSWORTH et al., 2007). However, table 4 shows that iron content increased significantly in e[CO_2]. At the present time, we do not have a specific explanation about that. Perhaps more studies about the nutritional thematic are necessary, especially when we talk about food security and food supply.

In theory, increased photosynthesis increases the availability of carbohydrates, which results in a gain in biomass and consequently in grain yield (AINSWORTH; LONG, 2005; HÖGY et al., 2009; KIMBALL et al., 2001). However, for the yield components presented, we must also consider the increase in average temperature (Figure 1; Figure 2) in both agricultural years. Numerous studies have reported that the weight of 1000 grains and crude protein is reduced in e[CO_2] when compared to a[CO_2] conditions. These are very important characteristics when it comes to species selection for a future scenario of climate changes. In this study, it was possible to observe that amaranth plants showed a smaller decrease in productivity when compared to quinoa plants (ZHANG et al.; 2021; LI et al., 2019; DAVIS and AINSWORTH, 2012).

5. CONCLUSIONS

- The elevated concentration of CO_2 is capable of altering the metabolism of amaranth (C_4) and quinoa (C_3) plants. Changes in growth pattern and photosynthetic pigments reflected changes in gas exchange and carbohydrate metabolism.
- Changes in photosynthetic parameters added to the higher content of carbohydrates and sucrolitic enzymes were able to boost the change in the nutritional status of the plant, causing the effect of dilution of nutrients considered essential such as nitrogen.
- Under high CO_2, it causes a decrease in productivity of quinoa grains.

REFERENCES

AERTS, R. Nutrient resorption from senescing leaves of perennials: are there general patterns? **Journal of Ecology**, p. 597-608, 1996.

AINSWORTH, E. A.; LONG S. P. What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. **New Phytologist**, v.165, p. 351-371, 2005.

AINSWORTH, Elizabeth A. et al. Does elevated atmospheric [CO_2] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves?. **Journal of Experimental Botany**, v. 58, n. 3, p. 579-591, 2007.

ALMEIDA, R. M. et al. A alta produção primária contrasta com a intensa emissão de carbono em um reservatório tropical eutrófico. **Fronteiras em microbiologia** , v. 7, p. 717, 2016.

ANTOLIN, L. A. S; HEINEMANN, A. B.; MARIN, F. R. Impact assessment of common bean availability in Brazil under climate change scenarios. **Agricultural Systems**, v. 191, p. 103174, 2021.

BENINCASSA, M. M. P. Análise de crescimento de plantas: noções básicas. 2. ed. Jaboticabal: Funep, 41p. 2003.

BLOOM, A. J. et al. CO_2 enrichment inhibits shoot nitrate assimilation in C3 but not C4 plants and slows growth under nitrate in C3 plants. **Ecology**, v. 93, n. 2, p. 355-367, 2012.

BUNCE, J. A. Variation in yield responses to elevated CO_2 and a brief high temperature treatment in quinoa. **Plants**, v. 6, n. 3, p. 26, 2017.

BUSCH, Florian A.; SAGE, Rowan F. The sensitivity of photosynthesis to O_2 and CO_2 concentration identifies strong Rubisco control above the thermal optimum. **New Phytologist**, v. 213, n. 3, p. 1036-1051, 2017.

CONROY, J.; HOCKING, P. Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. **Physiologia Plantarum**, v. 89, n. 3, p. 570-576, 1993.

CHANDRA, A. K. et al. Global production, demand, and supply. In: **Millets and Pseudo Cereals**. Woodhead Publishing, 2021. p. 7-18.

CROUS, K. Y. et al. Nitrogen and phosphorus retranslocation of leaves and stemwood in a mature Eucalyptus forest exposed to 5 years of elevated CO_2. **Frontiers in plant science**, v. 10, p. 664, 2019.

DAVIS, A. S.; AINSWORTH, E. A. Weed interference with field-grown soyabean decreases under elevated [CO_2] in a FACE experiment. **Weed Research**, v. 52, n. 3, p. 277-285, 2012.

DE CAMPOS, M. C. R et al. Downregulation of net phosphorus-uptake capacity is inversely related to leaf phosphorus-resorption proficiency in four species from a phosphorus-impoverished environment. **Annals of Botany**, v. 111, n. 3, p. 445-454, 2013.

DONG, J. et al. Effects of elevated CO_2 on nutritional quality of vegetables: a review. **Frontiers in plant science**, v. 9, p. 924, 2018.

DORNELES, K. R.; POSSO, D. A.; REBHAHN, I.; DEUNER, S.; PAZDIORA, P. C.; AVILA, L. A.; DALLAGNOL, L. J. Respostas morfofisiológicas e rendimento de grãos do trigo mediados pelo aumento da concentração de CO_2 atmosférico. **Revista Brasileira de Ciências Agrárias**, v. 14, n. 1, p. 1-7, 2019.

ESCUDERO, A. et al. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. **Oecologia**, v. 90, n. 1, p. 80-87, 1992.

GEISSLER, N. et al. Elevated atmospheric CO_2 concentration leads to different salt resistance mechanisms in a C3 (Chenopodium quinoa) and a C4 (Atriplex nummularia) halophyte. **Environmental and Experimental Botany**, v. 118, p. 67-77, 2015.

GONZALEZ-MELER, M. A. et al. Experimental air warming of a Stylosanthes capitata, vogel dominated tropical pasture affects soil respiration and nitrogen dynamics. **Frontiers in plant science**, v. 8, p. 46, 2017.

GRAHAM, D.; SMYDZUK, J. Use of Anthrone in the Quantitative Determination of Hexose Phosphates. Analytical biochemistry, V.11, p.246-255, 1965.

HANDEL, V. Direct microdetermination sucrose. Analytical Biochemistry, v. 22, n.2, p. 280-283, 1968.

HANNAH, L. **Climate change biology**. Academic Press, 2021.

HATFIELD, J. L.; WALTHALL, C. L. Climate Change: Cropping System Changes and Adaptations. **Encyclopedia of Agriculture and Food Systems**. v. 2, p. 256-565. 2014.

HÖGY, P. et al. Effects of elevated CO_2 on grain yield and quality of wheat: results from a 3-year free-air CO_2 enrichment experiment. **Plant Biology**, v. 11, p. 60-69, 2009.

HOUSHMANDFAR, A. et al. The relationship between transpiration and nutrient uptake in wheat changes under elevated atmospheric CO_2. **Physiologia plantarum**, v. 163, n. 4, p. 516-529, 2018.

KIMBALL, B. A. Crop responses to elevated CO_2 and interactions with H2O, N, and temperature. **Current Opinion in Plant Biology**, v. 31, p. 36–43, 2016.

KILLINGBECK, K. T. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. **Ecology**, v. 77, n. 6, p. 1716-1727, 1996.

KLOTZ, K. L.; FINGER, F. L.; SHELVER, W. L. Characterization of two sucrose synthase isoforms in sugarbeet root. **Plant Physiology and Biochemistry**, v. 41, n. 2, p. 107-115, 2003.

KUAI, B.; CHEN, J.; HÖRTENSTEINER, S. The biochemistry and molecular biology of chlorophyll breakdown. **Journal of Experimental Botany**, v. 69, n. 4, p. 751-767, 2018.

LEAKEY, A. D. B. et al. Elevated CO_2 effects on plant carbon , nitrogen , and water relations: six important lessons from FACE. **Journal of Experimental Botany**, v. 60, n. 10, p. 2859–2876, 2009.

LEEGOOD, R. C.; SHARKEY, T., VON CAEMMERER, S. Photosynthesis: Physiology and Metabolism. 1. ed. Kluwer Academic Publishers, 2000. 1-8 p.

LETCHER, T. M. Global warming—a complex situation. In: **Climate Change**. Elsevier, 2021. p. 3-17.
LI, T. et al. Elevated CO 2 improves root growth and cadmium accumulation in the hyperaccumulator Sedum alfredii. **Plant and Soil**, v. 354, n. 1, p. 325-334, 2012.

LI, X. et al. Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress. **Plant, Cell and Environment**, v. 38, n. 10, p. 2171–2192, 2015.

LI, L.; WANG, X.; MANNING, W. J. Effects of Elevated CO_2 on Leaf Senescence, Leaf Nitrogen Resorption, and Late-Season Photosynthesis in Tilia americana L. **Frontiers in plant science**, v. 10, p. 1217, 2019.

LIU, J. et al. Elevated CO_2 induces alteration in lignin accumulation in celery (Apium graveolens L.). **Plant Physiology and Biochemistry**, v. 127, p. 310-319, 2018.

MA, N. et al. Petal senescence: past the point of no return. **Journal of Experimental Botany.** v. 69 , 719 – 732. 2018.

MAKINO, A.; MAE, T. Photosynthesis and plant growth at elevated levels of CO_2. **Plant and Cell Physiology**, v. 40, n. 10, p. 999-1006, 1999.

MANNERSA R.; VARELA-ORTEGA, C.; ETTEN; JACOB. Protein-rich legume and pseudo-cereal crop suitability under present and future European climates. **European Journal of Agronomy, v. 113, 125974, ISSN 1161-0301, 2020.**

MONTEIRO, J.E.B.A.; SENTELHAS, P.C.; CHIAVEGATO, E.J.; GUISELINI, C.; SANTIAGO, A.V. & PRELA, A. Estimação da área foliar do algodoeiro por meio de dimensões e massa das folhas. Bragantia, 64:15-24, 2005

NIE, M. et al. Altered root traits due to elevated CO_2: a meta-analysis. **Global Ecology and Biology**. v.22, 1095–1105. 2013.

OLIVEIRA-FILHO, A. F. **Produção e qualidade de sementes de quinoa em função do arranjo espacial.** (Tese) Universidade Federal de do Ceará. 2017.

OMAR, M. E. D. M.; MOUSSA, A. M. A.; HINKELMANN, R. Impacts of climate change on water quantity, water salinity, food security, and socioeconomy in Egypt. **Water Science and Engineering**, v. 14, n. 1, p. 17-27, 2021.

PANG, J.; ZHU, J. G.; XIE, Z. B.; LIU, G.; ZHANG, Y. L.; CHEN, G. P. A new explanation of the N concentration decrease in tissues of rice (*Oryza sativa* L.) exposed to elevated atmospheric pCO2. **Environmental and experimental botany**,v. 57, p. 98–105, 2006.

PAZZAGLI, P. et al. Effects of CO_2 elevation and irrigation regimes on leaf gas exchange, plant water relations, and water use efficiency of two tomato cultivars. **Agricultural Water Management**, v. 169, p. 26-33, 2016.

POORTER, H. et al. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. **Functional Plant Biology**, v. 39, n. 11, p. 839-850, 2012.

PRITCHARD, S. G.; ROGERS, H. H. Spatial and temporal deployment of crop roots in CO2-enriched environments. **The New Phytologist**, v. 147, n. 1, p. 55-71, 2000.

REED, S. C. et al. Stoichiometric patterns in foliar nutrient resorption across multiple scales. **New Phytologist**, v. 196, n. 1, p. 173-180, 2012.

REICH, P. B. et al. Unexpected reversal of C3 versus C4 grass response to elevated CO2 during a 20-year field experiment. **Science**, v. 360, n. 6386, p. 317-320, 2018.

ROCHA, M de M. **Características da planta (Árvore do conhecimento-Feijão Cauipi)**. Agência Embrapa de Informação tecnologica (Ageitec). 2019. Disponível em: <https://www.agencia.cnptia.embrapa.br/gestor/feijao-caupi/arvore/CONT000fqgx1wv202wyiv80bhgp5pl9ecv20.html#:~:text=O%20h%C3%A1bito%20de%20crescimento%20determinado,para%20a%20base%20da%20planta>. Com acesso em: Agosto de 2021.

RUNION, G. B. et al. Varied growth response of cogongrass ecotypes to elevated CO_2. **Frontiers in plant science**, v. 6, p. 1182, 2016.

SANTOS, J. I. Aumento da concentração de CO_2 atmosférico e seus efeitos na interação planta daninha/cultura da soja. (Dissertação) Universidade Federal de Viçosa. 2018.

SEIFERMAN-HARMS, D. The light-harvesting and protective functions of carotenoids in photosynbthetic membranes. **Physiology Plantarum**, v. 69, p. 561-568, 1987.

SONG, N. et al. Elevated CO_2 increases Cs uptake and alters microbial communities and biomass in the rhizosphere of Phytolacca americana Linn (pokeweed) and Amaranthus cruentus L.(purple amaranth) grown on soils spiked with various levels of Cs. **Journal of environmental radioactivity**, v. 112, p. 29-37, 2012.

SOUZA, A. P. et al. Elevated CO_2 increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. **Plant, cell & environment**, v. 31, n. 8, p. 1116-1127, 2008.

SUDDERTH, E. A.; STINSON, Kristina A.; BAZZAZ, F. A. Host-specific aphid population responses to elevated CO_2 and increased N availability. **Global Change Biology**, v. 11, n. 11, p. 1997-2008, 2005.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. **6. ed**. Porto Alegre: ArtMed, 2017. 888 p.

TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J. Análise de solo, plantas e outros materiais. 1995. **Boletim Técnico** nº5, 147p.

THOMPSON, M. et al. Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. **Frontiers in Physiology**, v. 8, p. 578, 2017.

TINGEY, D. T.; PHILLIPS, D. L.; JOHNSON, M. G. Elevated CO_2 and conifer roots: effects on growth, life span and turnover. **The New Phytologist**, v. 147, n. 1, p. 87-103, 2000.

TOZZI, F. R. O.; GHINI, R. Impacto do aumento da concentração atmosférica de dióxido de carbono sobre a ferrugem e o crescimento do cafeeiro. **Pesquisa Agropecuária Brasileira**, v. 51, n. 8, p. 933-941, 2016.

VERGUTZ, L. et al. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. **Ecological Monographs**, v. 82, n. 2, p. 205-220, 2012.

XIE, H. et al. A field experiment with elevated atmospheric CO 2-mediated changes to C_4 crop-herbivore interactions. **Scientific reports**, v. 5, n. 1, p. 1-9, 2015.

XU, Z. et al. Effects of elevated CO_2, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. **Planta**, v. 239, n. 2, p. 421-435, 2014.

XU, H. et al. Effects of elevated CO_2 and increased N fertilization on plant secondary metabolites and chewing insect fitness. **Frontiers in plant science**, v. 10, p. 739, 2019.

ZHANG, D. et al. Increased carbon uptake under elevated CO_2 concentration enhances water-use efficiency of C4 broomcorn millet under drought. **Agricultural Water Management**, v. 245, p. 106631, 2021.

ZHU, X. et al. A superacid-catalyzed synthesis of porous membranes based on triazine frameworks for CO_2 separation. **Journal of the American Chemical Society**, v. 134, n. 25, p. 10478-10484, 2012.

WAND, S.J.; MIDGLEY, G.; JONES, M.H.; CURTIS, P.S. Responses of wild C_4 and C3 grass (Poaceae) species to elevated atmospheric CO_2 concentration: a meta-

analytic test of current theories and perceptions. **Global Change Biology**, v. 5, n. 6, p. 723-741, 1999.

WANG, J. et al. Responses of wheat and rice grain mineral quality to elevated carbon dioxide and canopy warming. **Field Crops Research**, v. 249, p. 107753, 2020.

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

WESTOBY, M. et al. Plant ecological strategies: some leading dimensions of variation between species. **Annual review of ecology and systematics**, v. 33, n. 1, p. 125-159, 2002.

YEMM, E. M.; COCKING, E. C. Estimation of amino acids by ninhydrin. Analyst, v.80, p. 209-213, 1955.

CHAPTER 2

Effects of elevated CO_2 on secondary metabolism: insights from a new perspective

1. INTRODUCTION

Considered the main greenhouse gas (GHG), concentration of atmospheric carbon dioxide (CO_2) has increased progressively since the Industrial Revolution. The last IPCC report revealed that the National Oceanic and Atmospheric Administration (NOOA) has observed an increase of 1,8 parts per million (ppm) of CO_2 in the atmosphere compared to 2021, reaching maximum values of 420.99 ppm (IPCC, 2022).

This rise in CO_2 is accompanied by other serious climate changes, such as an increase in temperature, alterations in solar radiation patterns, variations in the precipitation regime and others. These abiotic factors are considered important regulators of the metabolic functions of plants, which have CO_2 as the principal source of for photosynthetic carbon fixation and biomass accumulation (XU et al., 2013).

Evidence that the effects of increased CO_2 extend well beyond primary metabolism has been elucidated over the years in vegetative and reproductive stage of plants. Transcriptomics studies performed in Arabidopsis (*Arabidopsis thaliana*), Poplar (*Populus* spp.), soybean (*Glycine max*), and wheat (*Triticum aestivum*) show us that high CO_2 impacts genes involved in secondary metabolism, hormone-dependent processes, redox regulation, and pathogenesis-related (PR) responses (AINSWORTH et al., 2006; LI et al., 2008; LEAKEY et al., 2009; TALLIS et al., 2010; KANE et al., 2013; NIU et al., 2016; MHAMDI; NOCTOR, 2016). Certain secondary metabolites (phenylpropanoids) also were increased in tobacco (*Nicotiana tabacum*) grown at high CO_2 (MATROS et al., 2006) environments.

Even though the response of plants to e[CO_2] is considered dependent on its species or genotype, plants are expected to decrease production of reactive oxygen species (ROS) in C_3 plants (PADHAN et al., 2020). However, Qiu et al. (2008) observed an increased abundance of leaf protein carbonylation, a potent marker of oxidative stress in Arabidopsis and soybean plants exposed to e[CO_2]. Cheeseman

(2006) discovered that e[CO_2] grown leaves of soybean showed a higher abundance of H_2O_2 when compared to C4 plants. It is speculated that interaction of bicarbonate with iron or heme groups triggers ROS generation in e[CO_2] conditions (ARAI et al., 2005). Furthermore, evidence suggests alterations in cyclic electron transport, a reaction that is especially important as a source of ATP in the vascular bundle sheath chloroplasts of some plants which have the C_4 type of carbon fixation. This process is responsible for changes in the redox regulation (CHAPMAN et al. 1980; LEEGOOD et al. 1981; KUBICKI et al; 1996; TAKABAYASHI et al., 2005; ISHIKAWA et al., 2016).

Non-enzymatic antioxidants help redox metabolism by interrupting a free-radical chain reaction. The non-enzymatic compounds such as soluble phenolic compounds (and also flavonoid), as well as the antioxidant activity of betalains are well documented in several works and using diverse methodologies, e.g., scavenging of the 2,2-diphenyl1-picrylhydrazyl (DPPH) (MIGUEL, 2018; LUCHO et al., 2019[a]; LUCHO et al., 2019[b]).

Amaranth (*Amaranthus cruentus* L.) and Quinoa (*Chenopodium quinoa* Willd.) are broad leaf plants (non-grasses) that belong to the order *Caryophyllales* and family *Amaranthaceae* and *Chenopodiaceae* respectively (DAKHILI et al., 2019; NYONJE et al., 2021). These crops present an advantage, since their stems and leaves are edible. They are considered an inexpensive and abundant source of digestive fiber, protein containing methionine and lysine, vitamin C, carotenoids, and minerals. It is also an abundant source of antioxidant pigments, such as betacyanin, betaxanthin, betalain, amaranthine and bioactive phytochemicals, including flavonoids and phenolic acids. These bioactive components of natural origin have the capacity to quench ROS (KHANAM; OBA, 2013).

These crops have been extensively consumed by humans and animals in the Andes for millions of years (HARIADI et al., 2011), and have been studied recently for their ability to develop in adverse conditions such as high temperature, drought and salinity, mainly caused by climate change (ABUGOCH, 2009, RUIZ et al., 2014). However, most of the papers mention research related to the antioxidant capacity of grains of improving human health, but not the impacts on plant physiology. Therefore, the aim of this research was to evaluate the effects of e[CO_2] in some aspects of secondary metabolism of amaranth and quinoa plants at the transition phase from vegetative growth to flowering.

2. MATERIAL AND METHODS

Plant material and growth conditions

The experiments were conducted in the agricultural years of 2019/2020 and 2020/2021, in Open Top Chambers (OTC) belonging to the Herbology Center of the Federal University of Pelotas, located in the municipality of Capão do Leão - RS. These chambers are equipped with sensors, an automated CO_2 concentration control center, coolers responsible for homogenizing the air inside them, a system of gas injection and distribution valves in each chamber, maintaining concentrations of 400 (control) and 700 ppm (high CO_2), respectively. The OTC has a useful area of $4m^2$ and 2.15 m in height, coated with a 150-micron thick, transparent polyethylene plastic film and equipped with a top-opening reducer to deflect the air and prevent the dilution of the desired concentration of CO_2 inside the chamber. Carbon dioxide (Messer®) used was 99.9% pure and was supplied through a storage cylinder (capacity of 25 kg CO_2) coupled to the injection and distribution system of the chambers. The internal temperature of the OTC's was monitored daily using a data logger (HOBO Pro®), installed at the height of the canopy of the plants.

Seeds of the cultivar BRS Alegria (amaranth) and BRS Piabiru (quinoa) were seeded in polystyrene trays on commercial substrate (Plantmax®). After the appearance of the second pair of true leaves, the seedlings were transplanted into 8-L polyethylene pots filled with soil, which was previously analyzed for its physical and chemical attributes, amended and fertilized according to technical recommendations (EMBRAPA 1999). Only one plant was kept per pot after their complete development.

Table 5: Physical and chemical characteristics of the soil collected in the experimental area of Palma, Campus Capão do Leão, UFPel.

pH H_2O 01:01	Ca^{2+}	Mg^{2+}	Al^{3+}	Effective CEC	% SOC	% Clay	Clay Class	K^+	P^*
	$cmol_c\ dm^{-3}$				m/v			mg dm^{-3}	
4.8	1.5	0.6	1.4	3.78	1.38	16	4	30	6

* Phosphorus(P) was extracted using Mehlich solution

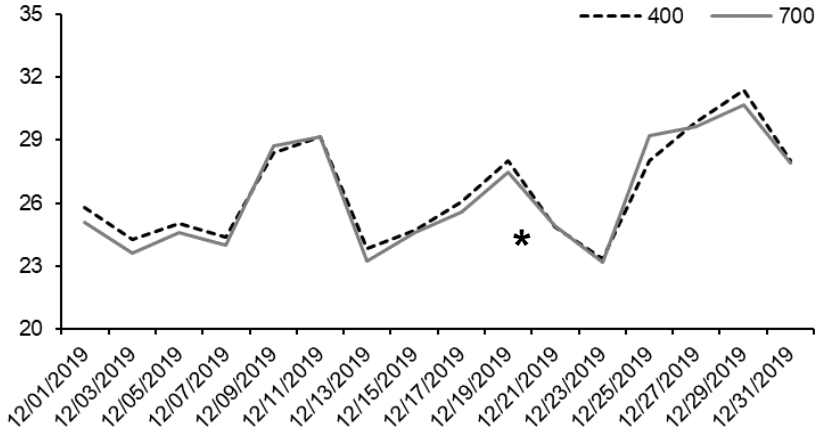

Figure 10: OTC's average indoor ambient temperature during the month of December 2019 – 400- 400 ppm CO_2 and 700 - 700 ppm CO_2. *Transition period between vegetative and flowering.

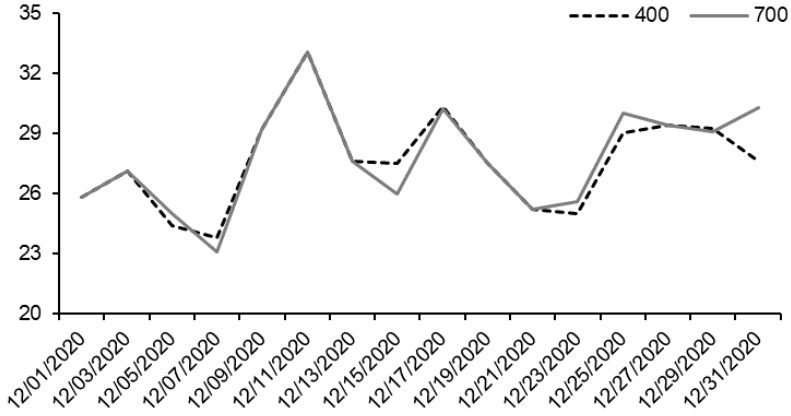

Figure 11: OTC's average indoor ambient temperature during the month of December 2020 – 400 ppm CO_2 and 700 ppm CO_2. *Transition period between vegetative and flowering

Antioxidant Activity: The ability of the extracts to scavenge the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was determined according to Pérez-Tortosa et al. (2012). Briefly, 50 µl of a series of diluted thyme extracts were added to 1 ml of a 100 µM methanol solution of DPPH. An absorbance at 517 nm was measured after a 30 min incubation period at room temperature in the dark and the readings were compared. The absorbance readings were compared to a calibration curve constructed using caffeic acid (0–1500 µM). The results were expressed as micromoles of reduced DPPH per gram fresh weight using an extinction coefficient of 12,500 M^{-1} cm^{-1} at 517 nm.

Total Soluble Phenol Contents: Levels of total soluble phenols were determined using the Folin–Ciocalteu reagent with gallic acid (0–3000 µM) as a standard, as described by López-Orenes et al. (2013). In brief, 190 µL of ultra-pure

water and 12.5 µL of Folin–Ciocalteu reagent were mixed and then added to 10 µL of diluted amaranth and quinoa extracts (sample:70% ethanol, 1:10). After that, 75 µL of 20% Na_2CO_3 were added to the reaction media and the tubes were incubated for 2.5 h in the dark at room temperature. Thereafter, the absorbance of solutions was determined at 765 nm. Total soluble phenol contents were expressed as µmol of gallic acid equivalent per gram (dry weight).

Flavonoid Contents: Total soluble flavonoid content was determined according to Kim et al. (2003) by mixing 37.5 µL of the samples, 37.5 µL of methanol, 75 µL of 5% Na2NO2 and 75 µL of 10% AlCl3. The reaction media were incubated in the dark for 6 min, and then 125 µL of 1N NaOH were added. A standard curve was constructed at 510 nm by using known concentrations of rutin (quercetin-3-rutinoside, 0–3000 µM, regression equation). Flavonoid contents were expressed as µmol of rutin equivalent per gram (dry weight).

Betalains: The extraction of betanin and betanidin was performed with two different types of extractor buffer. Betanidin was extracted by using a 10 mM sodium acetate and methanol (70/30%) buffer, plus 10 mM sodium ascorbate (pH 5.0). For betanin, 10 mM potassium phosphate buffer were utilized, plus 10 mM sodium ascorbate (pH 6.0), without addition of organic solvent. 125 mg of fresh weight were used in both analyses, macerated in a mortar and the extract was centrifuged at 10,000 g for 20 minutes at 4ºC as described by Gandía-Herrero et al. (2005). The molar extinction coefficient used for the calculation of betanidine was €= 54000 M-1 cm-1 and for betanin €= 65000 M^{-1} cm^{-1}, at a wavelength of 536 nm. The results were expressed as mg of betanin in 100 g of fresh mass and mg of betanin in 100 g of fresh weight.

Betaxanthins followed the protocol described in Reis et al. (2017), with the same methodology previously described for betanin. Betaxanthin concentration was obtained through the molar extinction coefficient €= 48000 M^{-1} cm^{-1}, at a wavelength of 480 nm and the result, expressed in mg of mg of betaxanthin per 100 grams of fresh weight.

For the extraction of amaranthine, leaf tissues were macerated in a mortar with 10 mM phosphate buffer, pH 6.0, plus sodium ascorbate at the same concentration. The homogenate was centrifuged at 10,000 g for 20 minutes at 4ºC, according to Reis et al. (2017). The concentration of amaranthine was determined taking into account

the molar extinction coefficient €= 56600 M cm^{-1}, at a wavelength of 536 nm. The result was expressed in mg of amaranthine per 100 grams of fresh weight.

Experimental design: The experimental design was completely randomized, totaling 10 plants of each crop per treatment. The data obtained was analyzed for homoscedasticity by the Bartlett test and for normality by the Shapiro Wilk test. Considering the assumptions, the analysis of variance (ANOVA) was carried out using the statistical software R (ExpDes.pt / www.r-project. org). Afterward, when F was significant, the means were compared to the control by the t-test (P≤0.05).

3. RESULTS

Antioxidant Activity

DPPH scavenging assay (Figure 12) showed that plants cultivated in e[CO_2] had a significantly increase in antioxidant capacity for both crops. Data obtained for amaranth and quinoa in e[CO_2] corresponded to 8,12 (± 0,67) and 9,10 (± 1,04) respectively, indicating the deactivation of free radicals.

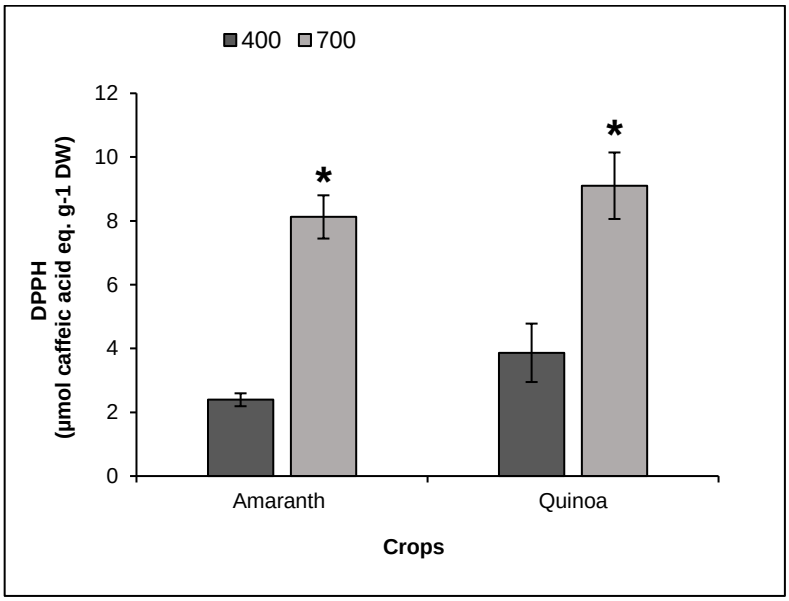

Figure 12: Antioxidant capacity evaluated by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) from leaves of amaranth and quinoa in transition stadium between vegetative and flowering, grown in the presence of different CO2 concentrations (400 and 700ppm). The extracts evaluated were obtained by means of the crop years 19/20 and 20/21. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=10).

Total Soluble Phenol and Flavonoid Contents

Elevated CO_2 significantly increased total phenolic (TPC-13A) and flavonoid (Figure 13B) contents in leaf tissues of amaranth and quinoa plants compared to plants grown under ambient CO_2 condition (Figure 4). A more pronounced response was observed for quinoa plants, showing up to 44.5% increases in flavonoids.

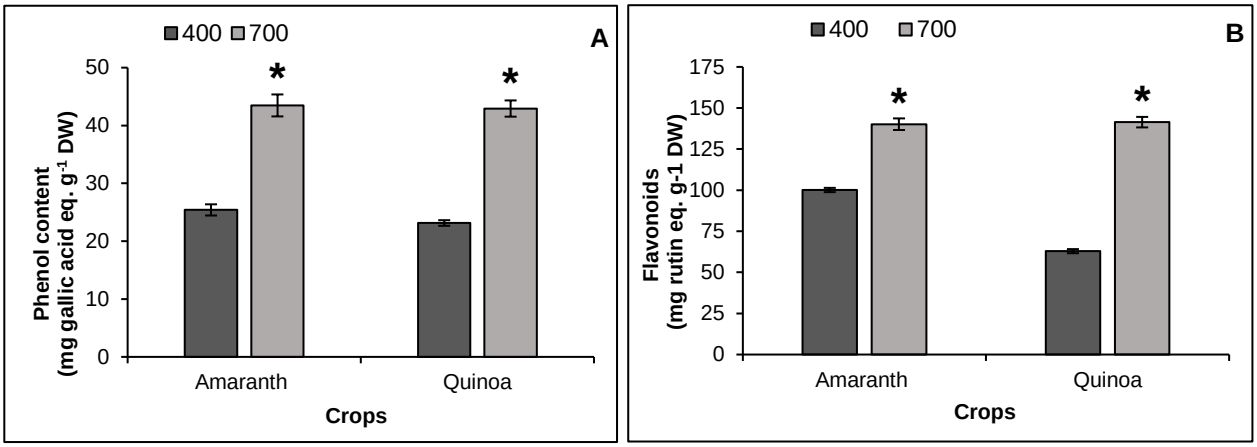

Figure 13: Total Soluble Phenol (A) and Flavonoid Contents (B) from leaves of amaranth and quinoa in transition stadium between vegetative and flowering, grown in the presence of different CO_2 concentrations (400 and 700ppm). The extracts evaluated were obtained by means of the crop years 19/20 and 20/21. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test ($P \leq 0.05$, n=10).

Betalains

Although the betaxanthin pigment (Figure 14A) showed significant differences for both cultures grown in e[CO_2], it was possible to observe different responses between the species. In addition to this, amaranth plants only had an increase in betanidin production (Figure 14B), while the quinoa plants showed changes in the levels of amaranthine (Figure 14C).

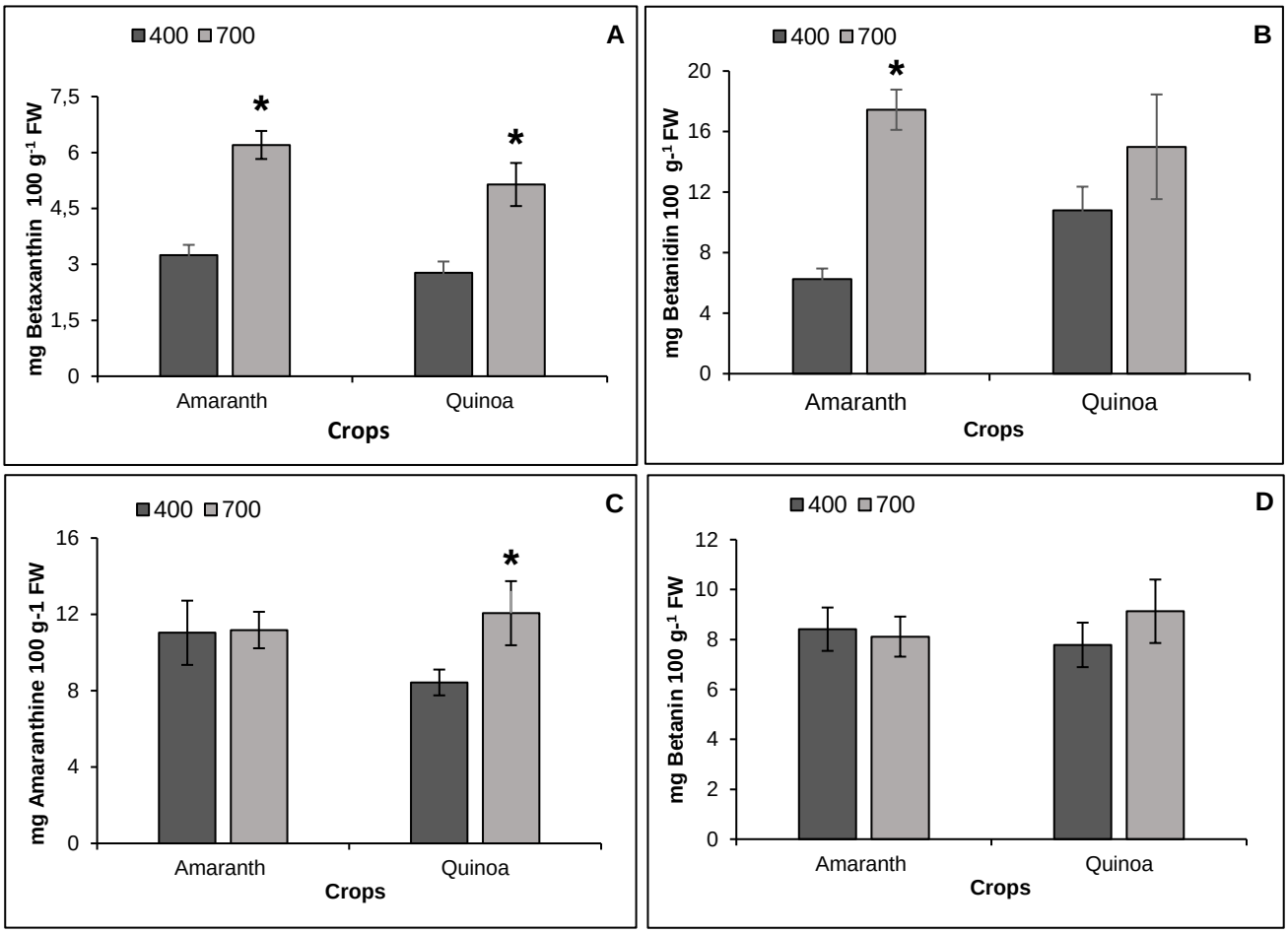

Figure 14: Betalain pigments contents: betaxanthin (A); betanidin (B); amaranthine (C) and betanin (D) from leaves of amaranth and quinoa in transition stadium between vegetative and flowering, grown in the presence of different CO_2 concentrations (400 and 700ppm). The extracts evaluated were obtained by means of the crop years 19/20 and 20/21. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test ($P \leq 0.05$, n=10).

4. DISCUSSION

The classic mechanism that concerns "how plants perceive increased CO_2" mostly of the time revolves around C_3 plants in vegetative or reproductive stage (there are few studies with C_4 plants). Despite that, the real key event of the life cycle in annual plants is the transition phase from vegetative growth to flowering, because of the relocation of resources to improve and guarantee species perpetuation (PALIT et al., 2020; SILVA; HANSSON; JOHANSSON, 2021). Considering only direct effects of increased CO_2 substrate on the ratio of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation to oxygenation reactions in C_3 plants, increases on carbon gain and enriched levels of compounds such as sugars and other carbohydrates have already been elucidated (FOYER; NOCTOR, 2020).

Recently, Dietz et al. (2016) have greatly enhanced our knowledge about the biochemical and physiological mechanisms of C_4 photosynthesis by examining the transcriptome, proteome and metabolome at the cell or tissue level. However, all this knowledge is mostly derived from C_3 plants and shows us that photosynthesis can be considered as a series of redox reactions, and it is mutually linked with the cellular redox status (TURKAN et al., 2018).

C_3 and C_4 photosynthesis share fundamental metabolic processes, such as the Calvin-Benson cycle, light-harvesting complexes, and electron transport components. Most C_4 plants fix the CO_2 in mesophyll cells with PEPC (phosphoenolpyruvate carboxylase) also release it in the bundle sheath cells where Rubisco is localized. As a result, the Calvin-Benson cycle occurs (LEEGOOD, 2002; TAIZ; ZEIGER, 2017).

The regulation of cyclic electron flow (CEF) is also enigmatic, although a range of models suggest that it is regulated by reactive oxygen species, the redox state of NADPH, and the ratio of ATP/ADP (CASANO et al., 2001; FINAZZI et al., 2002; LASCANO et al., 2003; JOLIOT$_a$ et al., 2004; BREYTON et al., 2006; JOLIOT$_b$ et al., 2006; LIVINGSTON et al., 2010; IWAI et al., 2010; TERASHIMA et al., 2012 ; TAKAHASHI et al., 2013 STRAND et al., 2016).

Plant secondary metabolites (SMs) are derivatives of primary metabolites produced by plants because of diverse physiological changes such as redox alterations. There are three major groups of SMs in plants based on their biosynthetic

pathway. These groups include nitrogen-containing compounds (betalains), phenolic compounds and flavonoids (ASHRAF et al., 2018).

As observed in this study, radical scavenging capacity analyzed by the levels of DPPH (Figure 12) in plant extracts of amaranth and quinoa was higher in e[CO_2]. The effect of antioxidants on DPPH scavenging is noticeable due to their hydrogen donating ability. Besides that, DPPH is a model of a stable lipophilic radical. Thus, these increases suggest that (I) changes in redox metabolism possibly derivate from alterations by the CEF and (II) amaranth and quinoa plants possess an efficient radical scavenging ability in e[CO_2] conditions (WANG et al., 2003; GHASEMZADEH et al., 2010; GHASEMZADEH et al., 2012; DOBRIKOVA et al., 2022).

Secondary metabolism is linked to primary metabolism by rates at which substrates are diverted from primary pathways and funneled into the secondary biosynthetic routes. In our study, it was possible to recognize an increase in TPC (Figure 13A) and flavonoid content (Figure 13B). This result is related to their properties, such as redox which enables them to act as antioxidants. They are the most important groups of secondary metabolites and bioactive compounds in plants (KIM; JEONG; LEE, 2003; CHEESEMAN, 2006. HUANG and BIE, 2010; VALLVERDÚ-QUERALT et al., 2014; DONG et al., 2018; PALIT et al., 2020; SACHDEV et al., 2021).

Betalains are pigments synthesized from tyrosine and constitute a class of secondary metabolites found exclusively in species of the order Caryophyllales (SEPULVEDA-JIMÉNEZ et al., 2004; MIGUEL, 2018). Even though flavonoids are considered the main mechanisms of secondary metabolism, betalains are found in greater availability and also have high antioxidant activity. Furthermore, its molecules have high stability, remaining active in adverse environments such as climate change. (KANNER et al., 2001; STINTZING; CARLE, 2004). Whereas the condensation of betalamic acid with cyclo-dopa [cyclo-3-(3,4-dihydroxyphenylalanine)] or its glucosyl derivatives originate violet betacyanins, resulting glycosides of betacyanins can be linked to acylation groups, leading to several structures such as betanin, amaranthin and others (HERBACH; STINTZING; CARLE, 2006; KHAN; GIRIDHAR, 2015; SIGURDSON; TANG; GIUSTI, 2017; MIGUEL, 2018). Since they are compounds derived from nitrogen, their actions are linked to the bioavailability of this nutrient for the plant (data presented in chapter 1). Although our research showed a different trend for each species, there was a general increase in betaxanthin, Betanidin and amaranthine compounds (Figure 14A, 14B, 14C) which demonstrated a high need for

the action of antioxidant compounds (CAI; SUN; CORKE$_a$ 2003; CAI; SUN; CORKE$_b$, 2005).

Other authors (GLISZCZYŃSKA-ŚWIGŁO; SZYMUSIAK; MALINOWSKA, 2006; SHAO et al., 2013; ESCRIBANO et al., 2017) also reported antioxidant activity of amaranth and quinoa extracts through their capacity for scavenging free radicals (DPPH) and reducing power. These activities were related to phenol content and not to betalains, justifying the distinct trend for each crop.

5. CONCLUSIONS

- Amaranth and quinoa plants grown in an e[CO_2] environment show alterations in secondary metabolism;

- e[CO_2] changes the redox stage of amaranth and quinoa in transitional stadium between vegetative and flowering, which may impact grain production;

- Amaranth and quinoa plants also showed a potent antioxidant capacity (based on DPPH, TPC, and flavonoids content);

- Betalains synthesis may increase in front of e[CO_2];

REFERENCES

AINSWORTH, E. A. et al. The effects of elevated CO_2 concentration on soybean gene expression. An analysis of growing and mature leaves. **Plant Physiology**, v. 142, n. 1, p. 135-147, 2006.

ALVAREZ-JUBETE, L. et al. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. **Food chemistry**, v. 119, n. 2, p. 770-778, 2010.

ARAI, H. et al. Effect of bicarbonate on iron-mediated oxidation of low-density lipoprotein. **Proceedings of the National Academy of Sciences**, v. 102, n. 30, p. 10472-10477, 2005.

ASHRAF, M. A. et al. Environmental stress and secondary metabolites in plants: an overview. **Plant metabolites and regulation under environmental stress**, p. 153-167, 2018.

BREYTON, C. et al. Redox modulation of cyclic electron flow around photosystem I in C_3 plants. **Biochemistry**, v. 45, n. 45, p. 13465-13475, 2006.

CAI, Y.; SUN, M.; CORKE, H. Antioxidant activity of betalains from plants of the *Amaranthaceae*. **Journal of agricultural and food chemistry**, v. 51, n. 8, p. 2288-2294, 2003

CAI, Y.; SUN, M.; CORKE, H.. Characterization and application of betalain pigments from plants of the *Amaranthaceae*. **Trends in Food Science & Technology**, v. 16, n. 9, p. 370-376, 2005.

CASANO, L. M.; MARTIN, M.; SABATER, B. Hydrogen peroxide mediates the induction of chloroplastic Ndh complex under photooxidative stress in barley. **Plant Physiology**, v. 125, n. 3, p. 1450-1458, 2001.

CHAPMAN, K. S. R.; BERRY, J. A.; HATCH, M. D. Photosynthetic metabolism in bundle sheath cells of the C_4 species Zea mays: sources of ATP and NADPH and the contribution of photosystem II. **Archives of Biochemistry and Biophysics**, v. 202, n. 2, p. 330-341, 1980.

CHEESEMAN, J. M. Hydrogen peroxide concentrations in leaves under natural conditions. **Journal of experimental botany**, v. 57, n. 10, p. 2435-2444, 2006.

DAKHILI, S. et al. Quinoa protein: Composition, structure and functional properties. **Food chemistry**, v. 299, p. 125161, 2019.

DE FARIA, A. P. et al. The increase of current atmospheric CO_2 and temperature can benefit leaf gas exchanges, carbohydrate content and growth in C_4 grass invaders of the Cerrado biome. **Plant Physiology and Biochemistry**, v. 127, p. 608-616, 2018.

DOBRIKOVA, A. et al. Combined Impact of Excess Zinc and Cadmium on Elemental Uptake, Leaf Anatomy and Pigments, Antioxidant Capacity, and Function of

Photosynthetic Apparatus in Clary Sage (*Salvia sclarea* L.). **Plants**, v. 11, n. 18, p. 2407, 2022.

DONG, J. et al. Effects of elevated CO_2 on nutritional quality of vegetables: a review. **Frontiers in plant science**, v. 9, p. 924, 2018.

ESCRIBANO, J. et al. Characterization of betalains, saponins and antioxidant power in differently colored quinoa (*Chenopodium quinoa*) varieties. **Food chemistry**, v. 234, p. 285-294, 2017.

FINAZZI, G. et al. Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. **EMBO reports**, v. 3, n. 3, p. 280-285, 2002.

GHASEMZADEH, A.; JAAFAR, H. Z. E.; RAHMAT, A. Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (*Zingiber officinale* Roscoe.) varieties. **Molecules**, v. 15, n. 11, p. 7907-7922, 2010.

GHASEMZADEH, A. et al. Combined effect of CO2 enrichment and foliar application of salicylic acid on the production and antioxidant activities of anthocyanin, flavonoids and isoflavonoids from ginger. **BMC Complementary and Alternative Medicine**, v. 12, n. 1, p. 1-10, 2012.

GLISZCZYŃSKA-ŚWIGŁO, A.; SZYMUSIAK, H.; MALINOWSKA, P. Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity. **Food additives and contaminants**, v. 23, n. 11, p. 1079-1087, 2006.

HERBACH, K. M.; STINTZING, F. C.; CARLE, R. Betalain stability and degradation—structural and chromatic aspects. **Journal of food science**, v. 71, n. 4, p. R41-R50, 2006.

HUANG, X.; BIE, Z. Cinnamic acid-inhibited ribulose-1, 5-bisphosphate carboxylase activity is mediated through decreased spermine and changes in the ratio of polyamines in cowpea. **Journal of plant physiology**, v. 167, n. 1, p. 47-53, 2010.

IBRAHIM, M. H., JAAFAR, H. Z. Enhancement of leaf gas exchange and primary metabolites under carbon dioxide enrichment up-regulates the production of secondary metabolites in *Labisia pumila* seedlings. **Molecules** 16, 3761–3777. 2012.

IPCC, 2022: *Climate Change: Impacts, Adaptation, and Vulnerability.* Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. **Cambridge University Press**. 2022.

IWAI, M. et al. Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. **Nature**, v. 464, n. 7292, p. 1210-1213, 2010.

JOLIOT, P.; BÉAL, D.; JOLIOT, A. Cyclic electron flow under saturating excitation of dark-adapted Arabidopsis leaves. **Biochimica et Biophysica Acta (BBA)-Bioenergetics**, v. 1656, n. 2-3, p. 166-176, 2004.

JOLIOT, P.; JOLIOT, A. Cyclic electron flow in C_3 plants. **Biochimica et Biophysica Acta (BBA)-Bioenergetics**, v. 1757, n. 5-6, p. 362-368, 2006.

KANE, K. et al. Long-term growth under elevated CO_2 suppresses biotic stress genes in non-acclimated, but not cold-acclimated winter wheat. **Plant and Cell Physiology**, v. 54, n. 11, p. 1751-1768, 2013.

KHAN, M. I.; GIRIDHAR, P. Plant betalains: Chemistry and biochemistry. **Phytochemistry**, v. 117, p. 267-295, 2015.

KHANAM, U. K. S.; OBA, S. Bioactive substances in leaves of two amaranth species, *Amaranthus* tricolor and *A. hypochondriacus*. **Canadian Journal of Plant Science**, v. 93, n. 1, p. 47-58, 2013.

KIM, D. O.; JEONG, S. W.; LEE, C. Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. **Food chemistry**, v. 81, n. 3, p. 321-326, 2003.

KUBICKI, A.; STEINMÜLLER, K.; WESTHOFF, P. Differential transcription of plastome-encoded genes in the mesophyll and bundle-sheath chloroplasts of the monocotyledonous NADP-malic enzyme-type C_4 plants maize and Sorghum. **Plant molecular biology**, v. 25, n. 4, p. 669-679, 1994.

KUBICKI, A. et al. Differential expression of plastome-encoded ndh genes in mesophyll and bundle-sheath chloroplasts of the C_4 plant Sorghum bicolor indicates that the complex I-homologous NAD (P) H-plastoquinone oxidoreductase is involved in cyclic electron transport. **Planta**, v. 199, n. 2, p. 276-281, 1996.

LASCANO, H. R. et al. The activity of the chloroplastic Ndh complex is regulated by phosphorylation of the NDH-F subunit. **Plant Physiology**, v. 132, n. 1, p. 256-262, 2003.

LEAKEY, A. D. B. et al. Genomic basis for stimulated respiration by plants growing under elevated carbon dioxide. **Proceedings of the National Academy of Sciences**, v. 106, n. 9, p. 3597-3602, 2009.

LEEGOOD, R. C. et al. Photosynthetic electron transport in the bundle sheath of maize. **FEBS Letters**, v. 126, n. 1, p. 89-92, 1981.

LI, P. et al. Arabidopsis transcript and metabolite profiles: ecotype-specific responses to open-air elevated [CO_2]. **Plant, Cell & Environment**, v. 31, n. 11, p. 1673-1687, 2008.

LIVINGSTON, A. K. et al. Regulation of cyclic electron flow in C_3 plants: differential effects of limiting photosynthesis at ribulose-1, 5-bisphosphate carboxylase/oxygenase and glyceraldehyde-3-phosphate dehydrogenase. **Plant, Cell & Environment**, v. 33, n. 11, p. 1779-1788, 2010.

LUCHO, S. R. et al. Plant growth regulators as potential elicitors to increase the contents of phenolic compounds and antioxidant capacity in stevia plants. **Sugar Tech**, v. 21, n. 4, p. 696-702, 2019.

LUCHO, S. R. et al. Salt stress-induced changes in in vitro cultured Stevia rebaudiana Bertoni: effect on metabolite contents, antioxidant capacity and expression of steviol glycosides-related biosynthetic genes. **Journal of Plant Growth Regulation**, v. 38, n. 4, p. 1341-1353, 2019.

MATROS, A. et al. Growth at elevated CO_2 concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with potato virus Y. **Plant, Cell & Environment**, v. 29, n. 1, p. 126-137, 2006.

MHAMDI, A.; NOCTOR, G. High CO_2 primes plant biotic stress defences through redox-linked pathways. **Plant Physiology**, v. 172, n. 2, p. 929-942, 2016.

MIGUEL, Maria Graça. Betalains in some species of the Amaranthaceae family: A review. **Antioxidants**, v. 7, n. 4, p. 53, 2018.

NIU, Y. et al. Physiological and transcriptome responses to combinations of elevated CO2 and magnesium in *Arabidopsis thaliana*. **PLoS One**, v. 11, n. 2, p. e0149301, 2016.

NYONJE, W. A. et al. Precision phenotyping and association between morphological traits and nutritional content in Vegetable Amaranth (Amaranthus spp.). **Journal of Agriculture and Food Research**, v. 5, p. 100165, 2021

PADHAN, Birendra K. et al. CO_2 Elevation accelerates phenology and alters carbon/nitrogen metabolism vis-à-vis ROS abundance in bread wheat. **Frontiers in plant science**, v. 11, p. 1061, 2020.

PALIT, P. et al. An integrated research framework combining genomics, systems biology, physiology, modelling and breeding for legume improvement in response to elevated CO_2 under climate change scenario. **Current plant biology**, v. 22, p. 100149, 2020.

PÉREZ-TORTOSA, V. et al. Antioxidant activity and rosmarinic acid changes in salicylic acid-treated *Thymus membranaceus* shoots. **Food chemistry**, v. 130, n. 2, p. 362-369, 2012.

QIU, Q. S. et al. Increased protein carbonylation in leaves of Arabidopsis and soybean in response to elevated [CO_2]. **Photosynthesis Research**, v. 97, n. 2, p. 155-166, 2008.
REIS, A. et al. Callus induction and betacyanin quantification by HPLC/MS-MS in *Alternanthera brasiliana* (L.) Kuntze. **Hoehnea**, v. 44, n. 1, p. 90-95, 2017.

SACHDEV, S. et al. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. **Antioxidants**, v. 10, n. 2, p. 277, 2021.

SHAO, L. et al. Differential responses of photosystem II activity to photooxidation in red and green tissues of *Amaranthus tricolor* leaves. **Pakistan Journal of Botany.**, 45(6): 1905-1912, 2013.2013.

SIGURDSON, G. T.; TANG, P.; GIUSTI, M. Mónica. Natural colorants: Food colorants from natural sources. **Annual review of food science and technology**, v. 8, p. 261-280, 2017.

STRAND, D. D. et al. Redox regulation of the antimycin A sensitive pathway of cyclic electron flow around photosystem I in higher plant thylakoids. **Biochimica Et Biophysica Acta (BBA)-Bioenergetics**, v. 1857, n. 1, p. 1-6, 2016.

TAKABAYASHI, A. et al. Differential use of two cyclic electron flows around photosystem I for driving CO_2-concentration mechanism in C_4 photosynthesis. **Proceedings of the National Academy of Sciences**, v. 102, n. 46, p. 16898-16903, 2005.

TAKAHASHI, H. et al. Cyclic electron flow is redox-controlled but independent of state transition. **Nature communications**, v. 4, n. 1, p. 1-8, 2013.

TERASHIMA, M. et al. Calcium-dependent regulation of cyclic photosynthetic electron transfer by a CAS, ANR1, and PGRL1 complex. **Proceedings of the National Academy of Sciences**, v. 109, n. 43, p. 17717-17722, 2012.

VALLVERDÚ-QUERALT, A. et al. A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay. **Food chemistry**, v. 154, p. 299-307, 2014.

WANG, S. Y.; BUNCE, J. A.; MAAS, J. L. Elevated carbon dioxide increases contents of antioxidant compounds in field-grown strawberries. **Journal of Agricultural and Food Chemistry**, v. 51, n. 15, p. 4315-4320, 2003.

CHAPTER 3

Transgenerational parental effect caused by CO_2 enrichment on seeds and seedlings of pseudocereals

1. INTRODUCTION

Atmospheric carbon dioxide (CO_2) concentration ([CO_2]) is one of the most important factors aggravating climate change in recent years. The year of 2022 was marked by news showing that carbon dioxide peaked at 421 parts per million in May, pushing the atmosphere further into territory not seen for millions of years (NOOA, 2022)

One of the most significant concerns regarding these climate change impacts on human welfare is food security and energy and Pseudoceareals can be already used for the first purpose and with potential for the second. In the Brazilian Cerrado, under experimental conditions, quinoa plots obtained up to 8 tons per hectare during winter and under irrigation (SILVA et al 2021).These alterations have various effects on crop productivity by affecting plant photosynthesis, carbon metabolism, water and nutrient use efficiency (HU et al., 2021). Besides that, the establishment of the crops in the field is also important in the current scenario when it comes to food security. This process depends on the inherent characteristics of seeds and can define the success and failure of production. For this reason, it is essential to use seeds of good physiological quality, which is determined by the interaction of four basic components: genetic, physical, physiological and sanitary (COMIRAN, 2017).

Seed development can be considered as a discontinuous, stepwise process where several different phases occur in succession to ensure the formation of a functional reproductive unit. Typically, seed germination begins with dry mature seed imbibition and ends with radicle protrusion. This process is not an isolated biological process for the dry seed, but a successive process combining seed development/desiccation and seedling establishment (HAN; YANG, 2015). This entire process is very complex and highly coordinated due to the fact that it integrates genetic, metabolic and physiological signaling pathways, which can be affected by both endogenous signals and environmental stimuli such as CO_2 (SABELLI, 2012).

Plants under abiotic stress and/or different growing conditions such as e[CO2], can adapt in order to survive. Some changes caused by adaptation can be transferred to future generations, allowing for adaptive transgenerational (GALLOWAY, 2005; FIPKE et al., 2022). Whereas the effect of CO_2 enrichment (e[CO_2]) on plant growth and crop yield have been extensively studied data of seeds produced under different parental CO_2 conditions should also be considered when evaluating crop productivity under future climate conditions (BAI et al., 2003).

Transgenerational parental effects can happen due to two main causes: the direct transmission of genetic material from the father to descent through the possible routes, such as the transmission of cytoplasmic organelles throughout the cell partitioning process or the endosperm or hereditary chromosomal mutations; the transmission of non-genetic information obtained from environmental conditions induces changes in gene expression (LUZURIAGA; ESCUDERO; PEREZ-GARCIA, 2006).

Some studies show that the increase in atmospheric CO_2 can affect the quality of seeds produced in this condition, with subsequent impact on the germination process. However, it is also known that this transgenerational effect of CO_2 on germination is not always clear and is often dependent on the species and genotypes in question (MARTY; BASSIRIRAD, 2014).

Therefore, understanding seed germination responses to climate change of crops such as pseudocereals is important for predicting changes in species composition, and subsequently in the structure and function of agricultural plant systems. Seeds and seedlings traits, vigor, viability, germination and determination of the activity of acid phosphatase and α-amylase enzymes have strong adaptive implications for species distribution and abundance under future climates (HOVENDEN et al., 2008; JIMENEZ-ALFARO et al., 2016; LI et al., 2018).

Pseudocereals such as quinoa and amaranth have been recognized as superfoods of 21st century (PIRZADAH; MALIK, 2020). Interest in these crops has increased largely because they are rich in numerous compounds with beneficial properties for human health, including proteins, peptides, flavonoids, phenolic acids, fatty acids, vitamins, amino acids, dietary fibers, lignans and unsaturated fatty acids, among others (SPEHAR et al., 2003; SPEHAR, 2006; SPEHAR, 2007; MARTÍNEZ-VILLALUENGA et al., 2020).

Thus, the aim of this research was to investigate the transgenerational parental effect of elevated carbon dioxide on seed germination and metabolic activity of quinoa and amaranth seedlings.

2. MATERIAL AND METHODS

The experiment was conducted in the Plant Nutrition Laboratory belonging to the Department of Botany, Institute of Biology, Federal University of Pelotas (UFPel), in the city of Capão do Leão. The quinoa (*Chenopodium quinoa* Willd) and amaranth (*Amaranthus cruentus*) seeds used were obtained from studies conducted in Open Top Chambers (OTC) that were carried out in crop years 19/20 and 20/21 using the cultivars BRS Piabiru and BRS Alegria respectively. The seeds that originated parental plants were initially obtained from purified strains of Embrapa Clima Temperado.

Seeds of quinoa and amaranth were disinfected with sodium hypochlorite (2% active chlorine) for 5 min and then were sown on “germitest” paper moistened with water to a volume of 2.5 times their weight. Transparent plastic boxes (Gerbox®) were used and were kept in a germinating chamber regulated at 25 °C with a photoperiod of 12h.

Standard viability and vigor tests were conducted according to the Seeds Analysis Rules (BRASIL, 2009), as described below:

Germination test (G%): carried out with 200 seeds (four sub-samples of 50 seeds) for each repetition, in a total of four repetitions. The results were expressed in germination percentage.

First germination count (FGC%): carried out with the germination test, in which the first count for quinoa was performed three days after sowing and for amaranth this period was of five days according to the Seeds Analysis Rules (BRASIL, 2009). Results were expressed in percentages of normal seedlings.

Germination speed index (GSI)**:** carried out with the germination test, in which daily counts were performed after 2 mm radicle protrusion from the seed tegument until the number of seedlings remained steady. Calculation for the germination speed index was undertaken according to Maguire (1962). Quantification of crude protein:

Seed protein content: was determined using the Kjeldahl method, which is based on three steps: digestion, distillation and titration. 200 mg of grain flour were

used in duplicate, a measure of catalyst or digester mixture (sodium sulfate and copper sulfate pentahydrate in the proportion 7/1) and 5 mL of concentrated sulfuric acid, which were placed in test tubes of 15 mL. Then, the tubes were placed in the digester block at 100ºC and the temperature was adjusted until reaching 400ºC, aiming to break the organic bonds and conserve nitrogen in ammonia. This procedure was carried out for approximately three hours in a hood due to the exhaustion of gases that are formed by the oxidation of carbon contained in the organic matter and by the carbon dioxide that is given off.

After the digestion process was completed, 10 mL of distilled water was added. Then, the test tube was attached to the nitrogen distiller, heated to an average temperature of 70 ºC. 15 mL of sodium hydroxide was inserted and, by steam drag, the excess of ammonium sulfate was treated, releasing it. The ammonia resulting from the digestion process was captured in an Erlenmeyer flask containing boric acid and three drops of indicator solution (0.1% methyl red alcoholic solution and bromocresol green alcoholic solution), the process ended when 75 mL was reached of ammonium borate ($NH_4H_2BO_3$) and changing from pink to green.

Titration was performed with the aid of a burette, using hydrochloric acid (HCl concentration 0.1N) as a standard titration solution. The process is completed when green turns to pink (color change in the presence of the indicator).

The calculation for the determination of total nitrogen was as follows:

$$NT = (Va - Vb) \times F \times 0.1 \times 0.014 \times 100 / P1$$

Being:

- NT – Total nitrogen content in the sample, in percentage;
- Va – Volume of hydrochloric acid solution used in sample titration;
- Vb – Volume of hydrochloric acid solution used in blank titration;
- F – Correction factor for hydrochloric acid;
- P1 – Sample mass (in grams).

To determine the total seed protein content, the value of total nitrogen verified by the Kjeldahl method was multiplied by the factor conversion of nitrogen into protein, in this case the value used was 6.75 (amaranth) and 6.25 (quinoa). The formula below

was used to determine seed protein content: PT = NT x Fc where PT – Total protein; NT – Total nitrogen; Fc – Conversion factor.

Determination of the activity of acid phosphatase (AP) and α-amylase (α-A) enzymes: determined according to the method presented by Aoac (1965), with some adaptations. Extractions were carried out in seedlings from germination tests at 3 - 5 days for quinoa and 5 - 14 days for amaranth. 0,500 mg of seedlings macerated by a mortar, using 20 mL of potassium acetate buffer (50 mM, pH 5.0) were used and later centrifuged at 4000 rpm for 20 minutes, at 4 °C. Then, the supernatant was removed and placed in test tubes, which were stored in a refrigerator at 4ºC until the analyses were performed. For acid phosphatase, 0.2 mL of the extract, 0.8 mL of potassium acetate buffer (50 mM, pH 5.0), and 0.1 mL of p-nitrophenyl phosphate (0.018 M) were added to all test tubes. The tubes were incubated at 30 °C for five minutes. Afterward, 1 mL of sodium hydroxide (0.5 N) was added and read in a spectrophotometer at 400 nm, with the activity expressed in μmol p-NPP hydrolysis min-1g-1of fresh weight (FW). The determination of α- amylase the extract was kept at 70 °C for 20 min. After that, a 15-minute centrifugation was carried out in order to have the supernatant. Then, in each test tube, 0.2 mL of the extract, 0.8 mL of buffer solution, 1 mL of starch solution, and 1 mL of I2+KI were added. The reading was executed in a spectrophotometer at 620 nm and results were expressed in μg of hydrolyzed starch $min^{-1}g^{-1}$ FM.

Experimental design: The used experimental design was completely randomized. Data obtained were analyzed for homoscedasticity by the Bartlett test and for normality by the Shapiro Wilk test, and considering the assumptions, the analysis of variance (ANOVA) was carried out using the statistical software R (ExpDes.pt www.r-project. org/). Afterward, when F was significant, the means were compared to the control by the t-test (P≤0.05).

3. RESULTS

Germination tests

Germination percentage test (G%) (Figure 15A) of amaranth and quinoa seeds showed significant changes only for amaranth harvested in the 20/21 crop year. A

possible transgenerational parental effect was observed just on 700 ppm. On the other hand, despite the fact that G% did not present much difference, the seeds of both species and crop years showed discrepancies in first germination count (Figure 15B) and germination speed index (Figure 15C) (except for amaranth 19/20). Here, it was possible to observe a clear primary effect of CO_2 on amaranth and quinoa seeds.

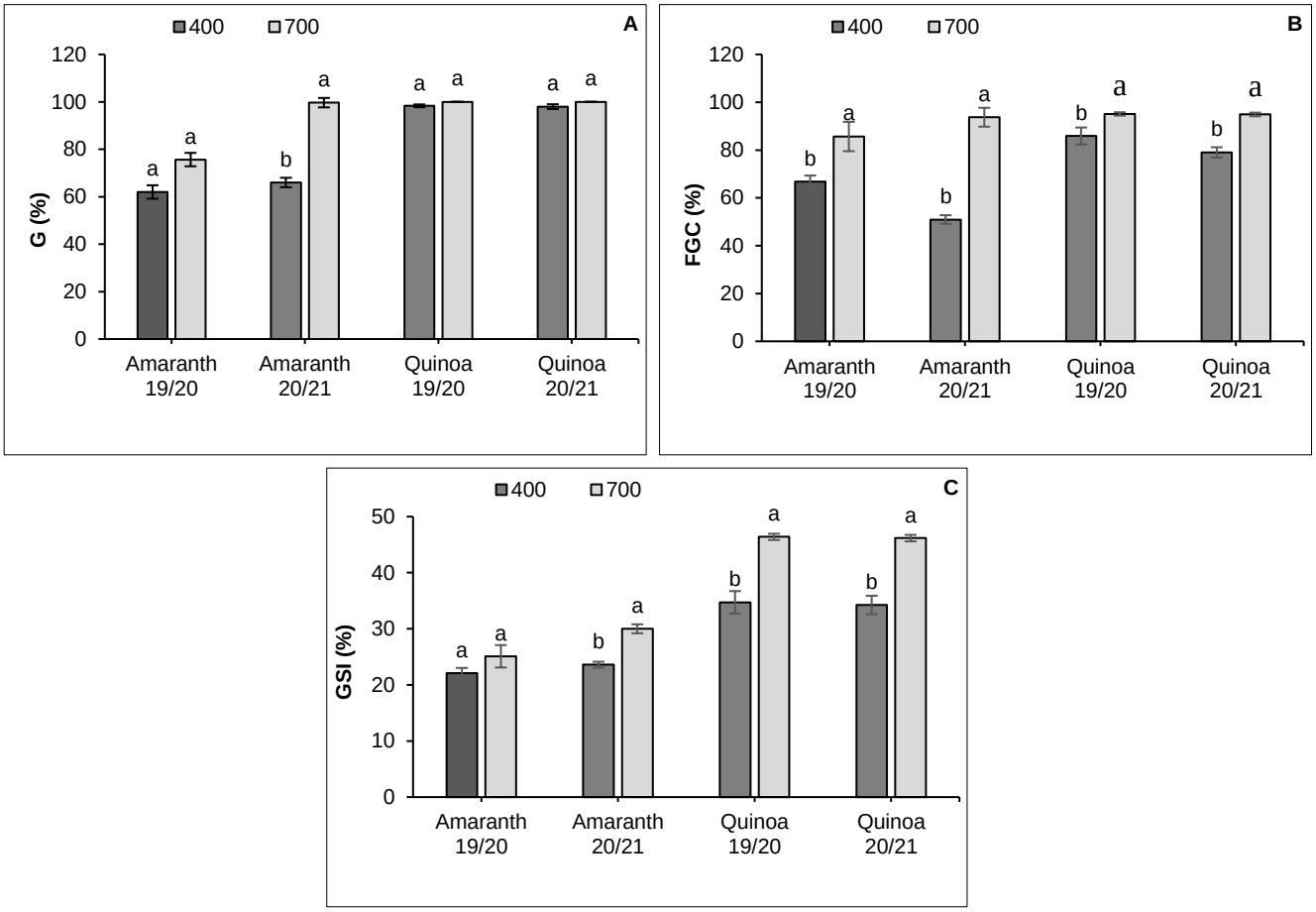

Figure 15: Transgenerational parental effect of CO_2 on germination parameters. (A) Germination -G%; (B) First Germination Count – FGC%; (C) Germination Speed Index - GSI of amaranth and quinoa seeds. Seeds were obtained of studies conducted in Open Top Chambers (OTC) that were carried out in crop years 19/20 and 20/21 using the cultivars BRS Piabiru and BRS Alegria. Plants grow in 400 and 700 parts per million (ppm) of CO_2 (control). Error bars correspond to the standard deviation. Letters compare 400 and 700 ppm of CO_2 by t-test ($P \leq 0.05$, n=200).

Seed protein content

Table 6 shows the protein content of the seed (SPC) obtained from amaranth and quinoa plants grown in different crop years, and two CO_2 concentrations. It was possible to observe that e[CO_2] contributed to a negative effect on the SPC of the seeds, which could possibly impact the enzymatic activity and initial growth.

Table 6: Transgenerational parental effect of CO_2 on seed crude protein content of amaranth and quinoa plants.

	Specie / Crop year			
Treatment	Amaranth 19/20	Amaranth 20/21	Quinoa 19/20	Quinoa 20/21
CO_2 (ppm)	**Seed protein content (%)**			
400	18.28±(1.35)a	17.99±(0.37)a	15.97±(0.97)a	15.94±(0.92)a
700	12.74±(0.62)b	11.87±(0.30)b	7.97±(0.89)b	7.79±(0.41)b
CV%	4.29	1.45	4.92	3.81

*Indicates significant difference by *t* test (P≤0.05, n=4); CV: coefficient of variation. Values in parentheses correspond to standard deviation.

Enzymatic activity

The acid phosphatase enzyme (Figure 16A-16B) and the α-amylase enzyme (Figure 16C-16D) from amaranth and quinoa seedlings showed similar results. Both species demonstrated a decrease in activity at different evaluation times (5 and 14 days for amaranth; 3 and 5 days for quinoa). However, it was noted that when compared to the parental effect of CO_2, there was a significant effect for those seeds which plants grew under 700 ppm.

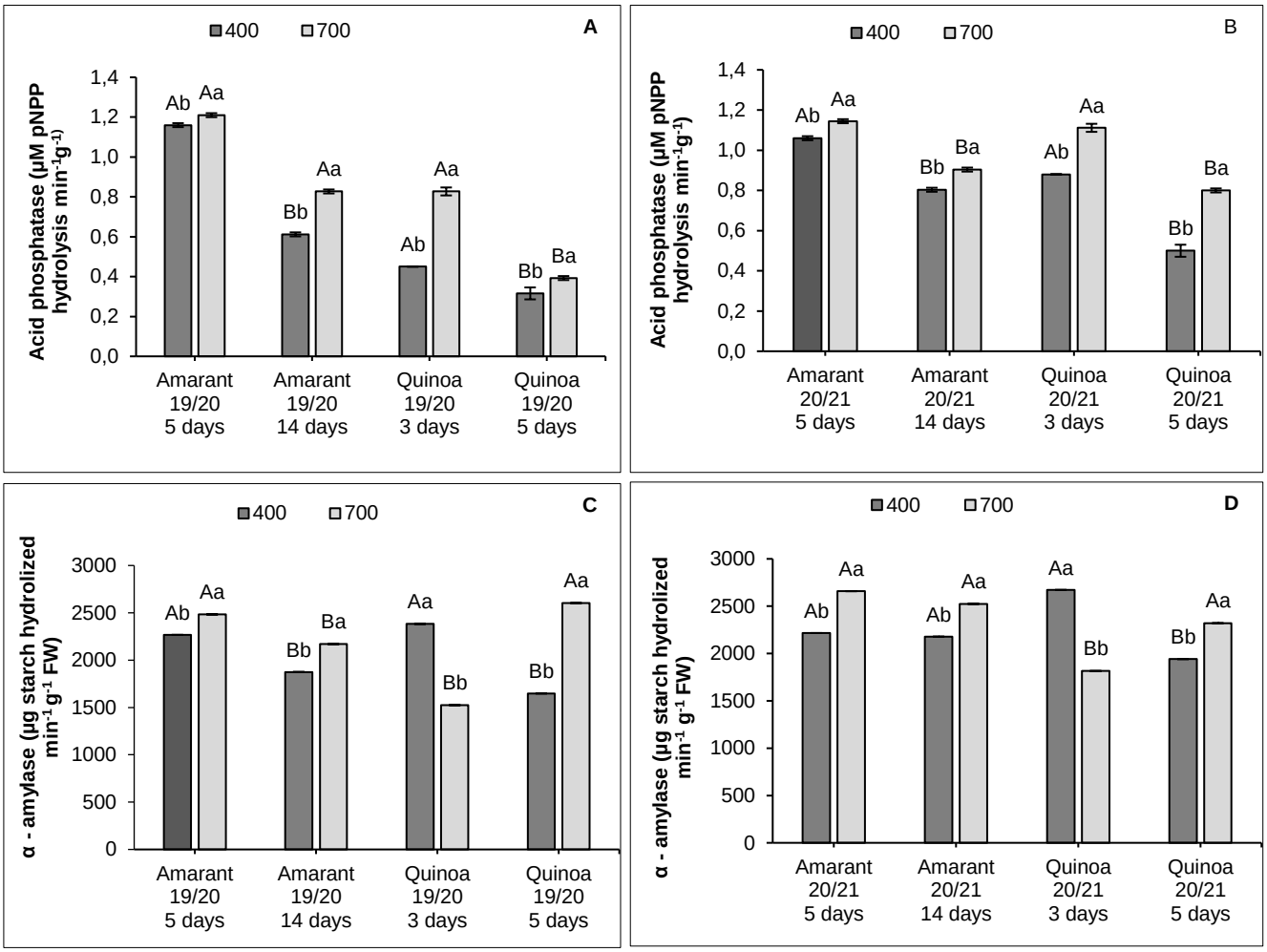

Figure 16: Transgenerational parental effect of CO_2 on enzymatic activity. (A) and (B) acid phosphatase activity (C) and (D) α-amylase activity amaranth and quinoa seedlings. Seeds were obtained of studies conducted in Open Top Chambers (OTC) that were carried out in crop years 19/20 and 20/21 using the cultivars BRS Piabiru and BRS Alegria. Plants grow in 400 and 700 parts per million (ppm) of CO_2 (control). Error bars correspond to the standard deviation. Capital letters compare the time of the evaluation, and lowercase letters compare 400 and 700 ppm of CO_2 by t-test (Means with the same letter within a parameter and entry are not significantly different at P≤0.05, n=6).

Pearson linear correlation

Using data from Pearson linear correlation, it was verified that germination had a high correlation with all data measured, not only viability and vigor but also biochemical parameters such as CSP and enzymatic activity (Table 7 and 8). Only the G% and FGC% did not show a high correlation for quinoa germination on the crop year of 19/20. Nevertheless, those parameters were significant by t-test.

Table 7: Pearson correlation for 8 variables evaluated for the transgenerational parental effect of CO_2 on seeds and Seedlings of amaranth. Crop year 19/20 below diagonal and Crop year 20/21 above diagonal.

	G%	***FGC(%)***	***GSI***	***AP (first)***	***AP (second)***	***α - A (first)***	***α - A (second)***	***CSP***
G%	1	0.99**	0.98**	0.99**	0.94**	0.95	-0.95**	0.99***
FGC(%)	0.90*	1	0.97**	0.99**	0.95**	0.94**	-0.99**	0.99**
GSI	0.76**	0.88*	1	-0.75**	0.77**	0.76**	-0.98*	0.98**
AP (first)	-0.80**	-0.94**	-0.75**	1	0.99**	0.99***	-0.99***	0.99*
AP (second)	0.81**	0.95**	0.77**	-0.99**	1	0.99**	-0.99**	0.99**
α - A (first)	0.80**	0.94**	0.76**	-0.99**	0.99**	1	-0.99**	0.99**
α - A (second)	0.80**	0.93**	0.73**	-0.99**	0.99**	0.99**	1	-0.98**
CSP	-0.81**	-0.94**	-0.78**	0.98**	-0.99**	-0.99**	-0.98**	1

*** p- value <0.001; ** p- value < 0.01; *p- value <0.05; ^{ns} not significant.

Table 8: Pearson correlation for 8 variables evaluated for the transgenerational parental effect of CO_2 on seeds and Seedlings of quinoa. Crop year 19/20 below diagonal and Crop year 20/21 above diagonal.

	G%	***FGC(%)***	***GSI***	***AP (first)***	***AP (second)***	***α - A (first)***	***α - A (second)***	***CSP***
G%	1	0.90*	0.90*	0.97*	-0.87*	-0.87	0.88*	0.82*
FGC(%)	0.60^{ns}	1	0.99***	0.98**	-0.99**	0.99**	0.99**	0.98**
GSI	0.98***	0.72^{ns}	1	0.98**	-0.99	-0.99**	0.99**	0.98**
AP (first)	0.93**	0.82*	0.98**	1	-0.99**	-0.99	0.99***	-0.99**
AP (second)	0.99***	0.59^{ns}	0.98**	0.93**	1	0.99***	-0.99**	0.99***
α - A (first)	-0.93**	-0.83*	-0.98***	-0.99***	-0.92**	1	0.99***	-0.99***
α - A (second)	0.93**	0.83*	0.98**	0.99***	0.93**	0.99***	1	-0.98**
CSP	-0.91**	-0.86*	-0.97**	-0.99***	-0.91*	-0.99**	-0.99**	1

*** p- value <0.001; ** p- value < 0.01; *p- value <0.05; ^{ns} not significant.

4. DISCUSSION

Seed germination and seedling development are considered critical phases in its life cycle. Several environmental factors such as temperature, water content, climate, soil and photoperiod have been reported in these processes (TEIXEIRA, et al., 2021). However, some authors (ZISKA & BUNCE, 1993; ANDALO et al., 1996; LEISHMAN et al., 1999; MOHAN et al., 2004; CLASSEN et al., 2010; MARTY; BASSIRIRAD, 2014) also reported little empirical evidence that e[CO_2] can have a 'direct' impact on a germinating seed, but like parental responses, this direct effect on germination is quite inconsistent between species.

Despite seed germination and seedling development being under genetic control (BAY et al., 2003; MARTY; BASSIRIRAD, 2014), parental environment such as availability of light, temperature, water, and nutrients might significantly influence the initial process. Characteristics like carbohydrate, crude protein content, gene expression, effects of DNA methylation patterns, histone modifications, hormones, etc. have been investigated as possible mechanisms that transfer parental environmental effects to the next generation (WULFF, 1986; TILLMAN-SUTELA et al., 1996; JOHANNES et al., 2009; BOYKO and KOVALCHUK, 2011 SCOVILLE et al., 2011; LAMICHHANE et al., 2018; MIRZAEI; HEMAYATI, 2021).

Both species were responsive to transgenerational parental effect of e[CO2], unlike some plants (*Arabidopsis thaliana* L; *Campanulastrum americanum*; *Austrodanthonia caespitosa)* which demonstrate negative effects on seed germination (ANDALO et al., 1996; GALLOWAY and ETTERSON, 2007; HOVENDEN et al., 2008). Regardless of the fact that amaranth and quinoa plants had not had an expressive effect on G%, our result elucidated that seeds obtain of plants cultivated under e[CO_2] may show higher initial growth (Figure 15 B and C).

Considering that the germination test may be less sensitive, the GSI should be considered to determine vigor. It is important to notice that the increase observed in FGC% and GSI may have altered the first metabolic activity that occurs together with seed rehydration. Respiratory rate might have been changed due to the parental effect of the plant cultivated under e[CO_2]. Besides that, this activity is reflected in the optimization of the activity and integrity of the mitochondria of viable embryos (BEWLEY and BLACK 1994). This was caused possibly due to an increase in water

absorption, causing a faster reactivation of metabolism (BEWLEY & BLACK, 1994; MARCOS FILHO, 2005).

The supply of metabolites for respiration and embryo growth of germinating seeds is based almost exclusively on the reserves existing in the seed. Crude protein and starch are quantitatively the material of most abundant storage in most seeds and this reserve is predominantly degraded by hydrolytic enzymes, such as α-amylase (DUA; SAWHNEY, 1991).

Seeds act as nitrogen sinks in the plant, the process of accumulating storage during seed development in the middle and late maturation stages, is crucial to determine crude protein (MÜNTZ et al., 2001). These reserves are essential for seed germination and seedling establishment (ERBAŞ et al., 2016; GOYOAGA et al., 2011; GU et al., 2016). In spite of that, when plants growing in ambient under e[CO_2], photosynthetic machinery is faster, causing a reduction in the nitrogen concentration and protein content of the seeds Chen et al., 2015). Our result shows us a decreased of CSP; however, the mechanism that how every plant responds to the seed's crude protein content is not clarified.

When it comes to enzymatic activity, we can infer that the changes related in this research on germination parameters are related to the activity of hydrolytic enzymes. They are involved in mobilizing reserves for embryo growth and subsequent radicle protrusion (BASKIN & BASKIN, 1998). Acid phosphatase participates in ester hydrolysis reactions and is also involved in the maintenance of cellular phosphate and its activity can affect the phosphate metabolism in seeds. Increases in these enzymes are normally caused by deterioration (CAMARGO et al. 2000).

A booster effect of triose phosphate synthesis is a direct effect of e[CO_2] in plants and posteriorly on seeds (POORTER, 2012). This is justified by the difference in results obtained when we compare seeds under e[CO_2] with seeds obtained of plants cultivated in 400ppm of CO_2.

α-Amylase has high activity at the beginning of the germination process and with the passage of time, it decreases. This trend is due to the reduction in the starch content as germination progresses (MAYER and POLJAKOFF-MAYBER, 1975; BEWLEY and BLACK, 1994). Nevertheless, our result demonstrated the opposite effect. As it was explained previously, a transgenerational parental effect of e[CO_2] promoted an increase in water absorption. The Pearson correlation (Table 7 and 8) suggests that the values of CSP are related to this event.

5. CONCLUSION

Plants grown under e[CO_2], such as amaranth and quinoa, have demonstrated a transgenerational parental effect on their progenies.

Acting as a reserve substance and catalyzing chemical reactions, the alterations in the germination parameters may have possibly happened due to the decrease in the protein content. These results impact enzymatic activity and the physiological quality of seedlings from both cultures.

REFERENCES

ANDALO, C. et al. Elevated CO_2 decreases seed germination in Arabidopsis thaliana. **Global Change Biology**, v. 2, n. 2, p. 129-135, 1996.

AOAC - Association of official agricultural chemists. Official methods of analysis. 10 ed. Editorial Board. Washington. 1965, 909 p. Disponível em: http://www.aoac.org/ Acesso em 20.03.2022.

BAI, Y. et al. Variations in germination and grain quality within a rust resistant common wheat germplasm as affected by parental CO2 conditions. **Environmental and Experimental Botany**, v. 50, n. 2, p. 159-168, 2003.
BASKIN, C. C.; BASKIN, J. M. **Seeds: ecology, biogeography, and evolution of dormancy and germination**. Elsevier, 1998.

BOYKO, A.et al. Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of Dicer-like proteins. **PloS one**, v. 5, n. 3, p. e9514, 2010.

BRASIL. Ministério da Agricultura e reforma Agrária. **Regras para análise de sementes.** Brasília: SNDA/DNDV/CLAV, 399 p. 2009.

CAMARGO, M. L. P. et al. Atividade enzimática em plântulas de *Eucalyptus grandis* provenientes de sementes envelhecidas artificialmente e naturalmente. **Ciência Florestal**, v. 10, n. 2, p. 113-122, 2000.

CLASSEN, A. T. et al. Climate change alters seedling emergence and establishment in an old-field ecosystem. **PLoS One**, v. 5, n. 10, p. e13476, 2010.

COMIRAN, M. et al. Toxidez por cobre: reflexos na qualidade de sementes e no desenvolvimento inicial de aveia preta. 2017.

FIPKE, M. V. et al. Transgenerational Effect of Drought Stress and Sub-Lethal Doses of Quizalofop-p-ethyl: Decreasing Sensitivity to Herbicide and Biochemical Adjustment in *Eragrostis plana*. **Agriculture**, v. 12, n. 3, p. 396, 2022.

GALLOWAY, L. F.; ETTERSON, J. R. Transgenerational plasticity is adaptive in the wild. **Science**, v. 318, n. 5853, p. 1134-1136, 2007.

HAN, C.; YANG, P. Studies on the molecular mechanisms of seed germination. **Proteomics**, v. 15, n. 10, p. 1671-1679, 2015.

HOVENDEN, M. J. et al. Warming and elevated CO_2 affect the relationship between seed mass, germinability and seedling growth in *Austrodanthonia caespitosa*, a dominant Australian grass. **Global Change Biology**, v. 14, n. 7, p. 1633-1641, 2008.

HU, S. et al. Response of rice growth and leaf physiology to elevated CO_2 concentrations: A meta-analysis of 20-year FACE studies. **Science of The Total Environment**, v. 807, p. 151017, 2022.

JIMÉNEZ-ALFARO, B. et al. Seed germination traits can contribute better to plant community ecology. **Journal of Vegetation Science**, v. 27, n. 3, p. 637-645, 2016.

JOHANNES, F. et al. Assessing the impact of transgenerational epigenetic variation on complex traits. **PLoS genetics**, v. 5, n. 6, 2009.

LAMICHHANE, J. R. et al. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. **Plant and soil**, v. 432, n. 1, p. 1-28, 2018.

LEISHMAN, M. R.; SANBROOKE, K. J.; WOODFIN, R. M. The effects of elevated CO_2 and light environment on growth and reproductive performance of four annual species. **The New Phytologist**, v. 144, n. 3, p. 455-462, 1999.

LI, J. et al. Seed traits and germination of native grasses and invasive forbs are largely insensitive to parental temperature and CO_2 concentration. **Seed Science Research**, v. 28, n. 4, p. 303-311, 2018.

LUZURIAGA, A. L.; ESCUDERO, A.; PÉREZ-GARCÍA, F. Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (*Cruciferae*). **Weed research**, v. 46, n. 2, p. 163-174, 2006.

MARTÍNEZ-VILLALUENGA, C.; PEÑAS, E.; HERNÁNDEZ-LEDESMA, B. Pseudocereal grains: Nutritional value, health benefits and current applications for the development of gluten-free foods. **Food and Chemical Toxicology**, v. 137, 2020.

MARTY, Charles; BASSIRIRAD, Hormoz. Seed germination and rising atmospheric CO_2 concentration: A meta-analysis of parental and direct effects. **New Phytologist**, v. 202, n. 2, p. 401–414, 2014.

MAYER, A. M. e POLJAKOFF-MAYBER, A. The germination of seeds. Oxford: Pergamon Press. 2.ed. 1975. 192p

MIRZAEI, M. R.; HEMAYATI, S. S.. The Effect of Environment and Maternal Plant on Germination Traits of Sugar Beet Seeds and an Approach to Select the Superior Genotype. **Agricultural Research**, p. 1-7, 2021.

MOHAN, J. E.; CLARK, J. S.; SCHLESINGER, W. H. Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO_2. **Global Change Biology**, v. 10, n. 2, p. 233-247, 2004.

PIRZADAH, T. B.; MALIK, B. Pseudocereals as super foods of 21st century: Recent technological interventions. **Journal of Agriculture and Food Research**, v. 2, p. 100052, 2020.

POORTER, H. et al. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. **Functional Plant Biology**, v. 39, n. 11, p. 839-850, 2012.

SABELLI, P. A. Seed development: a comparative overview on biology of morphology, physiology, and biochemistry between monocot and dicot plants. **Seed development: OMICS technologies toward improvement of seed quality and crop yield**, p. 3-25, 2012.

SANTOS, M. M. et al. Germinação e alteração de reservas em sementes de amendoim-bravo (*Pterogyne nitens*) sob estresse térmico. **Revista Brasileira de Biociências**, v. 17, n. 1, 2019.

SCOVILLE, A. G. et al. Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. **New Phytologist**, v. 191, n. 1, p. 251-263, 2011.

SPEHAR, C. R. Adaptação da quinoa (Chenopodium quinoa Willd.) para incrementar a diversidade agrícola e alimentar no Brasil. Cadernos de Ciência & Tecnologia, Brasília-DF, v. 23, n. 1, p. 41-62, 2006.

SPEHAR, C. R.; Quinoa: alternativa para a diversificação agrícola e alimentar. Planaltina, DF: Embrapa Cerrados, p. 71-103, 2007.

TILLMAN-SUTELA, E. et al. Lipids and their seasonal variation in the nucellar layers of Scots pine (*Pinus sylvestris*) seeds. **Canadian Journal of Botany**, v. 74, n. 9, p. 1416-1424, 1996.

WULFF, R. D. Seed size variation in *Desmodium paniculatum*: III. Effects on reproductive yield and competitive ability. **The Journal of Ecology**, p. 115-121, 1986.

ZISKA, L.; BUNCE, J. A. The influence of elevated CO_2 and temperature on seed germination and emergence from soil. **Field Crops Research**, v. 34, n. 2, p. 147-157, 1993.

CHAPTER 4

Flooding conditions alter the redox state and the metabolism of pseudocereals

1. INTRODUCTION

Extreme weather events have been occurring more and more frequently. These phenomena include: (1) changes in the atmosphere increasing UV radiation and greenhouse gases (GHGs); (2) droughts; (3) increases in temperature (4) changes in precipitation patterns, which can trigger degradation and decreased fertility over time, due to increased erosion, acceleration of the nutrient cycle, intensification of flooding, etc. (HORWATH; KUZYAKOV, 2018; WANG et al., 2020).

There is growing evidence that these changes and impacts are leading to an understanding of the potential pressures on the ability to ensure an adequate food supply for the human population (HATFIELD; WALTHALL, 2014). Therefore, understanding how plants behave in the face of these changes is important, especially when considering food safety and food supply.

Pseudocereals such as *Amaranthus cruentus* (Amaranth) and *Chenopodium quinoa* Willd (Quinoa), both dicotyledonous, herbaceous and components of the Amaranthaceae family, have C_4 and C_3 metabolism, respectively. These species have been extensively studied for their ability to develop in environments of high temperature, drought and salinity, since both cultivars present an exceptional nutritional value (ABUGOCH, 2009, RUIZ et al., 2014; JAYME-OLIVEIRA et al., 2017; DA SILVA et al., 2021) and have been important food sources for humans and animals in the Andes for millions of years (HARIADI et al., 2011).

Currently, it is only possible to find one single research concerning amaranth conducted in Russia. Balakhina and collaborators (2019) show us that amaranth is sensitive to hypoxia combined with nighttime low temperatures. When it comes to quinoa, only three papers explore quinoa responses to flooding and irrigation regime. There is a paper that shows alteration in matter partitioning, performed in Argentina (GONZÁLEZ et al., 2009); different irrigation intervals were evaluated in Saudi Arabia where the research focus was on growth. Yield results indicated that they are not affected by the irrigation regime (ALGOSAIBI et al., 2017); the last analysis was

conducted in Brazil and elucidated that quinoa has a high recovery capacity, especially when flooding stress occurs at vegetative stage (KOLESNY et al., 2017). The incipience of research with these plants demands more attention in stress tolerance studies for flooding, in order to help researchers with the identification of routes or genes to genetic enhancement of these crops.

It is important to highlight that in Brazil, the presence of lowlands ecosystems, formed by plains of rivers, lakes and lagoons, have a common characteristic: formation in varied conditions of drainage deficiency (alluvial and hydromorphic soils), resulting in flooding or waterlogging. In Rio Grande do Sul, these ecosystems occupy extensive areas, representing about 13 million hectares of the total area of the State. Relief varies from flat to gently undulated, being found in the Internal and External Coastal Plains and on the Coast South, mainly along the Patos and Mirim Lagoons, in the plains of the Central Depression rivers, such as the Sinos, Taquari, Caí and Jacuí rivers, and in the Campanha and West Frontier region, along the Ibicuí, Santa Maria, Quaraí rivers and other smaller ones (Figure 9), generally at low altitudes (0-200 m) (STRECK, et al.; FLORES; SCHNEIDER, 2018).

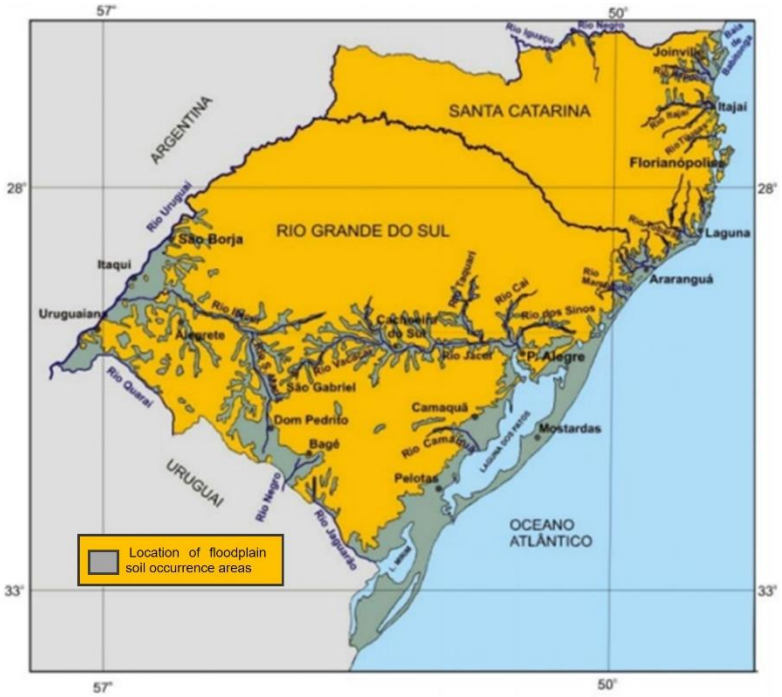

Figure 17: Location of floodplain soil occurrence areas in Rio Grande do Sul and Santa Catarina (adapted from IBGE, 1986) In: STRECK, et al.; FLORES; SCHNEIDER, 2018).

During flooding, the first event that takes place is soil water saturation, which characterizes this event. However, the mechanisms which trigger the response are

often presumed by-products of root zone flooding like variations in soil pH and redox potential. As soil becomes reduced, the amount of iron and iron oxides also diminishes and a modification of proton (i.e. pH) and cation balances occurs. The partial pressure of CO_2 may buffer carbonate thus lowering pH. Moreover, the pH modifications may have undesirable effects through aluminum, manganese or iron toxicity, calcium deficiency, reduced mineralization, or reduced turnover of organic soil matter, reflecting in altered plant metabolism (DAT et al., 2004).

Because they are sessile, flooding stress caused by increases in the precipitation constrains plants cultivation in new environments. As a result, plants have developed several adaptive strategies to deal with the challenges posed by flooding. Though, we can consider that this depends on the type, severity, and combination of environmental traits because the stresses require specific and flexible combinations of signaling components to trigger adaptation and acclimatization responses (BONARES et al., 2020; BERTERO, 2021).

The most detrimental physiological effect of flooding is a drastic decline in the diffusion of O_2 and CO_2 molecules available to the plant (SOLTANI et al., 2017). The first sign of damage on plants is the reduction in the production of ATP generated by anaerobic metabolism (hypoxic). Next, cytosol acidification inhibiting aquaporin (POSSO et al., 2020) happens, and as a result reduced water transport from roots may cause a decrease in the speed of all metabolic processes in the plant, with direct effects on stomatal conductance (stomata closure), photosynthesis and transpiration (HE et al., 2018; SOLTANI et al, 2018; BARICKMAN et al., 2019). Degradation of pigments, such as chlorophyll (a and b) and carotenoids (GARCIA et al., 2020; POSSO et al., 2018; FLOREZ-VELASCO et al., 2015; KUMAR et al., 2013) has been observed, as much as inhibition in growth rates (DA-SILVA and DO AMARANTE, 2020).

The damage in the photosynthetic apparatus due to the over-reduction caused by an imbalance between the two phases of photosynthesis causes the generation of reactive oxygen species (ROS), which induces or active the antioxidant system (catalase, ascorbate peroxidase and superoxide dismutase), the production of hydrogen peroxide and lipid peroxidation because of an increase in the electrolyte leakage (PARADISO et al., 2016).

During flooding, limited oxygen availability shifts the metabolism of plants towards a less efficient pathway of energy production. Consequently, increased levels of pyruvate accumulated due to glycolysis may enhance the fermentative enzymes

(BORELLA et al., 2019; GOYAL; KAUR; KAUR,2020). Furthermore, flooding may cause a large amount of carbohydrate consumption in plants, leading to energy shortage (QI et al., 2020). Therefore, the status of the sugar reserve and the activities of sucrose hydrolyzing enzymes are important determinants of flooding tolerance in crop plants (HOSSAIN and UDDIN 2011). As these climatic factors will change simultaneously, understanding how plants will respond and adapt to a new environment due to the increases in precipitation is an essential first step to understanding the full impact that multiple climate change factors, such as flooding, will have on plant crops (LEAKEY et al., 2009; SILVA; HANSSON; JOHANSSON, 2021). Therefore, the aim of this work was to evaluate the effects of flooding and recovery conditions on the physiology and metabolism of leaves and roots of amaranth and quinoa plants.

2. MATERIAL AND METHODS

Plant material and growth conditions

The experiment was carried out in greenhouse belonging to the Department of Botanic of the Federal University of Pelotas, located in the municipality of Capão do Leão - RS. The internal temperature of the greenhouse was monitored daily using a data logger (AK172 Akso).

Seeds of the cultivar BRS Alegria (amaranth) and BRS Piabiru (quinoa) were seeded in 8-L polyethylene pots filled with soil, which was previously analyzed for its physical and chemical attributes, amended and fertilized according to technical recommendations (EMBRAPA 1999), keeping three plants per pot after the complete establishment of the plants.

Plants were subjected to soil flooding on December 26, when started the plant transition period between vegetative and flowering. The evaluations and sampling occurred after 48 and 96 hours of flooding and during the recovery at 72 hours after the soil drainage. During harvesting, roots were carefully washed and homogeneous and leaves that were used to carry out the gas exchange were harvested, weighed and stored frozen (-80 °C) until biochemical analysis.

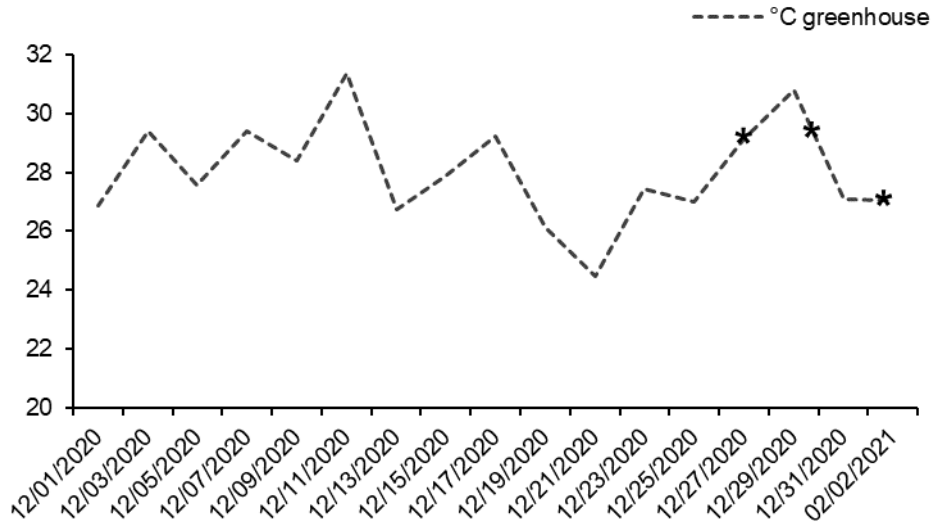

Figure 18: Greenhouse average indoor ambient temperature during the month of December 2020/January 2021. *Evaluations during the transition period between vegetative and flowering.

Table 9: Physical and chemical characteristics of the soil collected in the experimental area of Palma, Campus Capão do Leão, UFPel (2020/2021).

pH H_2O 01:01	Ca^{2+}	Mg^{2+}	Al^{3+}	Effective CEC	% SOC	% Clay	Clay Class	K^+	P^*
	$cmol_c\ dm^{-3}$				m/v			$mg\ dm^{-3}$	
5.2	2.3	1.7	0.2	4.4	1.93	18	4	33	10.7

* Phosphorus(P) was extracted using Mehlich solution

Extraction and quantification of photosynthetic pigments: The photosynthetic pigments were quantified according to the methodology proposed by Wellburn (1994). For this purpose, leaf discs of two young, expanded leaves were used, in four repetitions per treatment. The leaves were cut into small segments, using 0.01 g of fresh sample inserted into test tubes containing 3.5 mL of dimethylsulfoxide (DMSO) neutralized with 5% calcium carbonate. Then, the tubes were incubated in a water bath at a temperature of 65ºC for 1 hour, protected from light and then cooled in the dark until reaching room temperature. After, absorbance readings at 480 nm, 649 nm and 665 nm were taken in Molecular Devices spectrophotometer. Chlorophyll a, b, total and carotenoid contents were calculated based on the equations: *Chlorophyll a* =

(12.47 x A665) - (3.62 x A649); *Chlorophyll b* = (25.06 x A649) - (6.5 x A665); The results were expressed in mg g^{-1} FW.

Leaf gas exchange: The analysis leaf gas exchange was performed using an IRGA infrared gas analyzer (LI6400, Licor), the evaluation was carried out between 8:30 and 10:00 AM. The concentration of CO_2 in the chamber was matched for each treatment (400 and 700ppm) and the photon flux density was regulated to 1500 µmol of photons m-2 s^{-1} with a light source attached to the measuring chamber. Net CO_2 assimilation (*A*), Stomatal conductance (g_s), Internal concentration of CO_2 (*Ci*) and Transpiration rate (*E*) were measured in the middle third of the youngest expanded leaf.

Antioxidant enzymes activity: To determine the activity of antioxidative enzymes, leaves and roots (±0.2 g) were ground powdered in liquid nitrogen using a mortar and pestle and homogenized with 30% (w:v) polyvinylpyrrolidone (PVPP) and 100 mM potassium phosphate buffer, pH 7.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 20 mM ascorbic acid (AsA). Then, the homogenate was centrifuged at 12.000 g at 4 °C for 20 min, and the supernatant was used to determine the enzyme activity. Protein was determined by the method of Bradford (1976).

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by monitoring the inhibition of the nitro blue-tetrazolium (NBT) (GIANNOPOLITIS and RIES, 1977) coloration in a reaction medium consisting of enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 0.1 µM EDTA, 75 µM NBT and 2 µM riboflavin at 560 nm.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed through ascorbate oxidation at 290 nm following the method of Nakano and Asada (1981). The assay mixture contained crude enzyme extract, 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM AsA and 0.1 mM hydrogen peroxide (H_2O_2).

Catalase (CAT; EC 1.11.1.6) activity was assayed by monitoring the decline in the absorbance at 240 nm following the method of Azevedo et al. (1998). The incubation mixture contained crude enzyme extract, 100 mM potassium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2.

H_2O_2 content and lipid peroxidation: Leaves and roots (0.2 g) were ground with 0.1% (w/v) trichloroacetic acid (TCA) using a mortar and pestle. The homogenate was centrifuged at 12,000 g at 4 °C for 20 min and the supernatant was used to

determine the H_2O_2 content according to Velikova et al. (2000) which was samples were incubated in a reaction medium containing 10 mM potassium phosphate buffer (pH 7.0) and 1.0 M potassium iodide at 30 °C for 10 min. The absorbance was measured at 390 nm. A calibration curve was obtained with H_2O_2 standard. Lipid peroxidation was determined using thiobarbituric acid (TBA) according to Cakmak and Horst (1991) which determines malondialdehyde (MDA) as an end-product of lipid peroxidation. The molar extinction coefficient (ε=155×103 M^{-1} cm^{-1}) was used to calculate the amount of MDA–TBA complex (red pigment).

Determination of sucrolytic enzymes: Leaf samples (0.4 g) were ground until a fine powder was obtained in the presence of liquid N_2. The extraction of the neutral/alkaline invertase (CINV) and the acid invertase enzymes (CWINV and VINV) followed the methodology described by Zeng et al. (1999), with minor modifications. In each sample, 1.5 mL of extractor medium containing potassium phosphate buffer (200 mM, pH 7.5), PMSF (1 mM), $MgCl_2$ (5 mM), DTT (1 mM) and ascorbic acid (50 mM) was added and then centrifuged 18,000 × g for 20 min at 4°C. The supernatant solution was collected to measure soluble invertase activity (VINV and CINV) and the precipitate was collected to measure insoluble invertase (CWINV). In addition to the reagents used for the soluble invertases, NaCl (1 M) and Tritonne-X-100 (1%) were also added for CWINV. The enzyme extract (500 µL) was added to 1000 µL assay medium containing 500 µL sodium acetate buffer (pH 4.5 for VINV and CWIN activity and pH 7.5 for CINV activity), 200 mM sucrose and 5 mM $MgCl_2$. The incubation temperature was 37°C, and 200 µL aliquots were collected after 10 and 40 min to determine enzymatic activity. Enzymatic activity was evaluated by quantifying reducing sugars produced according to the dinitrosalicylic acid (DNS) method described by Miller (1959). All enzyme activities were determined in triplicate and expressed in micromoles of glucose per gram of fresh weight per min (µmol glucose g^{-1} FW min^{-1}).

Experimental design: The experimental design used was completely randomized, consisting of three plants per pot (experimental unit) and 10 replicates per treatment. The data obtained were analyzed for homoscedasticity by the Bartlett test and for normality by the Shapiro Wilk test, and considering the assumptions, the analysis of variance (ANOVA) was carried out using the statistical software R (www.r-project. org/). Afterward, when F was significant, the means of flooded or recovery were compared to the control by the t-test ($P \leq 0.05$).

3. RESULTS

Photosynthetic pigments

The results presented in Figure 19 demonstrated a reduction in photosynthetic pigments in amaranth and quinoa plants. Quinoa plants had a significant difference (p≤0.05) for CHL*b* (Figure 19B) in 48h of flooding. However, this decrease was more apparent in 96 hours of flooding to amaranth and other pigments. During the recovery, the levels of CHL*a* and CHL*b* for both crops were near to the control, while the carotenoids still presented low levels in quinoa plants.

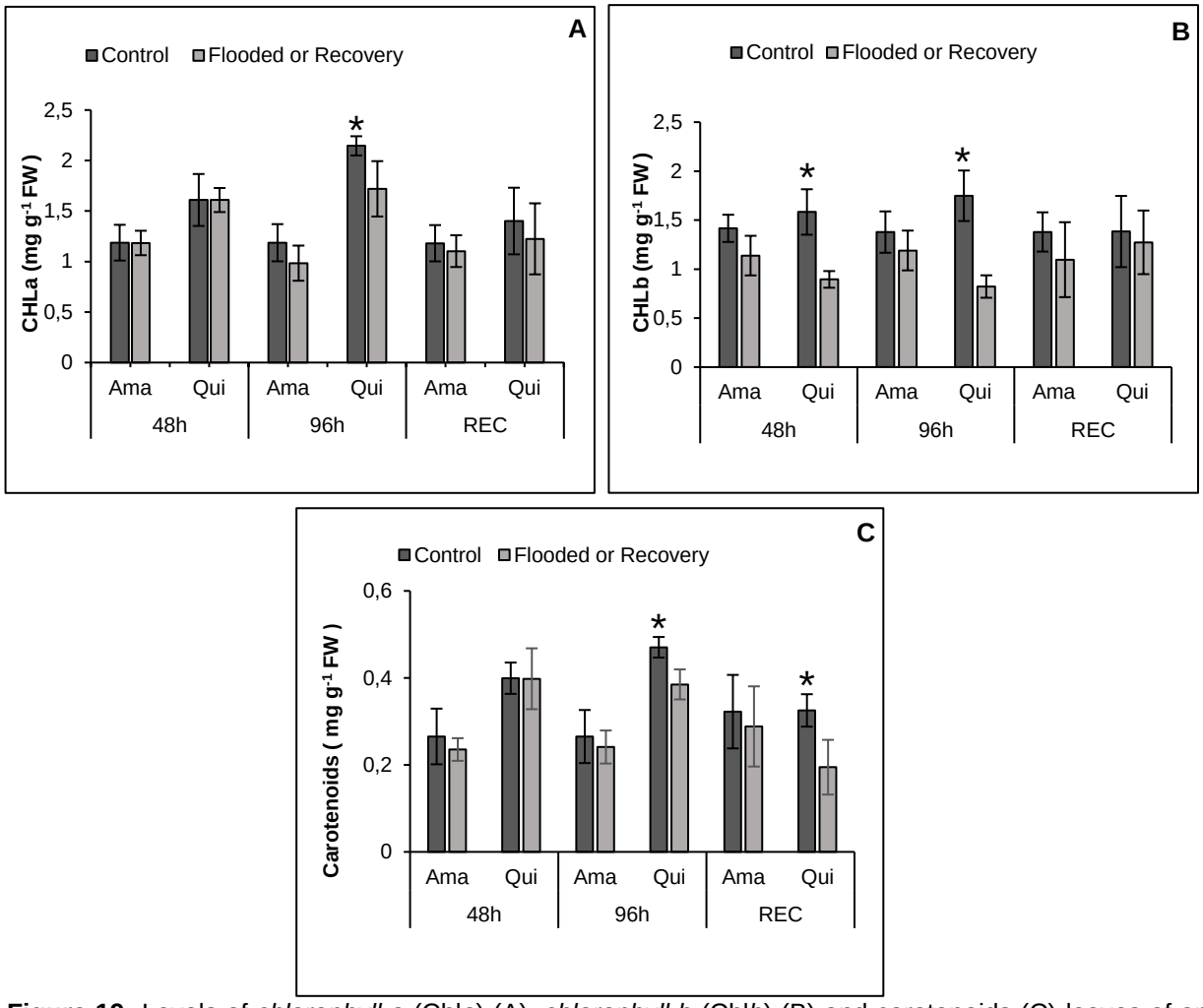

Figure 19: Levels of *chlorophyll a* (Chl*a*) (A), *chlorophyll b* (Chl*b*) (B) and carotenoids (C) leaves of amaranth and quinoa plants in transition stadium between vegetative and flowering subjected to flooding of the root system. Ama corresponds to Amaranth plants and Qui corresponds to Quinoa plants. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

Leaf gas exchange

Flooding mostly decreased leaf gas exchange in amaranth and quinoa plants (Figure 20). The *A* was lower just for quinoa plants subjected to flooding for 48 and 96h (Figure 20A). The same situation was observed after 72 hours of recovery.

Significant changes in stomatal conductance (Figure 20B) were observed for amaranth and quinoa at 48h. However, at 96h of flooding and after the recovery period, only quinoa demonstrated alterations. The same trend was observed for Ci (Figure 20C). Transpiration rate (Figure 20D) shows us an interesting result. Only quinoa plants presented a significant result during flooding and recovery periods.

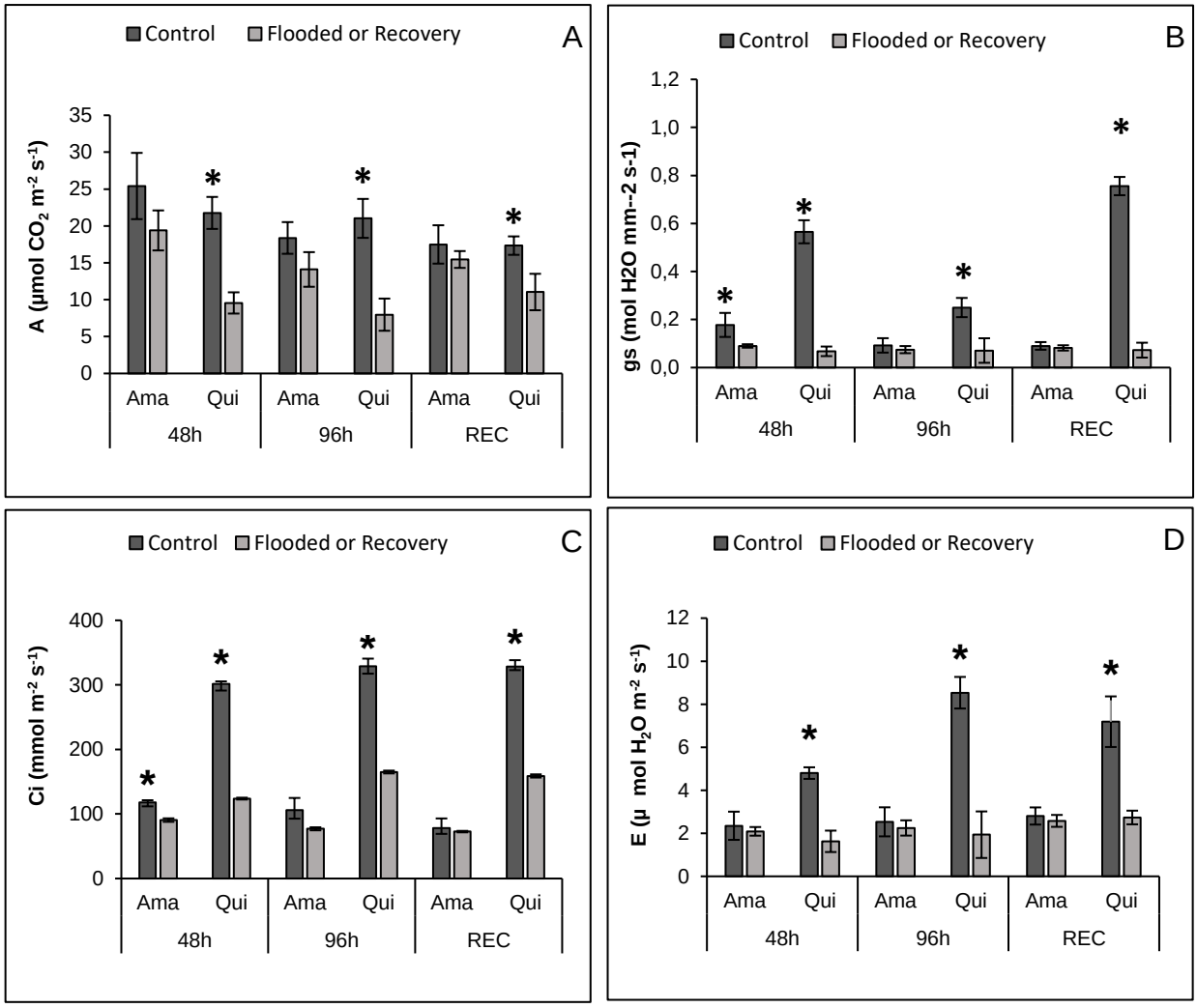

Figure 20: Gas exchange in leaves of amaranth and quinoa plants in transition stadium between vegetative and flowering subjected to flooding of the root system. (A) Net CO_2 assimilation; (B) Stomatal conductance; (C) Internal concentration of CO_2 and (D)Transpiration rate. flooding of the root system. Ama corresponds to Amaranth plants and Qui corresponds to Quinoa plants. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

Antioxidant enzymes activity

In leaves, SOD (Figure 21A), was more affected in quinoa plants than amaranth as of 96H and during the recovery. The activity of SOD had a significant (P≤0.05) increase in roots at 48H for both crops, while at 96H this result was observed just to quinoa. During recovery, there were no differences between control and flooded in the root, only in leaves. The activity of APX (Figure 21C, 21D) was modified condition-manner for leaves and roots of amaranth and quinoa. The activity of CAT (Figure 21E, 21F) had a significant (P≤0.05) increase in leaves and roots most of the time in flooded conditions. At 48H, 96H and during the recovery amaranth plants demonstrated a higher activity than control, while roots of quinoa plants had no difference at 72h of recovery.

H_2O_2 content and Lipid Peroxidation

The H_2O_2 content (Figure 22) in leaves (Figure 22A) showed a distinct behavior for each crop. Amaranth plants had higher levels of hydrogen peroxide in leaves during all those periods that were evaluated. This increase was observed on quinoa plants from 96h and during the recovery. On the other hand, roots of amaranth had a significant increase in H_2O_2 content (Figure 22B) amid recovery, while quinoa plants showed alterations over those periods. In spite of that, MDA was increased for both species during the all period.

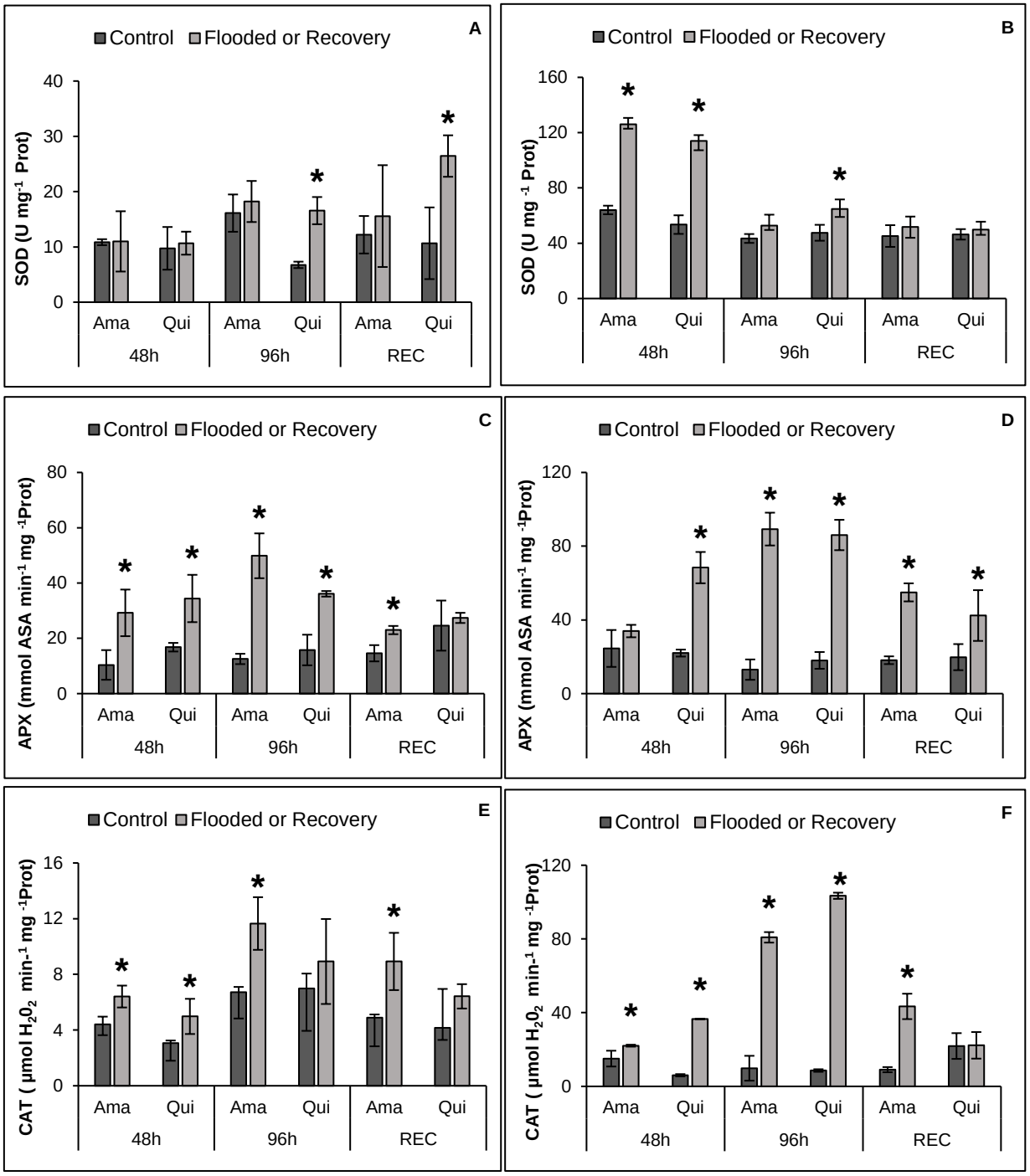

Figure 21: Activity of superoxide dismutase (SOD) (A, B); ascorbate peroxidase (APX) (C, D); catalase (CAT) (E, F) in leaves (A, C, E) and roots (B, D, F) of amaranth and quinoa plants in transition stadium between vegetative and flowering subjected to flooding of the root system. Ama corresponds to Amaranth plants and Qui corresponds to Quinoa plants Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

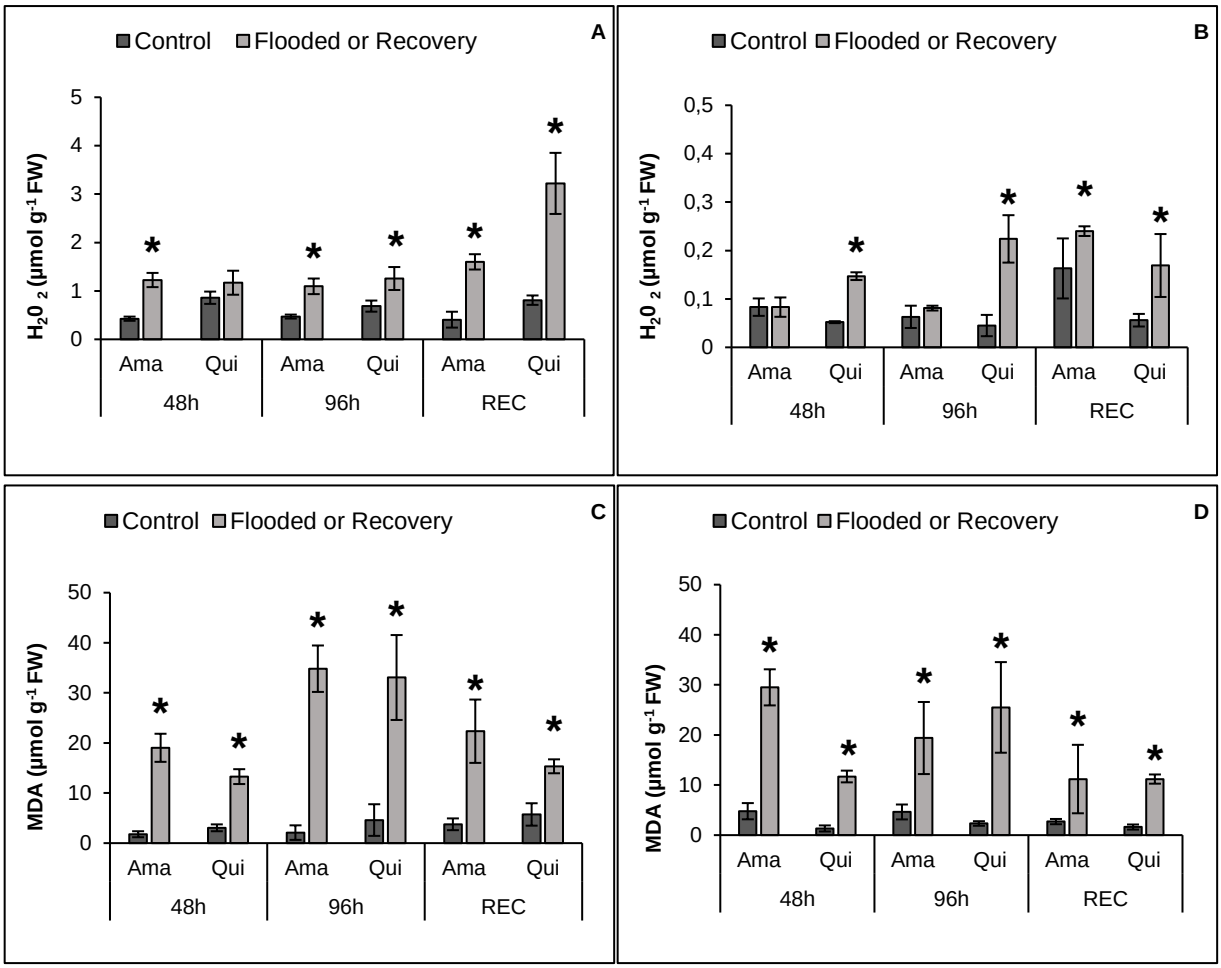

Figure 22: Level of hydrogen peroxide (H_2O_2) in leaves (A) and roots (B); Lipid peroxidation (MDA) in leaves (C) and roots (D) of amaranth and quinoa plants in transition stadium between vegetative and flowering subjected to flooding of the root system. Ama corresponds to Amaranth plants and Qui corresponds to Quinoa plants. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05, n=4).

Sucrolytic enzymes

Analysis of sucrolytic enzymes (Figure 23) showed that quinoa and amaranth plants had the activity of the soluble acid invertases (CINV-23A and VINV-23B) and cell wall acid invertase (CWINV-23C) reduced or nearly interrupted during flooded treatment. Amidst all periods of flooding or even after of recovery, results observed for the control plants were significantly (*P≤0.05; **P≤0.01) higher in the conditions in which the plant had an adequate water regime.

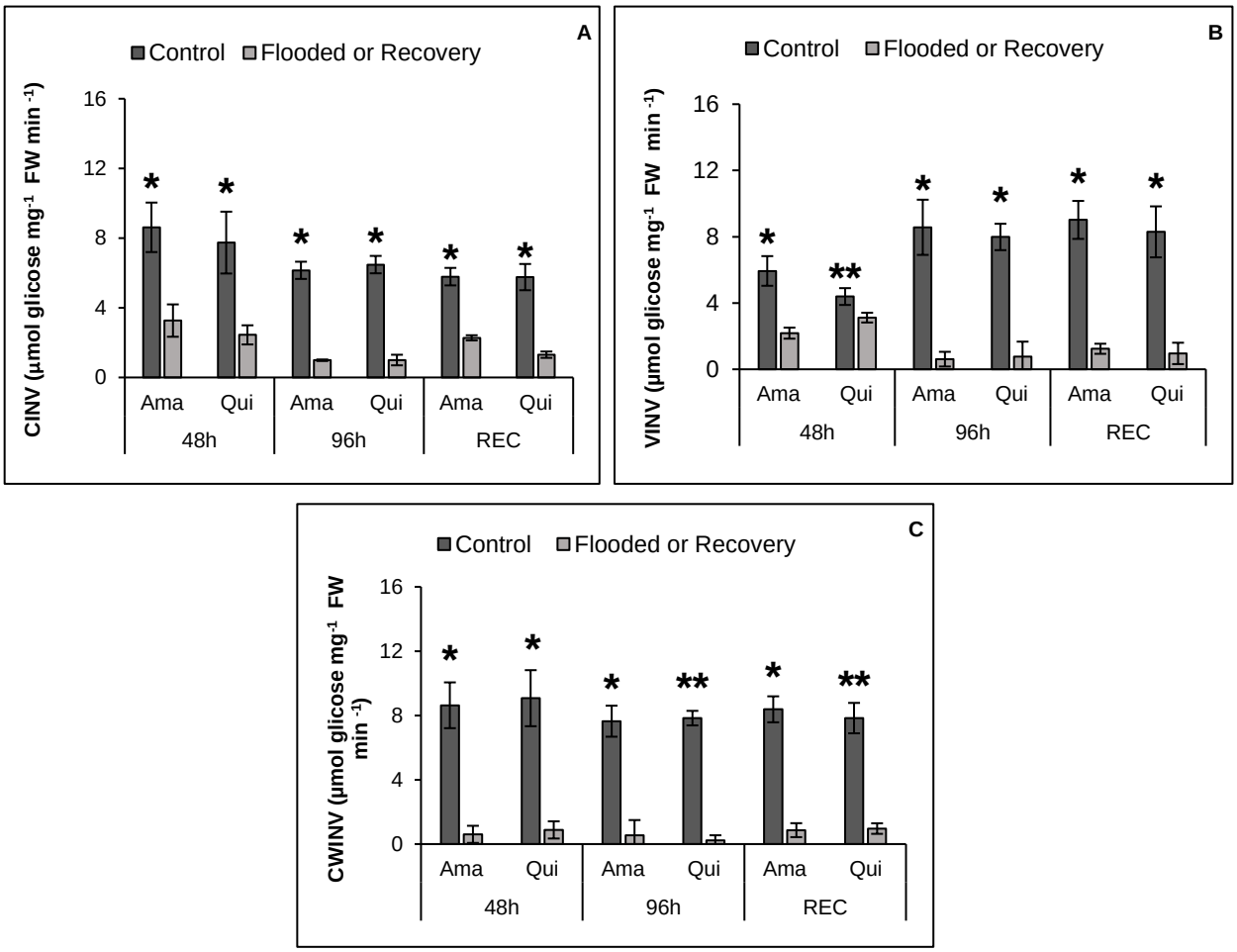

Figure 23: Sucrolytic enzimes - soluble acid invertases of cytosol (CINV–A); soluble acid invertases of vacuole (VINV-B); cell wall acid invertase (CWIV-C) of amaranth and quinoa plants in transition stadium between vegetative and flowering subjected to flooding of the root system. Ama corresponds to Amaranth plants and Qui corresponds to Quinoa plants. Error bars correspond to the 95% confidence interval. *Indicates significant difference by t-test (P≤0.05) **Indicates significant difference by t-test (P≤0.01) n=4.

4. DISCUSSION

Several studies reported results of flooding with considering main crops such as soybean (DA-SILVA and DO AMARANTE, 2020) wheat (DING et al., 2020), common bean (POSSO et al., 2020) and maize (TIAN et al., 2020). Nevertheless, studies about pseudocereals (amaranth and quinoa) and flooding are scarce.

In a growth chamber experiment for amaranth and a greenhouse study for quinoa (BALAKHNINA; GINS; FOMINA, 2019; GONZÁLEZ et al., 2009), some researchers noted a negative impact from flooding conditions in plants on the vegetative and reproductive stadium. They presented a reduction in several parameters such as photosynthetic pigments.

Reductions in chlorophyll content may be related to the ability to reconvert CHL*b* into CHL*a*, an important process during CHL breakdown, which is a common effect when plants are submitted to flooding conditions (DENGIMO, 2022; STASNIK; GROBKINSKY; JONAK, 2022) Several lines of evidence indicate that plants are capable of degrading only *CHLa,* but not *CHLb* (TANAKA; KOBAYASHI; TATSURU MASUDA, 2011). Therefore, the CHL*b*-to-*a* conversion is necessary. On one hand, it is vital to remove the phototoxicity of free CHL molecules that are uncoupled from the light-harvesting, CHL-binding complex proteins (LHCs) of the thylakoids in order to maintain cell viability and thus allow an efficient remobilization of nutrients in senescent organs/tissues. On the other hand, CHL breakdown has been repeatedly shown to be a prerequisite for the degradation of LHCs in senescent leaves and, as such, is important for accessing the second-largest pool of chloroplast nitrogen. In addition, by-products of CHL degradation are re-used for biosynthetic purposes, particularly phytol, which is salvaged into tocopherol during leaf senescence (KUAI; CHEN; HÖRTENSTEINER, 2018).

Because of the increase in the time of excited states of chlorophyll molecules from the reaction center of the photosystem and the reduction in the functionality of the electron transport chain caused by ROS generation in flooding conditions, the limitation of the production of NADPH and the parameters photosynthetic such as the net assimilation rate of CO_2 is heavily affected (POSSO et al., 2018). Here, we can associate the reduction in gas exchange and the transpiration rate duo the fact amaranth and quinoa plants showed stomatal closure induced by a reduction in water

absorption by roots and transport of water through xylem sap to the shoot (BARICKMAN et al., 2019).

The occurrence and deposition of calcium oxalate in leaves enables quinoa plants to retain humidity in leaves (SPEHAR, 2006). Although this characteristic is desirable in plant tolerance to drought (CARBONE-RISI, 1986), it is very damaging when in flooding conditions, as observed in this study (Figure 12).

In addition to the various negative effects of flooding, roots are suddenly exposed to lower oxygen levels resulting in redox imbalance and overproduction of reactive oxygen species (ROS) in mitochondria and chloroplasts. In response to that, activation of SOD (Figure 13), which is considered the first defense line in scavenging the ROS (TAIZ et al., 2017), was observed. In fact, here we had an interesting result. Apparently, at the first time (48h) of the stress condition, the leaves were less affected than the roots. Possibly, the plants opted to destine energy to scavenge H_2O_2 in roots first, due to the acidification of the cell cytosol (BANTI et al., 2013).

ROS levels have not decreased enough to maintain cellular homeostasis. Lipid peroxidation could not be avoided, despite high activities of antioxidant enzymes in leaves and roots. (DA-SILVA and DO AMARANTE, 2020). On the other hand, the transient increase in H_2O_2 levels in roots during reoxygenation can be correlated with the activity of the APX enzyme.

Plants commonly suffer from O_2 deprivation under flooding, as this condition might cause an energy crisis and induce down-regulated energy consumption. Reduced availability of O_2 as the final electron acceptor in the mitochondrial electron transport chain mediates a rapid reduction of the cellular ATP:ADP ratio and adenylate energy charge. In order to avoid this energy crisis, cells start the process of glycolysis and fermentation to generate ATP and regenerate NAD+, respectively (BAILEY-SERRES; VOESENEK, 2008).

In this study, CINV, VINV and CWINV decreased clearly, whereas possible SUS activity in cytosol may have supplied sugars to glycolysis, possibly supplying energy for maintaining the metabolism in plant under flooding conditions. In response to lower levels of O_2, mobilization of carbohydrates changes and improve starch to glucose conversion. Catabolism of sucrose has two distinct pathways in plants and might be changed to maintain an energetic balance. This process might occur unidirectionally and it is essentially irreversible by invertase (CINV, VINV, and CWINV) or by bidirectional UDP-dependent sucrose synthase, a reaction close to equilibrium.

Nevertheless, flooding stress can suppress invertase expression and promote sucrose synthase expression, switching to the more energy-saving pathway (GUGLIELMINETTI et al. 1995; ROLLETSCHEK et al. 2002; KREUZWIESER et al., 2014 WANG et al., 2014).

5. CONCLUSIONS

- Amaranth and quinoa plants showed severe metabolic changes when subjected to flooding in periods of 48 and 96 hours;
- The antioxidant system of this crop may have been more efficient in leaves and roots when compared to quinoa, which makes it possible to infer that amaranth plants would be able to reestablish themselves after a short period of hypoxia (96h);
- Increased on SUS activity was not able to adequately replace invertases in the progress of sucrose cleavage.

REFERENCES

ABUGOCH, L. et al. Stability of quinoa flour proteins (Chenopodium quinoa Willd.) during storage. **International journal of food science & technology**, v. 44, n. 10, p. 2013-2020, 2009.

ABUGOCH -JAMES, L. E. Quinoa (Chenopodium quinoa Willd.): composition, chemistry, nutritional, and functional properties. **Advances in food and nutrition research**, v. 58, p. 1-31, 2009.

ALGOSAIBI, A. M. et al. The effect of irrigation intervals on the growth and yield of quinoa crop and its components. **Journal of Agricultural Science**, v. 9, n. 9, p. 182-191, 2017.

AZEVEDO, R. A. et al. Response from elevated carbon dioxide to air and ozone fumigation in leaves and roots of wild type and a catalase-deficient mutant of barley. **Physiologia Plantarum**, v. 104, n. 2, p. 280-292, 1998.

BAILEY-SERRES, J.; VOESENEK, L. A. C. J. Flooding stress: acclimations and genetic diversity. **Annual review of plant biology**, v. 59, p. 313, 2008.

BALAKHNINA, T. et al. Oxidative stress development in the leaves of *Amaranthus cruentus* L. containing amaranthine under conditions of nighttime low temperatures, soil hypoxia and the combined effects of both stress factors. **Int. Agrophys**, v. 33, p. 511-516, 2019.

BANTI, V. et al. Low oxygen response mechanisms in green organisms. **International journal of molecular sciences**, v. 14, n. 3, p. 4734-4761, 2013.

BARICKMAN, T. C. et al. Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. **Plants**, v. 8, n. 6, p. 160, 2019.

BERTERO, H. D. Quinoa. In: SADRAS, Victor O.; CALDERINI, Daniel (Ed.). **Crop Physiology Case Histories for Major Crops**. Academic Press, p. 251-281. 2020.

BOANARES, D. et al. Oxidative metabolism in plants from Brazilian rupestrian fields and its relation with foliar water uptake in dry and rainy seasons. **Plant Physiology and Biochemistry**, v. 146, p. 457-462, 2020.

BORELLA, J. et al. Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. **Scientia Agricola**, v. 76, p. 51-62, 2019.

CAKMAK, I.; HORST, W. J. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). **Physiologia Plantarum**, v. 83, n. 3, p. 463-468, 1991.

CARBONE-RISI, J. J. M. **Adaptation of the Andean grain crop quinoa for cultivation in Britain** 1986. 123 f. Tese (Doutorado) - University of Cambridge, Cambridge, 1986.

DA-SILVA, C. J.; DO AMARANTE, L. Short-term nitrate supply decreases fermentation and oxidative stress caused by waterlogging in soybean plants. **Environmental and Experimental Botany**, v. 176, p. 104078, 2020.

DA SILVA, P. C. et al. Quinoa for the brazilian cerrado: Agronomic characteristics of elite genotypes under different water regimes. **Plants**, v. 10, n. 8, p. 1591, 2021.

DAT, J. F. et al. Sensing and signalling during plant flooding. **Plant Physiology and Biochemistry**, v. 42, n. 4, p. 273-282, 2004.

DENGIMO, Keme. Effects of Flood on the Growth and Some Aspects of Physiology of Five Genotypes of Cassava (Manihot esculentus). **African Scientist**, v. 21, n. 4, 2022.

DING, J. et al. Effects of waterlogging on grain yield and associated traits of historic wheat cultivars in the middle and lower reaches of the Yangtze River, China. **Field Crops Research**, v. 246, p. 107695, 2020.

FLÓREZ-VELASCO, N.; BALAGUERA-LÓPEZ, H. E.; RESTREPO-DÍAZ, H. Effects of foliar urea application on lulo (Solanum quitoense cv. septentrionale) plants grown under different waterlogging and nitrogen conditions. **Scientia horticulturae**, v. 186, p. 154-162, 2015.

FUKAO, Takeshi; BAILEY-SERRES, Julia. Plant responses to hypoxia–is survival a balancing act?. **Trends in plant science**, v. 9, n. 9, p. 449-456, 2004.

GARCIA, N. et al. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. **Environmental and Experimental Botany**, v. 172, p. 103975, 2020.

GIANNOPOLITIS, Constantine N.; RIES, Stanley K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, v. 59, n. 2, p. 309-314, 1977.

GONZÁLEZ, J. A. et al. Physiological responses of quinoa (*Chenopodium quinoa*) to drought and waterlogging stresses: dry matter partitioning. 2009.

GOYAL, K.; KAUR, K.; KAUR, G. Foliar treatment of potassium nitrate modulates the fermentative and sucrose metabolizing pathways in contrasting maize genotypes under water logging stress. **Physiology and molecular biology of plants**, v. 26, n. 5, p. 899-906, 2020.

GUGLIELMINETTI, L.; PERATA, P.; ALPI, A. Effect of anoxia on carbohydrate metabolism in rice seedlings. **Plant Physiology**, v. 108, n. 2, p. 735-741, 1995.

HARIADI, Y. et al. Ionic and osmotic relations in quinoa (Chenopodium quinoa Willd.) plants grown at various salinity levels. **Journal of experimental botany**, v. 62, n. 1, p. 185-193, 2011.

HATFIELD, J. L.; WALTHALL, C. L. Climate Change: Cropping System Changes and Adaptations. **Encyclopedia of Agriculture and Food Systems**. v. 2, p. 256-565. 2014.

HE, L. et al. The effect of exogenous calcium on cucumber fruit quality, photosynthesis, chlorophyll fluorescence, and fast chlorophyll fluorescence during the fruiting period under hypoxic stress. **BMC plant biology**, v. 18, n. 1, p. 1-10, 2018.

HORWATH, W. R.; KUZYAKOV, YAKOV. The Potential for Soils to Mitigate Climate Change Through Carbon Sequestration. In**: Climate Change Impacts on Soil Processes and Ecosystem Properties**. Chapter 3. p. 61-92. 2018.

HOSSAIN, Mohammad A. et al. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. **Frontiers in plant science**, v. 6, p. 420, 2015.

JAYME-OLIVEIRA, A. et al. Amaranth, quinoa, and millet growth and development under different water regimes in the Brazilian Cerrado. **Pesquisa Agropecuária Brasileira**, v. 52, p. 561-571, 2017.

KOLESNY, V. M. et al. Ação das enzimas antioxidantes em plantas de quinoa submetidas ao alagamento. XXVI Congresso de Iniciação Científica. (Anais) 3ª Semana Integrada UFPEL. 2017.

KREUZWIESER, Juergen; RENNENBERG, Heinz. Molecular and physiological responses of trees to waterlogging stress. **Plant, cell & environment**, v. 37, n. 10, p. 2245-2259, 2014.

KUAI, B.; CHEN, J.; HÖRTENSTEINER, S. The biochemistry and molecular biology of chlorophyll breakdown. **Journal of Experimental Botany**, v. 69, n. 4, p. 751-767, 2018.

KUMAR, P. et al. Yield, growth and physiological responses of mung bean [*Vigna radiata* (L.) Wilczek] genotypes to waterlogging at vegetative stage. **Physiology and Molecular Biology of Plants**, v. 19, n. 2, p. 209-220, 2013

LEAKEY, A. D. B. et al. Elevated CO_2 effects on plant carbon , nitrogen , and water relations: six important lessons from FACE. **Journal of Experimental Botany**, v. 60, n. 10, p. 2859–2876, 2009

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. **Plant and cell physiology**, v. 22, n. 5, p. 867-880, 1981.

PARADISO, A. et al. ROS production and scavenging under anoxia and re-oxygenation in Arabidopsis cells: a balance between redox signaling and impairment. **Frontiers in Plant Science**, v. 7, p. 1803, 2016.

PENG, Y. et al. Molecular and physiological responses in roots of two full-sib poplars uncover mechanisms that contribute to differences in partial submergence tolerance. **Scientific reports**, v. 8, n. 1, p. 1-15, 2018.

POSSO, D. A. et al. Root flooding-induced changes in the dynamic dissipation of the photosynthetic energy of common bean plants. **Acta Physiologiae Plantarum**, v. 40, n. 12, p. 1-14, 2018.

POSSO, D. A. et al. Nitrate-mediated maintenance of photosynthetic process by modulating hypoxic metabolism of common bean plants. **Acta Physiologiae Plantarum**, v. 42, n. 7, p. 1-17, 2020.

QI, X. et al. Sugar enhances waterlogging-induced adventitious root formation in cucumber by promoting auxin transport and signalling. **Plant, Cell & Environment**, v. 43, n. 6, p. 1545-1557, 2020.

ROLLETSCHEK, Hardy et al. Legume embryos develop in a hypoxic environment. **Journal of Experimental Botany**, v. 53, n. 371, p. 1099-1107, 2002.

RUIZ, K. B. et al. Quinoa biodiversity and sustainability for food security under climate change. A review. **Agronomy for sustainable development**, v. 34, n. 2, p. 349-359, 2014.

SILVA, W. T. A. F; HANSSON, M; JOHANSSON, J. Light competition and phenological adaptation of annual plants to a changing climate. **Climate Change Ecology**, p. 100007, 2021

SOLTANI, A. et al. Genetic architecture of flooding tolerance in the dry bean Middle-American diversity panel. **Frontiers in plant science**, v. 8, p. 1183, 2017.

SPEHAR, C. R. Adaptação da quinoa (*Chenopodium quinoa* Willd.) para incrementar a diversidade agrícola e alimentar no Brasil. **Cadernos de Ciência & Tecnologia**, v. 23, n. 1, p. 41-62, 2006.

STASNIK, P.; GROßKINSKY, D. K.; JONAK, C. Physiological and phenotypic characterization of diverse Camelina sativa lines in response to waterlogging. **Plant Physiology and Biochemistry**, v. 183, p. 120-127, 2022.
KREUZWIESER, Jurgen et al. Differential response of gray poplar leaves and roots underpins stress adaptation during hypoxia. **Plant physiology**, v. 149, n. 1, p. 461-473, 2009.

STRECK, E. V. et al.; FLORES, Carlos Alberto; SCHNEIDER, Paulo (Clbs.) **Solos do Rio Grande do Sul**. 3. ed., rev. e ampl. Porto Alegre: Emater/RS-Ascar,2018. 252 p. il. color. ISBN 978-85-98842-20-2.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. **6. ed**. Porto Alegre: ArtMed, 2017. 888 p.

TANAKA, R.; KOBAYASHI, K.; MASUDA, T. Tetrapyrrole metabolism in *Arabidopsis thaliana*. **The Arabidopsis book/American Society of Plant Biologists**, v. 9, 2011.

TIAN, L. et al. Effects of waterlogging stress at different growth stages on the photosynthetic characteristics and grain yield of spring maize (*Zea mays* L.) under field conditions. **Agricultural Water Management**, v. 218, p. 250-258, 2019.

VELIKOVA, V.; YORDANOV, I.; EDREVA, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. **Plant science**, v. 151, n. 1, p. 59-66, 2000.

ZENG, Y. et al. Rapid repression of maize invertases by low oxygen. Invertase/sucrose synthase balance, sugar signaling potential, and seedling survival. **Plant physiology**, v. 121, n. 2, p. 599-608, 1999.

ZHANG, P. et al. Physiological and de novo transcriptome analysis of the fermentation mechanism of *Cerasus sachalinensis* roots in response to short-term waterlogging. **BMC genomics**, v. 18, n. 1, p. 1-14, 2017.

WANG, X. et al. Dissecting the genetic mechanisms of waterlogging tolerance in Brassica napus through linkage mapping and a genome-wide association study. **Industrial Crops and Products**, v. 147, p. 112269, 2020.

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

FINAL CONSIDERATIONS

Anthropogenic activities, which have been intensified after the Industrial Revolution, are now considered the main cause of climate change. The intensification of the effects that would naturally occur has been getting worse. Changes in the frequency of extreme weather events that affect agricultural production have been observed more and more frequently every day.

As CO_2 is the main greenhouse gas and essential component for plant life on earth, it was possible to demonstrate that in this study the theory in which plants with C_4 metabolism would not undergo changes in the face of e[CO_2] needs revision.

In this research, theories addressing changes in growth, photosynthetic and carbohydrate metabolism parameters were confirmed. These are closely linked to sucrolytic enzymes, since they affect the source-sink relationship and nutritional dynamics.

On the other hand, some new perspectives were observed. Differences in secondary metabolism increase, in face to possible alterations in the cyclic electron flow, need to be better elucidated. Besides that, our research also demonstrated a transgenerational parental control on seeds in response to CO_2 enrichment.

In addition, amaranth and quinoa plants showed severe metabolic changes when subjected to flooding, and we speculate that, despite the decreased invertases activity in this condition, it is quite possible that an increased SuSy activity was not able to adequately replace invertases in the progress of sucrose cleavage.

Nowadays, more and more studies are focused only on research with e[CO_2] in addition to other biotic or abiotic conditions. Unfortunately, we currently do not have enough scientific basis to discuss alterations related only to the changes that the atmospheric increase in CO_2 can cause. Justifications such as "C_4 plants do not change under conditions of high CO_2" have been shown to be inefficient and generalist.