UNIVERSIDADE FEDERAL DE LAVRAS

DANIEL AMORIM VIEIRA

AGRONOMIC AND PHYSIOLOGICAL RESPONSES OF RICE TO SODIUM TREATMENTS AND HIGH CO_2

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia, área de concentração em Fisiologia Vegetal, para obtenção do título de Doutor.

Prof. Dr. João Paulo Rodrigues Alves Delfino Barbosa
Orientador

Profa. Dra. Flávia Barbosa Silva Botelho
Coorientadora

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Dr. João Paulo Rodrigues Alves Delfino Barbosa	UFLA
Dr. Paulo Eduardo Ribeiro Marchiori	UFLA
Dr. Claudivan Feitosa de Lacerda	UFC
Dra. Carla Pinheiro	ITQB
Dr. Vitor de Laia Nascimento	UFLA

Prof. Dr. João Paulo Rodrigues Alves Delfino Barbosa
Orientador

Profa. Dra. Flávia Barbosa Silva Botelho
Coorientadora

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This thesis work is dedicated to God, for the gift of life and to my dear Father Luís Vieira
who taught and educated me, always putting God first.
Also, it is dedicated to my beloved Marília Mickaele for her support and unconditional lovel help,
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RESUMO

O aumento das emissões de dióxido de carbono (CO_2) afeta, principalmente, as plantas que apresentam a via do C3, contribuindo para a redução da fotorrespiração e, consequentemente, redução da assimilação do nitrogênio (N). Um elemento que pode reduzir os efeitos do aumento do CO_2 na assimilação do N é o sódio (Na^+) atuando como cofator no transporte do piruvato, tendo papel direto na regeneração da Fosfoenolpiruvato carboxilase (PEPC). O arroz foi escolhido para o trabalho pela sua grande importância socioeconômica e também pelo aumento na demanda por arroz nos próximos anos. Nesse contexto, montamos dois experimentos com o objetivo de investigar como a disponibilidade de Na^+ pode afetar o crescimento e a produção de linhagens de arroz tropical de terras altas. O objetivo do primeiro experimento foi selecionar linhagens com maior tolerância à salinidade, sendo a linhagem que apresentou melhores respostas foi utilizada no segundo experimento que teve como objetivo avaliar a influência do sódio na modelagem da fotossíntese e na produtividade de grãos de arroz sob alto CO_2. O primeiro experimento foi conduzido em casa de vegetação com delineamento de blocos inteiramente casualizados (DBC) e esquema fatorial duplo: o primeiro fator sendo as dez linhagens de arroz de sequeiro e o segundo fator de variação as concentrações de NaCl (0, 3, 15 e 45 mmol por L) em 5 repetições, um indivíduo por unidade experimental, totalizando 200 parcelas. As características avaliadas foram: teor de clorofila total, trocas gasosas, teor de matéria seca da parte aérea e raiz, fenologia e variáveis de produção. Para o segundo experimento, conduzido em câmara de topo aberto, o delineamento experimental foi inteiramente casualizado, com os tratamentos: 0 mmol L^{-1} NaCl e 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} de NaCl e 400 µmol mol^{-1} de CO_2 (C1S2); 0 mmol L^{-1} de NaCl e 700 µmol mol^{-1} de CO_2 (C2S1) e 3 mmol L^{-1} de NaCl e 700 µmol mol^{-1} de CO_2 (C2S2). No primeiro experimento, ao final do ciclo, utilizando a produção de grãos por planta (GWP) como indicador de desempenho da planta sob salinidade, L6, L7 e L8 apresentaram os melhores resultados. L6 teve o maior GWP, enquanto L7 e L8 tiveram a menor redução no GWP com aumentos de NaCl, essas linhagens são recomendadas para testes adicionais para avaliar o impacto do estresse salino nas lavouras de arroz. No segundo experimento foi possível comprovar os efeitos do NaCl em condições de alto CO_2 sobre os processos fotossintéticos, nas condições de oxigênio a 2% sob o tratamento C2S2 para a variável J foi maior do que C2S1. O tratamento C1S2 aumentou J / Vcmax nas condições de 21% de oxigênio quando comparado ao tratamento controle, entretanto, com o fornecimento da dose de NaCl em condições de alto CO_2, houve diminuição da produção de grãos nessas condições. A dose de NaCl e a elevação de CO_2 isoladamente foram benéficas para a planta em relação à fotossíntese e rendimento de grãos para a linhagem de sequeiro CMG 2085.

Palavras-chave: Salinidade; Mudanças Climáticas; Rendimento de grãos; Oryza sativa L; Fisiologia de plantas.

ABSTRACT

The increase in carbon dioxide (CO_2) emissions mainly affects plants that present the C3 pathway, contributing to the reduction of photorespiration and, consequently, reduction of nitrogen (N) assimilation. An element that can reduce the effects of increased CO_2 on N assimilation is sodium (Na^+) acting as a cofactor in pyruvate transport, having a direct role in the regeneration of Phosphoenolpyruvate carboxylase (PEPC). Rice was chosen for the job because of its great socio-economic importance and also because of the increase in demand for rice in the coming years. In this context, we set up two experiments with the aim of investigating how Na^+ availability can affect the growth and production of tropical upland rice lines. The objective of the first experiment was to select lines with greater tolerance to salinity, and the line that showed the best responses was used in the second experiment, which aimed to evaluate the influence of sodium on photosynthesis modeling and rice grain yield under high CO_2. The first experiment was carried out in a greenhouse with a completely randomized block design (RBD) and a double factorial scheme: the first factor being the ten upland rice lines and the second variation factor the NaCl concentrations (0, 3, 15 and 45 mmol per L) in 5 repetitions, one individual per experimental unit, totaling 200 plots. The characteristics evaluated were: total chlorophyll content, gas exchange, shoot and root dry matter content, phenology and production variables. For the second experiment, conducted in an open-top chamber, the experimental design was completely randomized, with treatments: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} of NaCl and 400 µmol mol^{-1} of CO_2 (C1S2); 0 mmol L^{-1} of NaCl and 700 µmol mol^{-1} of CO_2 (C2S1) and 3 mmol L^{-1} of NaCl and 700 µmol mol^{-1} of CO_2 (C2S2). In the first experiment, at the end of the cycle, using the grain yield per plant (GWP) as an indicator of plant performance under salinity, L6, L7 and L8 showed the best results. L6 had the highest GWP, while L7 and L8 had the smallest reduction in GWP with increases in NaCl, these lines are recommended for further testing to assess the impact of salt stress on rice crops. In the second experiment it was possible to prove the effects of NaCl under high CO_2 conditions on photosynthetic processes, under 2% oxygen conditions under C2S2 treatment for variable J was greater than C2S1. The C1S2 treatment increased J / Vcmax under conditions of 21% oxygen when compared to the control treatment, however, with the supply of the dose of NaCl under high CO_2 conditions, there was a decrease in grain production under these conditions. The dose of NaCl and the elevation of CO_2 alone were beneficial to the plant in relation to photosynthesis and grain yield for the rainfed line CMG 2085.

Keywords: Salinity. Climate change. Grain yield. *Oryza sativa* L. Plant physiology

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LIST OF ABBREVIATIONS

CO_2	Carbon dioxide
Na^+	Sodium
Cl^+	Chloride
C	Carbon
N	Nitrogen
N_2	Nitrogen gas
K^+	Potassium
NaCl	Sodium Chloride
$NH_4)_2SO_4$	Ammonium Sulphate
MAP	Monoammonium Phosphate
KNO_3	Potassium Nitrate
PPFD	Photosynthetic Photon Flux Density
A	Net photosynthesis
gs	Stomatal conductance
E	Transpiration
WUE	Water Use Efficiency
CC	Chlorophyll content
FLL	Flag Leaf Length
TNP	Total number of panicles
PL	Panicle length
WHG	Weight of 100 grains
LDM	Leaf Dry Matter
RDM)	Root Dry Matter
SDM	Shoot Dry Matter
TDM	Total Dry Matter
RSR	Root/Shoot Ratio
APDM	Aerial Part Dry Matter
LA	Allocation to Feaf
GA	Allocation to Grain
SA	Allocation to Stem
RA	Allocation to Roots

HI	Harvest Index
GWP	Total Grain Weight per Plant
A/Ci	Curve Net CO_2 assimilation rate
Vc_{max}	Maximum carboxylation rate of ribulose biphosphate carboxylase-oxygenase (Rubisco)
J	CO_2 assimilation rate limited by RuBP regeneration
Rd	Leaf respiration in the light
A/PAR	The light curves
R_{dark}	Dark respiration
f	Apparent quantum yield
LCP	Light Compensation Point
A_{max}	Maximum net assimilation rate
Fv/Fm	Maximum quantum yield of photosystem II

SUMMARY

FIRST PART
Bibliography review

1. GENERAL INTRODUCTION

In recent years, there has been growing concern about the effects of climate changes on plant production, especially the effects of irregularity in the distribution of rainfall, increased air temperature and increased emission of greenhouse gases, especially carbon dioxide (CO_2). In the next few years an increase in the world population is expected, consequently increasing the demand for food. One of the crops that will need to increase its production is rice (*Oryza sativa* L.), due to its great socioeconomic importance and because is part of the diet of more than half of the world's population. This crop may have its production altered by climate change due to its C3 metabolism (KAZEMI et al., 2018).

Among the problems that will be aggravated by the effects of climate change is the salinity of soils (JAGADISH et al., 2012). There are some technologies to solve or alleviate the effects of salinity in agricultural crops, but they are quite costly. As around 20% of the world's arable soils have salinity problem, one solution for cultivation in salty areas is the selection of salt-tolerant genotypes to ensure food security for the world population (PORCEL et al., 2012).

In order to contribute to the solution of this problem, we carried out the first experiment with the aim of selecting salt-tolerant genotypes of upland rice from the germplasm bank of the breeding program of the Federal University of Lavras-MG, Brazil. Despite many investigations focused on different physiological characteristics associated with salinity tolerance during the initial phase of seedlings, little attention has been dedicated to the investigation of physiological characteristics during the production phase (HOSSAIN et al., 2015). In this way, we analyzed germination, survival, gas exchange, and production of the genotypes, in order to obtain a solid set of results regarding tolerance and production of upland rice lines.

Based on the results of this first work of selection of salt-tolerant genotypes, it was possible to select the lines L6 (CMG-2085), L7 (CMG F6 LAV1-7), and L8 (CMG 1896) as the most tolerant. Among that, CMG 1896 was the most productive and, even growing with 3 mmol. L^{-1} dose of sodium chloride (NaCl) and did not have its growth, development and production negatively affected. Low concentrations of sodium chloride (NaCl) are beneficial for the development of many cultures. For example, in some plants with C4 metabolism, the low salt concentration acts on the regeneration of the enzyme Phosphoenolpyruvate

Carboxylase (PEPC), and rice has an isoform located in the chloroplasts that acts on the assimilation of ammonium (MASUMOTO et al., 2010).

Therefore, we hypothesized that the supply of a low dose (3 mmol. L^{-1} of NaCl) would be beneficial to the CMG 2085 line, which was more tolerant and productive in the first experiment, increasing the nitrogen content of the leaf under high CO_2 conditions. Additionally, under conditions of high CO_2, nitrogen metabolism in rice is greatly affected, anticipating the leaf senescence and shortening the phenological cycle, which may affect the production of grains per plant. Thus, our research is of great importance to verify the effects of increased CO_2 concentration on rice, once it investigates the maintenance and/or increase of grain production per plant in a scenario of climate change.

Thus, the objective of the second experiment was to evaluate the effects of a low dose of NaCl on leaf nutrient content, growth, photosynthesis, chlorophyll a fluorescence and productivity of rice plants grown under two different CO_2 conditions. It was possible to prove the beneficial effects of NaCl under high CO_2 conditions on the leaf nitrogen content and also on the photosynthetic results, as the reduction in *Vcmax* (Maximum carboxylation rate of Rubisco) and *J* (CO_2 assimilation rate limited by RuBP regeneration) rates are directly related to Rubisco's (Ribulose biphosphate carboxylase-oxygenase) biosynthesis, since there is less need for this enzyme. motivated by greater activity, it contributed to a greater nitrogen efficiency and availability of this element for other processes, such as the increase in the allocation of photoassimilates to grains; however, with the supply of NaCl dose under high CO_2 conditions, there was a decline in grain production under these conditions. The dose of NaCl and the elevation of CO_2 alone were beneficial to the plant in relation to growth, photosynthesis and dry mass and grain production.

2 BIBLIOGRAPHY REVIEW

2.1 Socioeconomic importance of rice in Brazil and in the world

Rice belongs to the Poaceae family and is one of the most important food crops as it provides about one third of the total daily calories for the world population (ALI et al., 2014). Rice is one of the most cultivated cereals in the world, with great economic and social importance (HUONG et al., 2018). It is the food of more than three billion people, accounting for 50-80% of the daily calorie intake (AREF & RAD 2012). It stands out for its production and cultivation area: around 150 million hectares of rice are cultivated annually in the world, producing 590 million tons (LEMES et al., 2018).

In terms of economic value, it is considered a relevant food crop in many developing countries, mainly in Asia and Oceania, where 70% of the total population of developing countries and around two thirds of the world's undernourished population live. Rice is one of the best nutritionally balanced foods, it is responsible for providing a considerable recommended amount of zinc and niacin. Rice protein is biologically rich due to its high digestibility (88%) (ALI et al., 2014). It provides 20% of the energy and 15% of the protein per capita needed by man, and being an extremely versatile crop that adapts to different soil and climate conditions, it is considered the species that has the greatest potential to fight hunger in the world (NUNES, 2018).

Approximately 90% of rice grown worldwide is produced and grown in Asia. Latin America ranks second in production and third in consumption. As in Asia, rice is an important product in the economy of many Latin American countries because it is a basic item in the population's diet, as in the cases of Brazil, Colombia and Peru, or because it is an important product in international trade: for Uruguay, Argentina and Guyana, as exporters, and for Brazil, Mexico and Cuba, among others, as importers (NUNES, 2018).

Worldwide rice production has not kept pace with consumption growth. In the last six years, world production has increased by about 1.09% per year, while the population grew by 1.32% and consumption by 1.27%, with great concern regarding the stabilization of world production (NUNES, 2018). In the 2020/2021 crop, Brazil expects to produce 10.935 million tons of rice, a 2.21% reduction compared to the previous crop year. This is produced in an area of 1683.6 thousand hectares, with a productivity of 6905.0 kg/ha, allowing the country to supply its own consumption demand by the Brazilian population during this period (CONAB, 2021).

2.2 Climate change effects in the rice crop

In recent years, the concern about global change threatening plant growth and production has increased, largely due to impacts on food security. The threats include increased temperature, irregular rainfall and increased emission of greenhouse gases, especially carbon dioxide (CO_2), mainly released by anthropogenic activities. CO_2, despite being essential for the photosynthetic process in plants, is the main gas that is contributing to global warming (REHMAN et al., 2021).

Climate change has drastically affected the grain production of several crops, even with an increase in the concentration of CO_2 in recent years (WANG et al., 2016). The reduction in food availability is quite worrying, as an increase in the world population is expected in the coming years (VAN ITTERSUM et al., 2016) and food demand will drastically raise.

Among the crops that may be affected in the future by climate change is rice (*Oryza sativa* L.), which is part of the staple diet of more than half of the world's population (KAZEMI et al., 2018). Because rice is a grass that has C3 metabolism, it is influenced by the increase in CO_2 concentration, what could reduce the photorespiration that is responsible for the loss of 1/3 of the CO_2 absorbed by diffusion. Rice can respond satisfactorily to increased CO_2 levels, boosting photosynthesis, biomass and grain yields. This may be based in expansion of leaf area and allocation of nitrogen in the leaf; however, these gains could be greater if the shortening of the phenological phases did not occur, with early leaf senescence (DAHAL et al., 2014).

However, the increase of greenhouse gases, such as CO_2, is accompanied by the increase of air temperature, which is also responsible for the climate change. Air temperature may increase by 2°C by the end of the century XXI, based on simulations of general circulation models (GCMs), under the representative concentration pathway (RCP) (WEILU et al., 2020).

Air temperature is one of the factors that most limit grain production in rice under high CO_2 conditions. In scenarios of only high increases in CO_2, dry mass accumulation increases compared to current conditions. However, temperature increases alone or in combination with the enrichment of the CO_2 concentration, also including the reduction in water availability, remarkably reduce dry mass compared to current environmental conditions. Therefore, the increase in temperature leads to a decrease in rice productivity, regardless of whether it occurs alone or in combination with a high concentration of CO_2 (WANG et al., 2019).

In order to increase rice productivity in a climate change scenario, there are some possibilities. Such as the selection of genotypes, through the screening of variables of interest, which can be done at all stages of development, especially in the early stages and/or reproductive phases (ALI et al., 2014). Genotype selection through screening can be done at the field level or under laboratory conditions. In field conditions, the heterogeneity of soil physicochemical characteristics, climatic factors and other environmental factors that tend to modulate physiological processes are limited (SHANNON, 1997). Therefore, screening in the laboratory or in a greenhouse has advantages over field screening in relation to the initial selection processes, regarding the control of substrate, climate, fertilization and infestation of pests and diseases.

In addition to genotype selection, there are other possibilities to increase rice productivity under current and future climatic conditions. In recent years, many studies around the world have sought to insert the C4 pathway in rice, however, the complexity of this transformation, in addition to the long time to reach the goals, has made this insertion difficult (RIBOUA et al., 2013) Compared with C3 plants, such as rice, C4 plants are more efficient in improving photosynthesis and yield and by minimizing photorespiration, making them more suitable for survival in hot, dry environments with greater photosynthetic capacity, nitrogen use and use efficiencies of water (ZHU et al. 2010; ZHANG et al., 2017).

The insertion process of C4-PEPC from maize into rice made it possible to increase production and tolerance to abiotic stress (ZHANG et al., 2017). However, transgenics is a self-cost technology, so studies that have feasibility, practicality and low cost are needed in order to bring short-term answers regarding the maintenance or increase of productivity in the face of predicted climate changes.

Among the possible solutions to mitigate these effects, the use of Na^+ emerges as a viable option. It has promising effects in reducing the effects of photorespiration with increased CO_2 concentration, as it acts directly on ammonium assimilation by acting as a co-transport of pyruvate for regeneration (PEPC) and specifically in rice plants (MASUMOTO et al., 2010). Thus, studies evaluating the effects of selection of sensitive/tolerant genotypes to low to high NaCl concentrations and adding to this the effects of increased CO_2 on rice grain production.

2.3 Influence of salinity in the rice crop

The current estimates show that around 7% of the world's agricultural area is affected by salinity, which may increase to 20% in the future. One of the causes of land salinization is the use of artificial irrigation and inadequate soil management. Irrigated soils contribute about a third of the world's food production, however, much of the total area of irrigated land can be adversely affected by salinization (KOSOVÁ, 2013).

The intensity with which salt stress influences the growth and productivity of rice is determined by several factors, especially: the saline composition of the medium, the intensity and duration of stress, the edaphoclimatic conditions, the cultivar, and phenological stage of the crop (CARMONA et al., 2011). Salinity can affect the germination, growth, development and total dry mass production phases of plants, whereas in rice it can reduce the total number of panicles, number of grains per panicle and grain weight (LEMES et al., 2016).

Cultivated plants are classified as sensitive, moderately sensitive, moderately tolerant and tolerant or resistant, with regard to the degenerative action of salts on germination, growth and production. Rice is classified as moderately susceptible, with salt being harmful to its development from 40 mmol L^{-1} of NaCl. However, there are variations in salt tolerance among genotypes of the same species (WANG et al., 2019).

The reports of Na^+ ion toxicity for plants is quite common in the literature, however, at low concentrations this element may be beneficial in the range of concentrations that would be considered high for common nutritional ions, such as nitrate (NO_3^-), ammonia (NH_4^+) or potassium (K^+), and, for halophyte plants, levels can go far beyond that (LEMES et al., 2018; FLOWERS and COLMER, 2008). Many plants use the Na^+ ion when available in the environment as an osmotic molecule, to aid in rapid growth expansion, which may confer a competitive advantage in fast-growing species (KRONZUCKER et al., 2013). In fact, given its physicochemical similarity to K^+, the role of Na^+ as a generic “benign” osmotic in plant vacuoles is reasonable. For example, in sugar beets, a 95% replacement of K^+ in plant leaf tissue with Na^+ did not result in a measurable negative impact on osmotic potential (SUBBARAO et al. 1999).

In conditions of potassium deficient, Na^+ can be a substitute to some extent, even not being an essential nutrient to plants, but, as such, it can be considered a 'non-essential' or 'functional' nutrient (MAATHUIS, 2014). In plants with C4 metabolism, Na^+ is believed to facilitate the conversion of pyruvate to phosphoenolpyruvate (PEP), which occurs in mesophyll cells prior to the Calvin cycle (JOHNSTON et al. 1988).

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SECOND PART

Articles

Article I - Mapping phenotypic parameters linked to salt stress tolerance strategies in rice lines

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Mapping phenotypic parameters linked to salt stress tolerance strategies in rice lines

Daniel Amorim Vieira [1], Mayra Alejandra Toro-Herrera [1], João Paulo Pennacchi [1], Ane Marcela das Chagas Mendonça [2], Paulo Eduardo Ribeiro Marchiori [1], Flávia Barbosa Silva Botelho[3], João Paulo Rodrigues Alves Delfino Barbosa [1*]

[1]Universidade Federal de Lavras/ UFLA- Departamento de Biologia, Campus Universitário, CP 3037 - 37200-900 - Lavras, MG- Brasil

[2] Universidade Federal do Sergipe/ UFS- Programa de graduação em Desenvolvimento e Meio Ambiente, 49100-000- São Cristovão, SE, Brasil,

[3]Universidade Federal de Lavras/ UFLA - Departamento de Agricultura, Campus Universitário, CP 3037- 37200-900 - Lavras, MG, Brasil

* corresponding author: <jp.barbosa@dbi.ufla.br>

Abstract

Salinity is recognized as one of the main abiotic stresses, causing declines in the productivity of many crops, such as rice. Considering the importance of rice as a basic crop, the generalized pattern of salinization in soils around the globe and the sensitivity of the crop to saline soils, the aim of the present study was to evaluate the sensitivity / tolerance of upland rice strains. The upland rice lines used in this work were obtained from the Genetic Improvement Program of the Federal University of Lavras. The experiment was carried out in a greenhouse, with a block design and factorial scheme, with 10 lines and four NaCl treatments (0, 3, 15 and 45 mmol L^{-1}) on the substrate. Parameters related to the emergence rate, seedling survival rate, growth and architecture, biomass allocation, gas exchange and productivity were evaluated. Salinity levels influenced the emergence and survival rates, especially at the highest levels of NaCl, with strains L1, L4 and L5 showing survival rates below 60%. These strains were excluded from the experiment and the other seven strains were carried out until the end of the culture cycle. Grain weight per plant (GWP) was used as an indicator of greater tolerance to salinity and strains L6, L7 and L8 showed outstanding performance under treatments with increased NaCl. L6 had the highest GWP, while L7 and L8 had less pronounced decreases with increasing NaCl doses. These three strains can be recommended for further studies of the impacts of NaCl on rice production.

Key words: Stress abiotic, Climate change, *Oryza sativa* L., Sustainable agriculture, Plant physiology

Introduction

The culture of rice (*Oryza sativa* L.) has great socioeconomic importance, being the basic culture of more than half of the world population (Kazemi et al., 2018). Demand for rice is expected to increase by 60% with the observed pattern of world population growth by 2050 (Kromdijk and Long, 2016; FAO, 2019). This required increase in production is challenging, as the increase in demand is not in line with the reduction in crop productivity, mainly due to the negative impacts of adverse weather conditions and the limited opening of new agricultural areas (Aggarwal et al., 2019, Loboguerrero et al., 2019). Thus, the search for solutions to meet the demand for sustainable and environmentally friendly food production, is necessary and urgent (Chou et al., 2019).

In this context of climate change, salinity is one of the most common abiotic stresses generated by the combined action of human activity and climate. Salinity reaches about 20% of all agricultural land around the globe, being even more impactful in irrigated areas, where it reaches 33% of areas (FAO, 2018). The mineral elements that most contribute to salinity in agricultural fields are sodium (Na^+) and chloride (Cl^+), with Na mainly being present in upland rice growing areas (Maathuis, 2014, Reddy et al., 2017).

Palliative strategies to correct saline soils are often expensive and the use of salt-tolerant genotypes is the most common strategy for planting in these areas (Shahbaz, Ashraf, 2013; Hairmansis et al.,2014; Rasel et al., 2020 and Tin et al., 2021), making salinity important for achieving high grain yields (Momayezi et al., 2009). In this context, the lack of reliable screening techniques is one of the biggest limitations in the development of varieties with salinity tolerance (Gregorio et al., 2002).

Plants that are classified as tolerant to salinity generally have three main mechanisms to avoid or mitigate the impacts of stress: stem ions independent tolerance or osmotic tolerance, ion exclusion and tissue tolerance (Pires et al., 2015). Despite the importance of rice culture and the increase in areas affected by salinity in the future, the study of the responses of this culture to this abiotic stress is not fully understood (Pires et al., 2015).

Thus, the aim of this work was to evaluate the effects of fertilization in substrate with NaCl on the physiological aspects, growth and development and production of upland rice strains of the upland rice genetic improvement program at UFLA. More specifically, it aimed to select lines with greater tolerance to salinity and explain their strategies to overcome the limitations imposed by different levels of stress.

Material and Methods

Plant material, experimental design and conditions

The research experiment was carried out from November 2018 to March 2019 in a glasshouse on the Department of Biology of the Federal University of Lavras (UFLA) in Lavras-MG, Brazil (21º13’40’’S e 44º57’50’’W GRW). The experiment was in a block design, in a factorial scheme with two factors, rice lines and NaCl doses, with 5 replicates, in a total of 200 experimental units. The 10 lines were selected from Upland Rice Breeding Program of the UFLA and were identified as: L1 (CMGFG LAM 20-2), (L2 (CMG ERF 85-14), L3 (BRS ESMERALDA), L4 (BRS MG CAÇULA), L5 (CMG ERF 85-6), L6 (CMG 2085), L7 (CMG F6 LAV1-7), L8 (CMG 1896), L9 (CMG ERF 221-29) and L10 (CMG ERF 85-13). The Na doses were: 0, 3, 15 e 45 mmol NaCl, representing, respectively, 0, 0.18, 0.87 e 2.61 g of NaCl Kg^{-1} of substrate. The doses of NaCl were chosen based on the literature, going from a dose that could be beneficial (ranging from 3 to 15 mmol.L^{-1} of NaCl) up to a doses that has the potential to cause damage to the rice crop, (around 45 mmol.L^{-1}) (Cui et al., 1995; Reddy et al.,2017).

Ten seeds were sown in 4 L pots, filled with a substrate composed by clay (red oxisol) and sand (washed sand) in a 1:2 ratio. Fertilization was perform fortnightly following recommendation for upland rice (Alvarez et al., 1999): 10 g of ammonium sulphate ($(NH_4)_2SO_4$), 4 g of monoammonium phosphate (MAP) and 3.63 g of potassium nitrate (KNO_3) per plant (corresponding respectively to 120 kg ha^{-1} P_2O_5, 100 kg ha^{-1} N and 80 kg ha^{-1} K_20).

Irrigation was performed on a daily basis at 5 pm, through weighing lisimetry using a scale. The maximum retention capacity of the substrate was defined and water level was completed to 100% of the evapotranspiratory demand, in every irrigation. The imposition of the NaCl (Synth, model PA-ACS, molecular weight 53.44 g mol^{-1}) treatments was made by incorporating the specific quantities for each treatment in the substrate, when the pots were filled. A plastic recipient was placed on the bottom of each pot to capture the drained water. This water was reincorporated to the substrate on the following day irrigation, aiming to maintain the NaCl levels, avoiding leaching of the salt.

Seedling emergence and plant survival analysis

The evaluation of seedling emergence was performed 10 days after sowing, in the V1 stage (collar formation on first complete leaf on the main stem), according to the used growth and development scale (Counce et al., 2000). The emergence rate (ER, in %) was calculated, as the number of emerged seedlings from the 10 sown seeds in each pot. After emergence evaluation, thinning was performed with only one plant being left per pot, being each pot with one plant consider as an experimental unit. At 30 days after emergence, in the V6 stage (collar formation on sixth leaf on the main stem) (Counce et al., 2000), the survival rate was evaluated for all the lines and NaCl treatments, in five replicates. A minimum of 80% of survival rate was set as the threshold for selecting lines according to their capacity to tolerate salinity. Thus, the lines L1, L4 and L5, which presented survival rates of 60%, 20% and 40%, respectively, were considered to be sensitive to salinity and were not evaluate on the further steps of the experiment. However, the pots were kept in the experiment to avoid unbalance of experimental units.

Gas-exchange and chlorophyll index

Gas-exchange and chlorophyll index evaluation was performed at 30 and 60 days after emergence, on the respective, V6 and V13 stages (Counce et al., 2000). The gas-exchange evaluations were carried out between 9 and 11 am, using an infra-red gas analyser (IRGA LI-6400XT, LI-COR). The light level, measured in photosynthetic photon flux density (PPFD), was of 1000 µmol m^{-2} s^{-1}. The following parameters were measured: net photosynthesis (A, in µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, in mol H_2O m^{-2} s^{-1}), transpiration (E, in mmol H_2O m^{-2} s^{-1}). The instantaneous water use efficiency was calculated as the ratio A/E (WUE, in µmol CO_2 $mmol^{-1}$ H_2O), according to Esmaeilpour et al. (2016). Chlorophyll index (SPAD) was measured using a chlorophyll meter (atLEAF+, FT Green LTC). LTC). Gas-exchange and chlorophyll analysis were performed in the central area, of the youngest and fully expanded leaf, of the main tiller.

Leaf analysis, plant growth and yield components

At the end of the experimental period, 120 days after emergence, a number of parameters were evaluated in two main groups: developmental and biomass allocation traits. For the developmental traits, the following parameters were measured: flag leaf length (FLL, in cm), total number of panicles (TNP, unitless), panicle length (PL, in cm), and weight of 100 grains (WHG, in g) (at 13% moisture), according to Alvarez et al. (2012). The total grain weight per plant (GPW, in g) was also measured, at the same moisture as WHG. For the biomass allocation traits, the following parameters were measured: leaf dry matter (LDM, in g), root dry matter (RDM, in g), shoot dry matter (SDM, in g, as the sum of stem and leaf dry matter), total dry matter (TDM, in g, as the sum of root and shoot dry matter), root/shoot ratio (RSR, unitless, as RDM/SDM) and harvest index (HI, in g g^{-1}, as the ratio between grain and shoot dry matters). Samples for dry matter were placed on a forced air oven, at 65 ºC, until constant weight. Leaf samples were also collected for the evaluation of sodium content, in five replicates, for each combination of lines and NaCl doses. The replicates were mixed in a single sample which was analysed through the method of emission flame spectrometry to obtain leaf Na content (Malavolta et al.,1997) (Table 1).

Statistical Analysis

The normality of data was evaluated through the analysis of the residuals' distribution by Kruskal-Wallis test. Initially, analyses of variance were used to evaluate the effect of single factors and their possible interaction for each analysed parameter. After that, all variables were subjected to regression analysis. For the regression, first and second-degree polynomial models were fitted to describe the mathematical relationships between the measured traits and the NaCl doses. The percentage of change for parameters between 0 and 45 mmol L^{-1} of NaCl doses were calculated as the difference of the parameters divided by the value at the parameter at the 0 doses. For the data of foliar sodium content, additionally to the regression to soil Na levels, a Pearson correlation analysis to GWP was performed. Only statistically significant regressions ($p< 0.1$), are presented in this manuscript. Statistical analyses were conducted using the RStudio statistical software (v. 1.2.5033, RStudio). The main packages used were agricolae, ggplot2, multcomp, ggpubr and Rmisc.

Results

The Na doses applied to soil were differently managed by the cultivars in terms of transportation to the leaves (Fig. 1) with contrasting patterns being observed. For instance, L3 presented a prominent linear increase in leaf levels of Na with increases in Na in the soil. Other genotypes also presented the same pattern but with much smaller slope, potentially showing a mechanism to control the transport of Na from soil to leaf, as L2, L6, L9 and L10. On the other hand, L7 and L8 (although the regressions is not significant) showed a quadratic pattern were the levels of leaf Na increased until the level of 15 mmol L^{-1}in the soil, but, showed limited increase of Na in the leaf when the levels increased to 45 mmol L^{-1} in the soil (Fig. 1).

Regarding the emergence rate (Table 2) there was no significant interaction ($p < 0.05$) between the factors, but there was a significant difference ($p < 0.05$) for the strains and salt doses when analyzed separately. In this context, L10 had a lower emergence rate than L3, L5, L7 and L9. The highest emergence rate was observed at the 3 mmol L^{-1} NaCl dose, followed by the 0 and 15 doses. On the other hand, the lowest emergence rate was observed at the 45 dose. (Table 2).

The same pattern of no interaction ($p > 0.05$) between lines and salinity was observed for developmental and biomass allocation parameters. In gas-exchange parameters the interactions were analysed for lines, salinity and time with no significance results. By this reason, the analysis was focused on the linear regression for each parameter *versus* the salinity level, for each single line.

The GWP was used as an indicator of the level of tolerance/sensitivity to salt stress. The lines showed higher GWP at 0 and 3 mmol L^{-1} doses with significative reductions at 15 and 45 mmol L^{-1} doses (Table 2). In all salinity levels, L6 presented higher GWP than L2 and L10 (Table 2). In general, plants tended to decrease its GWP as the level of NaCl increased. The linear regressions among GWP and NaCl levels were significant for all the lines, with exception of L7 (Fig. 1). The Pearson correlation analysis between GWP and leaf Na, did not show significant results for the lines ($p = 0.1096$), However, the doses of salt were negatively correlated to GWP ($p = 0.0029$, correlation; $r = -0.54$).

L8 presented the lowest loss in GWP (-18%) from 0 to 45 mmol L^{-1} NaCl followed by L6, L9 and L2 (-34, -36 and -42%, respectively) and L3 and L10 (-60 and -69%, respectively). The different decrease patterns can be explained based on the behaviour of other parameters related to yield-components, biomass allocation and gas-exchange parameters, as highlighted in the sequence (Figure. 1, Table 3).

L8 presented an increase in PL (+6%) and a decrease in WHG (-16%), although for the last it was not as sharp as the decrease for L3 (-56%) and L10 (-93%) (Fig. 1, Table 3). L8 presented a decrease in TDM (-39%), however, the main reduction was related to the root biomass instead of the shoot biomass, what can be noticed by a pronounced decrease in RSR (-

45%) (Fig. 2, Table 3). Also, L8 presented and increase in the net photosynthesis, A (+14%), at the V13 stage (Fig. 3, Table 3).

From the group of intermediate losses in GWP, L6 decreased in PL (-10%) but maintained GWP in opposition to the other lines (Fig. 1, Table 3). It presented a decrease in LDM and TDM (-16% and -28%, respectively), but was not highly impacted in the allocation of biomass to the grain, what can be observed by the slope of HI (-14%) (Fig. 2, Table 3). Also, it presented and increase in A (+16%) without major changes in gs (-2%), at V6 stage (Fig. 3, Table 3). For L9, if compared to L6, it did not present decrease in PL, but had higher losses in WHG (-10%) and HI (-30%) (Figs. 1 and 2, Table 3). It has a decrease in A (-8%) and E (-14%), with WUE also decreasing (-6%) (Fig. 3, Table 3). L2 decrease in GWP may be related to the decrease in LDM (-22%) (Fig. 2, Table 3).

Considering the lines with the higher losses, L3 presented an increase in FLL (+27%) and A and gs at V13 (+34 and +20%, respectively) (Figs. 1 and 3, Table 3). However, this higher potential of carbon uptake may have not been translated in lower GWP losses by the decreases in NI (-35%), WHG (-56%) and HI (-27%) (Figs. 1 and 2, Table 3). In addition to that, the combination of higher FLL and E may be related to higher water loss. L10, which presented the higher losses in GWP, presented increases in FLL (+44%) and PL (+10%) (Fig. 1, Table 3). However, this was not enough to compensate the losses in WHG (-93%), TDM (-54%) and HI (-42%) (Fig. 1 and 2, Table 3). No differences were observed for leaf chlorophyll levels among lines, salinity levels and their interaction.

Discussion

Our first results showed that NaCl influenced the emergence rate and that the lines presented difference tolerance to this condition. Soil salinity affects seed germination and emergence by creating an osmotic potential on the outer part of the seed, preventing or limiting water intake to the inner part of the seed, or, by the toxic effect of Na^+ and Cl^- ions to the seed (Khajeh-Hosseini et al., 2003). There is a report of no influence of salinity in emergence rate of rice seeds, showing a potential tolerance, even in doses of 160.3 mmol. L^{-1} NaCl (Khan et al., 2014). However, we noticed that L10 presented lower emergence rate than L3, L5, L7 and L9 (Table 2).

For seedling survival, L1, L4 and L5 presented lower rates than 60%, at the highest NaCl levels, after 30 days of emergence (Table 2). This behaviour of higher seedling death reveals the sensitivity of the mentioned lines to salinity, at the early stages of plant development, and a possible tolerance of the other lines. However, survival and emergence tolerances to salinity may not be linked, once L5 presented high emergence rate, but lower survival and L10 presented the opposite behaviour, with lower emergence, but higher survival rate (Table 2). This indicates that the strategies to alleviate salinity effects may be different at each process. One of the plant strategies to mitigate salt stress at early stages in rice is related to the capacity to accumulate Na^+ and Cl^- ions in structural tissues and in the bigger vacuoles of older leaves, decreasing their accumulation in younger leaves (Wang et al., 2012). This may raise the carbon fixation by younger leaves and increase the chances of plant survival, at the seedling and early development stages (Reddy et al., 2017).

Salinity did not influence significantly ($p > 0.05$) the chlorophyll content of leaves at V6 and V13 stages. None differences were also observed among the lines. This result is corroborated by Kasemi et al. (2018), working with rice varieties in salinity conditions of 0, 30, 60 and 90 mmol L^{-1} de NaCl and also by Chiconato et al. (2019) for sugarcane plants in salinity levels of 0, 40, 80 and 160 mM NaCl. Under salinity conditions, the nitrogen metabolism is rearranged towards amino acids synthesis, aiming to alleviate the impact of the stress conditions, what can decrease chlorophyll content (Xu et al., 2016), as observed, in rice, by Amirjani (2012).

The ionic unbalance motivated by the excess of Na^+ and Cl^- ions on the soil and inside the plant cells, causes the reduction of water absorption leading to the changes in the physiological process, causing the so-called physiological drought (James et al., 2011; Nishimura et al., 2011). Some of these changes includes the membrane disruption, reduction in photosynthetic capacity and the lack of capacity of detoxification of the reactive oxygen species (ROS) due to the damage occasioned to the antioxidant enzymes (Rahnama et al., 2010; James et al., 2011). These processes may cause cell death and lead to initial senescence of old leaves, progressing to young leaves and, latest, collapse of the whole plant (Quintero et al., 2007; Siringam et al., 2011).

Considering carbon fixation and the processes related to it, the photosynthetic capacity of plants grown under salinity is reduced and dependent of the level and duration of the stressful condition (Munns, 2002), as well as the genotypic differences of the varieties/lines, and plant development stage (Dadkhah, 2013). In this study, the different salinity levels did not significantly change the gas-exchange parameters, although the factor time and lines were significant ($p < 0.001$). However, the regression analysis showed significant differences in gas exchange parameters as a function of the increase in NaCl levels (Fig. 3).

A general pattern of response to salinity, at the whole plant level, is the reduction in growth and development (Munns, Tester, 2008), linked to lack of carbon assimilation and lower source capacity (Soledad et al., 2012). This is related to the above-mentioned limitations in gas-exchange, impacting in availability of intercellular carbon for photosynthesis due to stomatal closure, motivated by lower leaf water potential. By this reason, the capacity to maintain or increase transpiratory rates and stomatal conductance, under saline conditions, is an indicator of tolerance to salinity (Harris et al., 2010; Hernandez et al.,2018; Razzaque et al., 2019).

In our study, L3 presented this characteristic of increased gs and E under higher salinity, what also increased A (Fig. 3). However, this was not enough to increase or maintain GWP at higher NaCl doses. This is due to the fact that other traits, as WHG, was drastically decreased (Fig. 1). This may be related to the trade-offs between photosynthesis and allocation from leaves to the grain, represented by lower HI (Fig. 2), in the late crop development stages (Sultana e Ikeda, 1999). Other trade-off between gas-exchange parameters is the possible change in WUE, mainly when A and E change in different scales with NaCl treatments. L9 presented a slow decrease in WUE (-6%, Table 2) what is in accordance to the results of Singh and Sasahara (1981), which reported no changes in WUE under salt treatments.

In cereals, and consequently in rice, the grain production is highly dependent of the photosynthetic capacity and the source-sink relations. Variations in the assimilation capacity and in the carbohydrate synthesis, accumulation and partition directly influence grain production (Biswal e Kohli, 2013). In rice, the three upper leaves, including the flag leaf, are the main sources of carbohydrates for the grain filling process, available from leaf photosynthetic assimilation and driven to the panicle (Yoshida, 1972). The importance of the flag leaf is even higher under abiotic stress conditions, as salinity (Slewinski, 2012). However, in our study, the two lines which showed significant increases in FLL with NaCl treatment intensification, L3 and L10, were the most penalized in GWP losses (Fig. 1, Table 3). This may be related to the fact that an increase leaf area may have led to a higher transpiration levels, increasing the water flow from root to shoot and consequently increasing the Na^+ and Cl^- ions concentration on leaf tissues. Through the foliar analysis of Na, which showed a negative correlation between GWP and the doses of salt, it is possible to notice the direct effects of salinity on the production of grains in rice, also corroborated by Wang et al. (2019). The higher decrease in GWP for L3 may be related to the incapacity to block Na transportation from soil to leaf, at higher levels. On the other hand, L7 and L8 may show a strategy of blocking Na transportation to the leaf, at the same higher levels, reducing its impact and helping in maintaining GWP.

In terms of biomass allocation, the shoot dry matter is intrinsically related to the rice crop performance (Yeo et al., 1990, Razzaque et al., 2019). The line L8, the one with the lowest significant loss in GWP, presented a sharp decrease in RDM, but a less pronounced decrease in SDM, what can be observed by the pattern of RSR (Fig. 2 and Table 3). Changing the balance between shoot and root proved to be a successful strategy to keep GWP, under salinity conditions. The reduction in RDM may have allowed a lower assimilation of NaCl from the soil, but without causing a pronounced reduction in SDM. In this way, the plant was able to have enough photosynthetic active structure to maintain source capacity to feed the sinks (grains) (Sultana; Ikeda, 1999). However, the pattern of reduction in root mass goes against the findings of Razzaque et al. (2019) which report the increased in root dry matter as a trait strongly linked to salt tolerance.

The potential benefits of Na^+ ions in plant metabolism, have been a matter of discussion, recently (Kronzucker et al., 2013; Maathuis, 2014). Some studies suggest that in low concentrations, Na^+ may be beneficial to the plant due to its common availability in soils, the osmotic control of plants and turgor maintenance (Pardo e Quintero, 2002; Subbarao et al., 2003; Wu, 2018), potentially improving cell expansion and promoting rapid growth as a competitive advantage (Kronzucker et al., 2013). It may also be a functional substitute to K^+, as both ions share similar functions in plant metabolism, reducing the dependence of potassium fertilizers which are normally more expensive (Maathuis, 2014, Wakeel et al., 2011).

Further advantages of the presence of NaCl in soils, in determined concentrations, are related to the ion exchange on the vacuole. When Na^+ is transported into the vacuole, K^+ ions may be transferred to the cytosol, through the H^+ proton pump. This may increase the availability of K^+ ions to crucial metabolic processes (Wakeel et al., 2011). Furthermore, Na^+ may act in the control of stomata movement, in substitution to K^+, although this process is not completely explained (Robinson et al., 1997; Roelfsema e Hedrich, 2005). There are reports of improved stomata control (Marschner, 1995) and water use efficiency (Gattward *et al*., 2012) in plants grown under a condition of Na^+/K^+ availability, instead of only potassium fertilization. Also, Pires et al. (2015) reported that the capacity to keep a low Na^+/K^+ ratio is not always the main trait for salt tolerance in rice.

It is important to highlight that this study did not aim to compare the benefits of Na^+ or its potential to substitute K^+, as the potassium fertilization was performed as recommended for the rice crop (Alvarez et al., 1999). However, we observed that some parameters did not show difference or even a beneficial change at the lower NaCl doses, 3 or 15 mmol L^{-1}. This can be exemplified by the behaviour of WHG for L3 or the gas-exchange parameters for L6 and L9, at V6, or L3, at V13. Of course, as this experiment was not planned to test this specific role of NaCl, the statistical methods as well as the NaCl concentrations were not the most appropriate to infer about those results.

We, finally, recommend the lines L6, L7 and L8 as potential genotypes to the studies of salt tolerance strategies in rice. Together with the possible Na^+ benefits at lower levels, other approaches may boost research in salt tolerance in rice: the potential map of jasmonate-related genes, which are recognized to play a major role in the tolerance to osmotic stress in rice (Tang et al., 2019) and the use of molecular approaches for gene identification (Liu et a., 2019).

Conclusion

In this study we mapped different patterns of behaviour of rice lines from the Upland Rice Genetic Breeding Program of UFLA, for many parameters linked to crop production. Lines L1, L4 and L5 showed increased death rate under the highest NaCl doses and showed to be saline-sensitive at the early developmental stages. At the end of the cycle, using GWP as an indicator of plant performance under salinity, L6, L7 e L8 presented the best results. L6 presented the highest GWP, while L7 and L8 presented the less pronounced decrease in GWP with NaCl increases. Those lines are recommended for further tests for evaluation of salt stress impact on the rice crop.

Authors' Contribution

Conceptualization: Vieira, DA; Toro-Herrera, MA; Pennacchi, JP; Mendonça, AMC; Botelho, FBS; Marchiori, PER; Barbosa, JPRAD **Data acquisition**: Vieira, DA; Toro-Herrera, MA. **Data analysis**: Vieira, DA; Toro-Herrera, MA; Pennacchi, JP; Barbosa, JPRAD **Design of Methodology**: Vieira, DA; Barbosa, JPRAD; Botelho, FBS; Marchiori, PER **Writing and editing**: Vieira, DA; Toro-Herrera, MA; Pennacchi, JP ; Mendonça, AMC; Botelho, FBS; Marchiori, PER; Barbosa, JPRAD.

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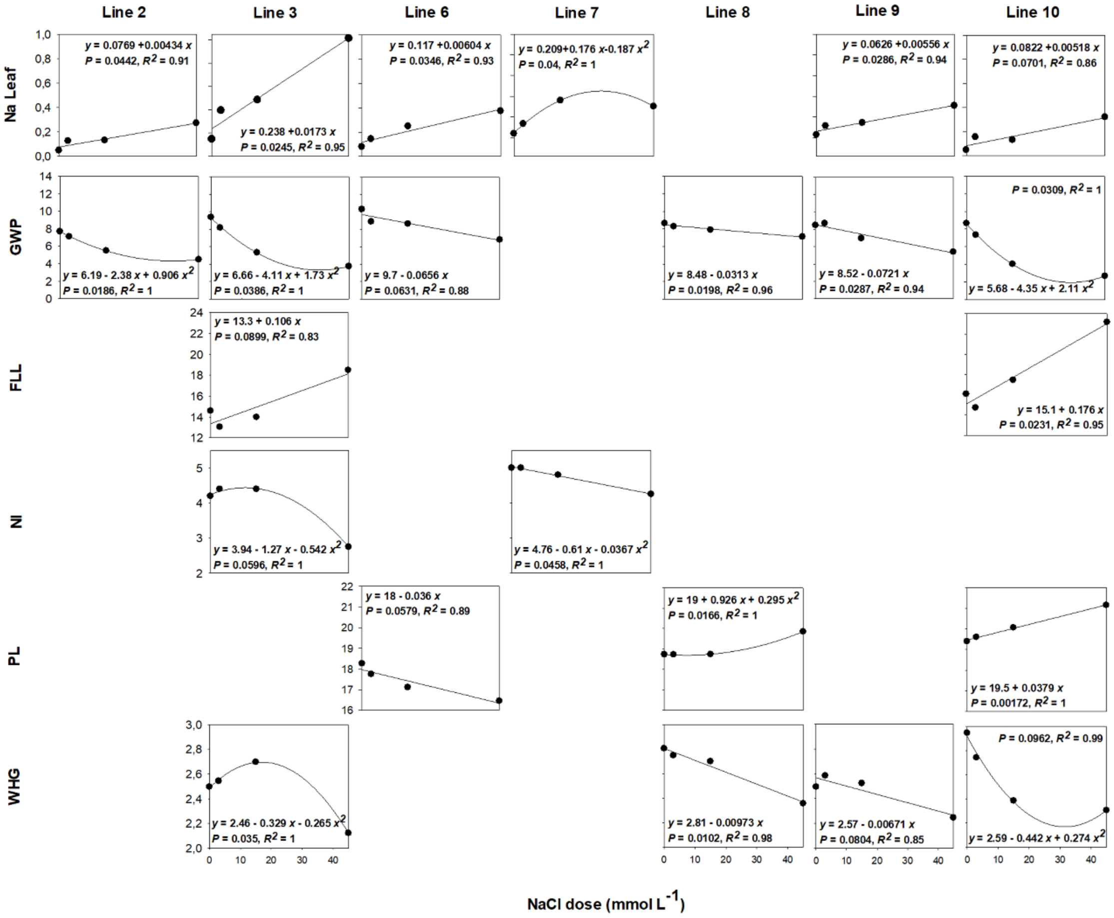


Figure 1. Linear regression of developmental parameters vs NaCl doses, for 7 rice lines. *p*, significance level of the linear regression, R^2, coefficient of determination of the linear

regression. Na Leaf, sodium leaf; GWP, grain weight per plant; FLL, flag leg length; NI, Number of inflorescences; PL, panicle length; WHG, weight of 100 grains. Only the linear regression with *p*<0.1 are shown in the figure.

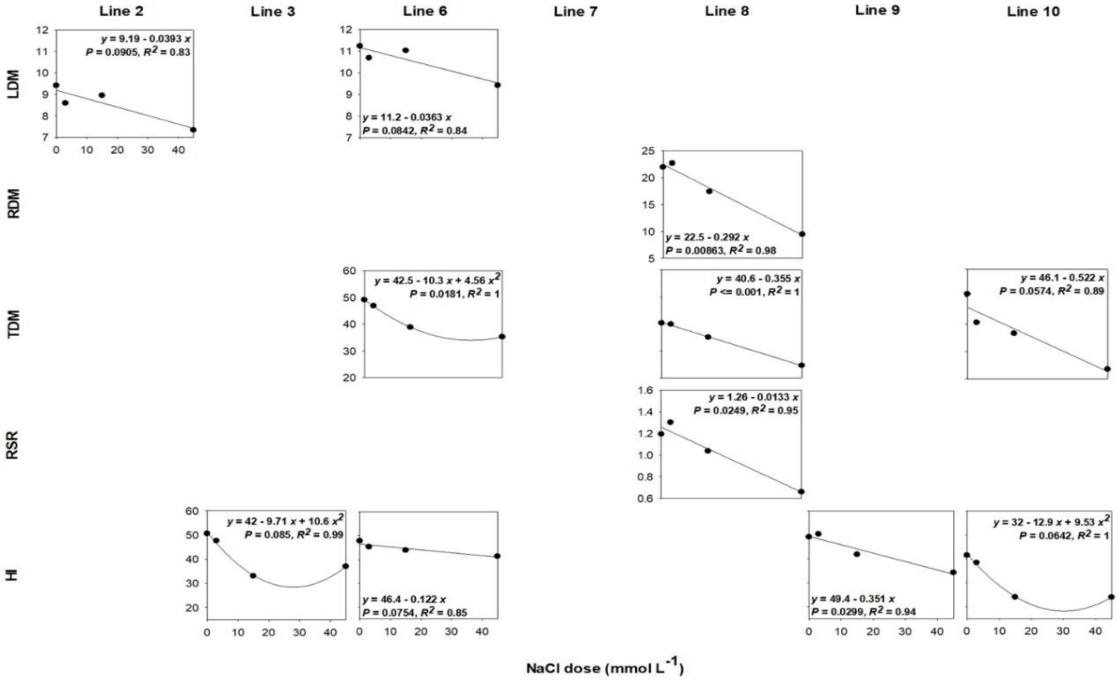


Figure 2. Linear regression of biomass allocation parameters vs NaCl doses, for 7 rice lines. *p*, significance level of the linear regression, R^2, coefficient of determination of the linear regression. LDM, leaf dry matter; RDM, root dry matter; SDM, shoot dry matter; TDM, total dry matter; RSR, root to shoot ratio; HI, harvest index. Only the linear regressions with *p*<0.1 are shown in the figure.

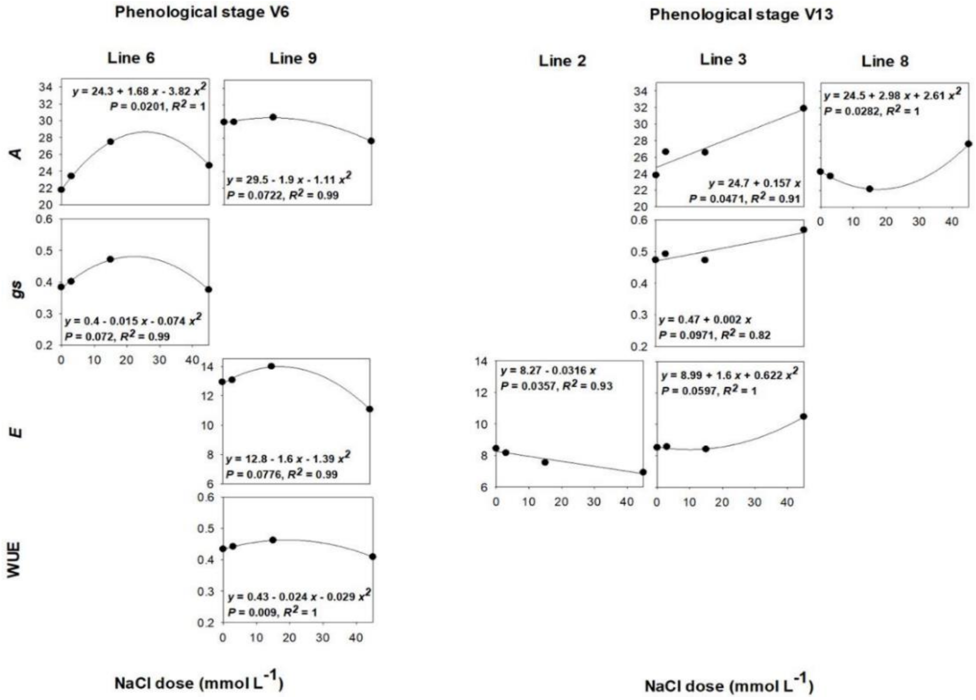


Figure 3. Linear regression of gas-exchange parameters vs NaCl doses, for 7 rice lines. p, significance level of the linear regression, R^2, coefficient of determination of the linear regression. A, net photosynthesis; gs, stomatal conductance; E, transpiration; WUE, water use efficiency. V6 and V13, collar formation on sixth and thirteenth leaf on the main stem, respectively (Counce et al., 2000). Only the linear regressions with p<0.1 are shown in the figure.

Table 1. Analysis of leaf sodium (Na) content in the harvest phase in the treatments of lines(L) and salinity(S).

Treatment	Na	Treatment	Na	Treatment	Na	Treatment	Na
L2S1	0.049	L6S1	0.080	L8S1	0.139	L10S1	0.048
L2S2	0.127	L6S2	0.144	L8S2	0.129	L10S2	0.155
L2S3	0.132	L6S3	0.249	L8S3	0.395	L10S3	0.131
L2S4	0.273	L6S4	0.374	L8S4	0.367	L10S4	0.321
L3S1	0.148	L7S1	0.039	L9S1	0.030		
L3S2	0.395	L7S2	0.135	L9S2	0.115		
L3S3	0.486	L7S3	0.360	L9S3	0.145		

L3S4	1.014	L7S4	0.303	L9S4	0.311

Table 2. Tukey *post-hoc* test for emergence rate (ER), survival rate (SR) at the highest NaCl doses and grain weight per plant (GWP) for two factors (Lines and Salinity)

	Parameter		
Factor	**ER (%)**	**SR (%)**	**GWP(g)**
Lines(L)			
L1	69.8 abc	60 -	-
L2	72.4 abc	80 -	6.28 bc
L3	75.2 ab	80 -	6.81 abc
L4	62.2 bc	20 -	-
L5	73.9 ab	40 -	-
L6	68.0 abc	100 -	8.67 a
L7	79.7	80 -	7.90 ab
L8	72.1 abc	80 -	8.03 ab
L9	77.0 a	100 -	7.38 abc
L10	60.5 c	100 -	5.68 c
Salinity(S)			
0	68.6 b	-	8.85 a
3	83.1 a	-	8 .24 a
15	73.2 b	-	6.32 b
45	59.5 c	-	5.37 b

Equal lowercase letters do not differ significantly between treatments; Different lowercase letters between treatments are significant by the Tukey test ($p<0.05$).

Table 3. Differences (in %) between parameters at 0 and 45 mmol L^{-1} of NaCl for 7 rice lines

		Lines						
Group	**Parameter**	**L2**	**L3**	**L6**	**L7**	**L8**	**L9**	**L10**
Morphological	**GWP**	-42	-60	-34	-13	-18	-36	-69
	FLL	-4	27	-5	28	13	20	44
	NI	-22	-35	4	-15	-8	12	-25
	PL	-2	-4	-10	-9	6	5	9
	TNP	-5	2	7	-4	-8	9	-4
	WHG	-6	-56	-1	-7	-16	-10	-93
	LDM	-22	-27	-16	-10	-18	3	-27
	RDM	-23	-69	-31	-9	-57	-14	-60
	SDM	-42	-60	-34	-13	-18	-36	-69
	TDM	-27	-60	-28	-10	-39	-15	-54
	RSR	7	-52	-14	-1	-45	4	-31
	HI	-13	-27	-14	-5	0	-30	-42
	A(V6)	16	-3	13	-1	-8	-8	-4
	gs(V6)	-9	-26	-2	-33	-10	-18	-16
	E(V6)	4	1	-10	-21	-3	-14	-9
	WUE(V6)	-10	-1	-21	-20	4	-6	4
	A(V13)	0	34	-13	6	14	-2	16
	gs(V13)	-28	20	-29	-15	24	-2	27
	E(V13)	-18	23	-15	-5	21	2	22
	WUE(V13)	-19	-11	1	-10	5	3	4

Negative and positive grey numbers represent, respectively, decreases and increases in the percentage of change between 0 and 45 mmol L^{-1} of NaCl for non-significant (p>0.1) linear regressions. Negative (red) and positive (green) numbers represent, respectively, decreases and increases in the percentage of change between 0 and 45 mmol L^{-1} of NaCl for significant (p<0.1) linear regressions. GWP, grain weight per plant; FLL, flag leg length; NI, Number of inflorescences; PL, panicle length; TNP, total number of panicles; WHG, weight of 100 grains; LDM, leaf dry matter; RDM, root dry matter; SDM, shoot dry matter; TDM, total dry matter; RSR, root to shoot ratio; HI, harvest index; A, net photosynthesis; gs, stomatal conductance; E, transpiration; WUE, water use efficiency. V6 and V13, collar formation on sixth and thirteenth leaf on the main stem, respectively (Counce et al., 2000).

Article II - Sodium can improve photosynthesis of upland rice under high CO_2

The present manuscript was prepared according to the standards of the journal Rice

Sodium can improve photosynthesis of upland rice under high CO_2

Daniel Amorim Vieira [1], Mayra Alejandra Toro-Herrera [1], João Paulo Pennacchi [1], Flávia Barbosa Silva Botelho[2], Paulo Eduardo Ribeiro Marchiori [1], João Paulo Rodrigues Alves Delfino Barbosa [1*]

[1] Department of Biology, Federal University of Lavras, Lavras, MG, Brazil
[2] Department of Agriculture, Federal University of Lavras, Lavras, MG, Brazil
* corresponding author: jp.barbosa@dbi.ufla.br

Abstract

Background: The effects of climate change are increasingly worrying the food security of the world population, due to the effects of plant production. Among the crops of great socioeconomic importance, rice deserves to be highlighted and, as it is a C3 metabolism plant, it will be greatly influenced by the increase in CO_2. The objective of this work was to evaluate the influence of sodium on photosynthesis modeling and grain yield in a rice strain under high CO_2 concentration. The experimental design was in completely randomized blocks in a double factorial scheme, with the first factor being the CO_2 concentrations (400 μmol mol^{-1} (C1) or 700 μmol mol^{-1} (C2)) and the second factor being the NaCl supplementation (0 mmol L^{-1} NaCl (S1) and 3 mmol L^{-1} NaCl (S2)). The following traits were evaluated: nitrogen (N), potassiun (K) and sodium (Na) leaf content; Gas-exchange parameters; Chlorophyll fluorescence and grain weight per plant (GWP). **Results:** Under conditions C1S2 there was a decrease in N content, treatment C2 increased K; in relation to the gas exchange parameters, in C2 under 2% O_2 condition, there was an increase of J under C2S2 compared to C2S1. For J / Vcmax C1S2 was higher than C1S1. In relation to Fv/Fm, C1S1 was greater than C1S2 and C2S1 greater than C2S2. In the parameters from A / PAR curves there was no difference between treatments. GWP C1S2 was greater than C1S1 and C2S1 was greater than C2S2. **Conclusions**: It was possible to prove that a low dose of NaCl influences the photosynthetic and grain production processes of an upland rice line.

Keywords: Sodium benefits; *Oryza sativa* L; Climate changes; Plant physiology; Grain production.

1. Background

Current predictions indicate that there is a need to increase, in the short term, plant-based production of food, fibre and energy, to meet population demands. As an environmental and economic requirement, there is a need for this increase in production to occur without the concomitant increase in the sustainable use of resources and without the exploration of new agricultural areas. The challenge for researchers and farmers is to increase production, meeting the environmental requirement, under adverse climate scenarios as: increased temperature, irregularities in the rain cycle, salinity problems and increased concentration of greenhouse gases in the atmosphere (FAO, 2019).

Rice (*Oryza sativa* L.) is one of the agricultural crops that may be affected by the mentioned climatic events. This crop is one of the most relevant to humankind's diet, as it is part of the basic diet of more than half of the world's population (Kazemi et al. 2018). In order to meet this growing demand for food, a significant increase in the production of this cereal will be necessary. Thus, studies aiming the development of practical application techniques for maintenance and/or increased productivity in the face of environmental challenges are of great importance. These techniques/methods may be associated with technological alternatives for cultivation that increase production or increase its efficiency in adverse environmental conditions, which are cheaper and require less time for research and implementation under field conditions (Dwivedi et al., 2016).

Considering the environmental conditions, plants that have C3-type photosynthetic metabolism, such as rice, can be favoured by the conditions of ongoing atmospheric composition changes, as the increase in Carbon dioxide(CO_2) concentration contributes to the suppression of the photorespiratory process that is responsible for the loss of about 1/4 of the CO_2 absorbed through photosynthesis. Leaf photosynthesis is the main biochemical process that supplies carbohydrates to different plant organs and even in the accumulation of photoassimilates for grain production, which is a product of interest in rice (Wang et al., 2019). However, photorespiration is of great importance in the assimilation of nitrogen (N) in plants, which can cause a counterbalance (Wang et al. 2016; Lam et al. 2012; Pérez-López et al. 2012).

Previous researches have shown that the increase in CO_2 concentration in the atmosphere, promoted a reduction in the N content throughout the plant, limiting the expected gains in these conditions for plants with C3 metabolism. This is caused by the fact that the reduction in nitrogen content promotes the shortening of the phenological phases and anticipation of leaf senescence, having a direct effect on photosynthesis efficiency, biomass production and allocation, and grain yield (Dahal et al. 2014).

The rational supplementation of ion sodium (Na^+) has been recognized as a potential technology that can contribute to the maintenance or increase of grain production by rice under conditions of increased CO_2 levels. This is explained by the fact that, in adequate concentrations, Na^+ can result in commonly beneficial effects, stimulating the growth of many plant species (Vieira et al., 2021: unpublished data; Pardo and Quintero, 2002; Rodríguez-Rosales et al. 2009; Schulze et al. 2012). This element is commonly found in the soil composition around the globe and plants use it to regulate the osmotic potential, absorb water and sustain cell turgor pressure (Pardo and Quintero, 2002; Kronzucker et al. 2013).

It is important to highlight the novelty of this work regarding the effects of low NaCl concentration on the photosynthetic process of rice under high CO_2 conditions. Thus, this research raises the hypothesis that, under conditions of high CO_2 and with adequate Na^+ availability, rice plants will be efficient in photosynthetic processes and more productive. The objective of this work was to evaluate the influence of sodium on photosynthesis modeling and grain yield in a rice strain under high CO_2 concentration.

2. Material and Methods

2.1 Plant material, experimental conditions, and experimental design

The study was conducted from November 2019 to March 2020 in a greenhouse of the Department of Biology on the campus of the Federal University of Lavras (UFLA), municipality of Lavras (21°13′40″S and 44°57′50″W GRW), southern region of the state of Minas Gerais, Brazil. The rice line used was the CMG 2085, developed by the upland rice cultivation program, in a partnership between the Brazilian Agricultural Research Corporation (EMBRAPA), the Agricultural Research Company of Minas Gerais (EPAMIG), and the Federal University of Lavras (UFLA).

The rice line and the NaCl concentrations were selected based on previous results found by Vieira et al. (submitted), as this line presented good productivity and was tolerant to the imposed conditions of salinity. This line has the following characteristics: cycle of 120 days from planting to harvest, upright architecture, and height of approximately 100 cm.

The experiment was conducted for approximately 120 days under two atmospheric CO_2 concentrations (400 μmol mol^{-1} (C1) and 700 μmol mol^{-1} (C2)) in combination with two NaCl concentrations in the substrate (0 mmol L^{-1} NaCl (S1) and 3 mmol L^{-1} NaCl (S2)). The CO_2 concentration conditions were made possible by cultivation in open-top chambers with a base diameter of 1.3 m, with an open top of 0.5 m in diameter, height of 1.8 m and volume of 1.3713 m^3. Structural and functional details of the chambers are in the appendix (Figure 1). The ambient conditions in the open top chamber, throughout the experiment were: a) for 400 µmol mol^{-1} were, average temperature of 26.71 ºC and average relative humidity of 77. 34% and b) for 700 µmol mol^{-1}, average temperature of 26.16 ºC and average relative humidity of 79.28%. The experimental design was completely randomized, with the treatments being supplemented with NaCl (0 mmol L^{-1} and 3 mmol L^{-1}) at the concentration of CO_2 (400 µmol mol^{-1}), being (C1S1 and C1S2), respectively, and with the supplementation of NaCl (0 mmol L^{-1} and 3 mmol L^{-1}) in the concentration of CO_2 (700 µmol mol^{-1}), being (C2S1 and C2S2), respectively.

2.2 Sowing, fertilization, irrigation and leaf nutrition analysis

Ten seeds were sown in 4 L pot (Citropote® type) filled with a substrate of 1:2 clay: sand (clay portion was latosol-red dystrophic soil and sand portion was washed sand). Physicochemical characterization of the substrate is shown in the appendix (Table 1SM).

The total fertilization for the cycle was based on the recommendation for upland rice (Alvarez et al., 2012): 10 g of ammonium sulphate ($(NH_4)_2SO_4$), 4 g of monoammonium phosphate, and 3.63 g of potassium nitrate (KNO_3) per plant (corresponding to 120 kg ha^{-1} of P_2O_5, 100 kg ha^{-1} of N, and 80 kg ha^{-1} of K_2O). The fertilization was fractionated and applied fortnightly, and the plants were fertilized until inflorescence emission, 60 days after seedling emergence, which corresponded to R4 (one or more flowers on the main stem panicle had reached anthesis) (Counce et al., 2000).

Irrigation was performed daily at 5 pm by using a weighing lysimeter with the aid of a scale to maintain a depth of 100% of the evapotranspiratory demand of the plant after determining the maximum water retention capacity of the substrate. The NaCl dose treatments were applied at the moment when the pots were filled with the substrate, avoiding a cumulative effect. The reagent used was NaCl (Synth, model PA-ACS), with a molecular weight of 53.44 g mol^{-1}.

To maintain the NaCl concentration, a transparent plastic dish was placed under each pot to store the excess water and the leached NaCl. At the time of irrigation, this solution was reintroduced into the pot. After 10 days of planting (in the seedling phase), thinning was performed to leave one individual per pot, which was considered an experimental unit.

Samples were collected from the shoots for foliar analysis in phenological phase V13 (formation of the collar on the 13th leaf of the main stem). The leaves were dried in an oven at 65°C and sent to the Laboratory of Plant Mineral Nutrition of the Soil Science Department of UFLA, where the chemical analysis of nitrogen (N), potassium(K), and sodium (Na) were performed according to the method described by Malavolta et al. (1997).

2.3 A/Ci Curve

The measurements were performed on a fully expanded flag leaf before the inflorescence emission (V13; Counce et al., 2000). The A/Ci curve (net CO_2 assimilation rate (A) versus calculated substomatal CO_2 concentration (Ci)) was evaluated under two oxygen conditions (2% oxygen through a mixture with inert nitrogen gas (N_2) and 21% oxygen under environmental conditions). The measurements of A/Ci curves were performed using LiCor LI-6800-XT, with leaf temperature of 30°C, photon flux density of 1500 μmol m^{-2} s^{-1} and the vapor pressure deficit between leaves and air of 2 kPa.

The curves were differently performed for each of the CO_2 treatments. For the C1S1 and C1S2 treatments, the curve was started at 400 μmol mol^{-1} of CO_2 and then reduced to 250, 150, and 50 μmol mol^{-1} of CO_2 with further increase to 400, 600, 800, 1000, 1200, 1500, and 1800 μmol mol^{-1} CO_2. For the C2S1 and C2S2 treatments, the initial point of the curve was of 700 μmol mol^{-1} of CO_2 with reduction to 500, 250, 150, and 50 and further increase to 700, 900, 1100, 1500, and 1800 μmol mol^{-1} CO_2. The Ethier and Livingston (2004). New version 11 December 2013 was used to obtain the maximum carboxylation rate of ribulose biphosphate carboxylase-oxygenase (Rubisco) (Vcmax, μmol CO_2 m^{-2} s^{-1}); the CO_2 assimilation rate limited by RuBP regeneration (light limited/RuBP limited) (J, mmol CO_2 m^{-2} s^{-1}); the leaf respiration in the light, also called day respiration (mitochondrial respiration related to growth and maintenance) (Rd, mmol of CO_2 $m^{-2}s^{-1}$) and the following calculated ratios J/Vc_{max}, J/Rd and Rd/Vc_{max}.

2.4 Light curve

The measurements were performed in the same flag leaf used for A/Ci curve, at the same developmental stage. Performed using LiCor LI-6800-XT, with leaf temperature of 30°C and the vapor pressure deficit between leaves and air of 2 kPa. The light curves (net CO_2 assimilation rate/photosynthetically active radiation, A/PAR) with 21% oxygen were performed at the Ci value of 400 µmol CO_2 mol $^{-1}$ for the treatments C1S1 and C1S2 and 700 µmol CO_2 mol^{-1} for the treatments C2S1 and C2S2. The photon flux density (PAR) value was changed stepwise: 2000, 1500, 1000, 600, 400, 200, 100, 60, 40, 20, and 0 photon flux density (µmol photon m^{-2} s^{-1}).

The following indices were obtained from the A/PAR curves: dark respiration (R_{dark}, mmol m^{-2} s^{-1}), apparent quantum yield (f, mol/mol), light compensation point (LCP, mmol m^{-2} s^{-1}), maximum net assimilation rate (A_{max}, mmol CO_2 m^{-2} s^{-1}) and curvature (q, admission). (Marshall e Biscoe, 1980; Thornley e Johnson, 1990).

2.5 Chlorophyll fluorescence

The measurements were performed using LiCor LI-6800-XT, in the same flag leaf used for A/Ci and A/PAR curves, at the same developmental stage. The fluorescence was measured in leaves adapted to the dark for 30 minutes. The maximum quantum yield of photosystem II (PSII, calculated as F_v/F_m).

2.6 Production of grain

Regarding the rice production component, the total weight of grains per plant was evaluated (13% moisture basis) (GWP), according to Alvarez et al. (2012).

2.7 Statistical analysis

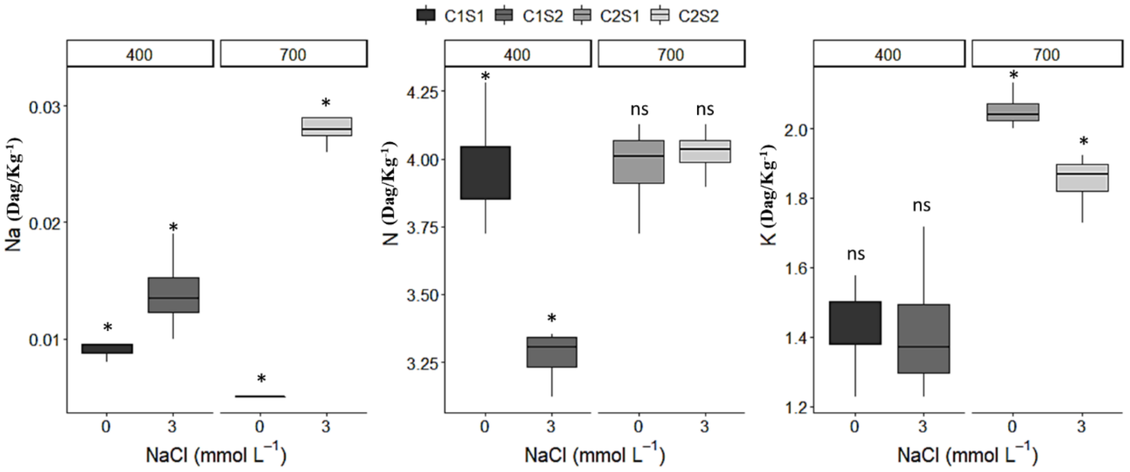
The biometric variables were analyzed in seven replicates, totalizing 28 plots. The leaf gas exchange variables were analyzed in four replicates, totalizing 20 plots. The variables obtained in the A/Ci and A/PAR curves, and chlorophyll fluorescence had four replicates, totalizing 20 plots. For the components of production, there were eight replicates, totalizing 32 plots. The experimental design was completely randomized, with the treatments: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S2). The data was submitted to the normality test (Shapiro-Wilk), homogeneity and analysis of variance. The mean values of each variable were compared using the t-student mean test using the minimum significant difference ($p < 0.05$, F test). The RStudio statistical software (version 3.5.3, 2019) was used.

3. Results

3.1 N, K and Na leaf content

In relation to leaf nutrient contents (figure 1). There was a significant difference for Sodium content ($p < 0.05$, F-test) being that the C1S2 treatment was greater than C1S1 and the C2S2 treatment was greater than C2S1. As for N, C1S1 was greater than C1S2, and there was no difference between C2S1 and C2S2. In relation to K, C1S1 and C1S2 did not differ from each other, but there was a difference in C2 conditions, with C2S1 being greater than C2S2.

Figure 1. Nutrient contents in V13: Leaf sodium content (Na); Leaf nitrogen content (N) (A) and leaf potassium content (K) (B) and leaf sodium content (Na) (C). The treatments were: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO$_2$ (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO$_2$ (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO$_2$ (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO$_2$ (C2S2).



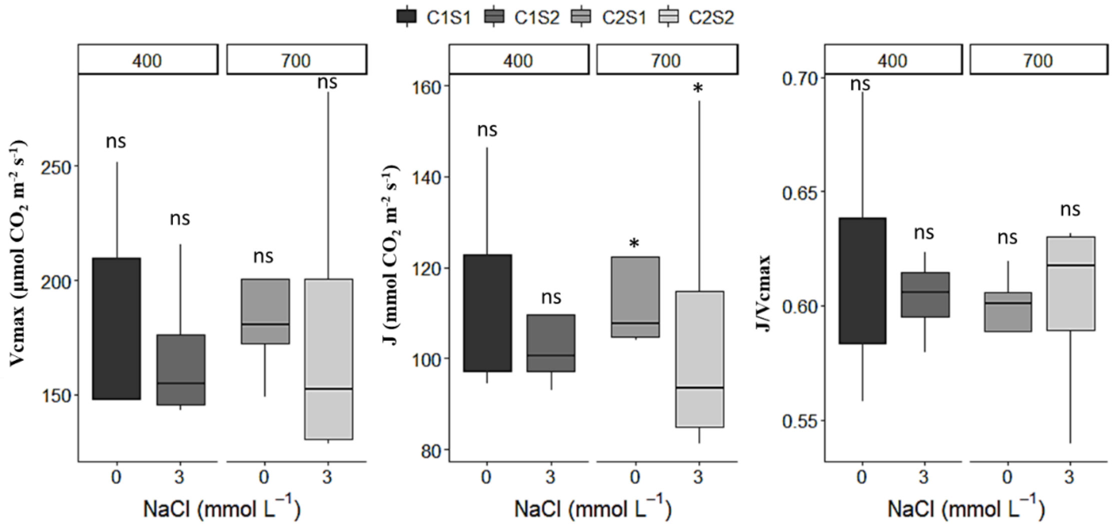
Data shown in Boxplot. The asterisk signifies a significant difference between the means at 5% significance and non-significant ns, by the t-student test. Value p(t)/p(W) of Na C1: 0.0264 and C2 0.01; p(t)/p(W) of N C1: 0.0008 and C2 0.3002 and p(t)/p(W) of K C1: 0.4785 and C2 0.0034.

3.2 Gas-exchange parameters

A representative average A/Ci curve at 2% of atmospheric oxygen is presented, in the appendix (Figure 2A). The photosynthetic parameters estimated by modelling of the curves are presented in figure 2.

The parameters Vcmax, J and J/Vcmax did not differ significantly ($p < 0.05$, F-test) for any of the factors. Rd and Rd/Vcmax did not differ for NaCl conditions, but showed a significant difference ($p < 0.05$, F-test) for CO_2 concentrations, with higher values observed for C1.

Figure 2. Photosynthetic parameters estimated by modeling the A/Ci curve under 2% oxygen conditions: Vcmax, J and J/Vcmax. The treatments used: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S2).



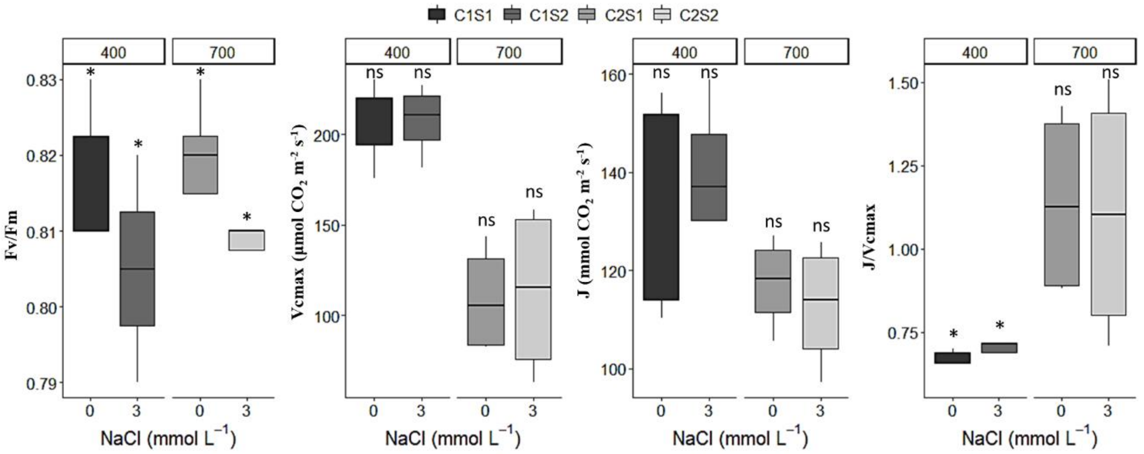
Data shown in Boxplot. The asterisk indicates significant difference between the means at $p<0.05$ and ns - non-significant, by the t-student test. Value *p*(t)/*p*(W) of Vcmax low O_2 C1: 0.27 and C2 0.38; *p*(t)/*p*(W) of J low O_2 C1: 0.32 and C2 0.1 and *p*(t)/*p*(W) of J/ Vcmax low O_2 C1: 0.4128 and C2 0.3865.

Under normal atmospheric conditions, with oxygen concentration at 21%, the representative average A/Ci curves are shown in the appendix (Figure 3A) and the estimated photosynthetic parameters in figure 3.

3.3 Chlorophyll fluorescence and photosynthetic parameters

For chlorophyll fluorescence (Fv/Fm), photosynthesis under conditions of 21% oxygen and the variable GWP are found in figure 4. There was a significant difference in Fv/Fm, with C1S1 being higher than C1S2 and in condition C2, C2S1 was greater than C2S2. The variables Vcmax and J did not show significant difference, but the J/Vcmax ratio in C1 was different, with C1S2 being greater than C1S1. In C2 there was no differences.

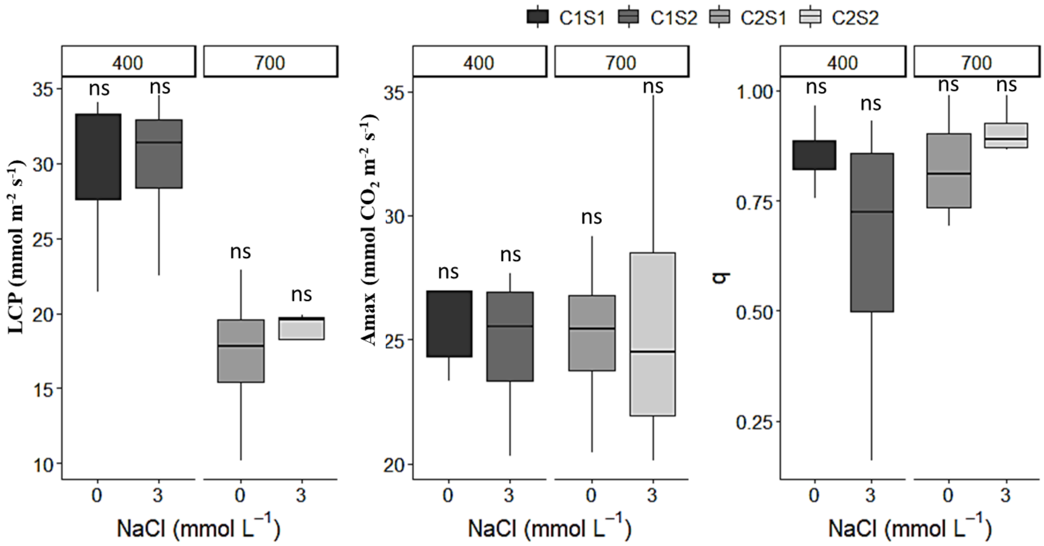
Figure 3. Chlorophyll fluorescence and photosynthetic parameters estimated by modeling the A/Ci curve under 21% oxygen: Vcmax, J and J/Vcmax. The treatments used: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S2).



Data shown in Boxplot. The asterisk indicates a significant difference between the means at p<0.05 and ns - non-significant, by the t-student test. Value *p*(t)/*p*(W) of Fv/Fm C1: 0.0854 and *p*(t)/*p*(W) C2: 0.0951; Value *p*(t)/*p*(W) of Vcmax C1: 0.4606 and C2 0.4471; *p*(t)/*p*(W) of J C1: 0.296 and C2 0.2974 and *p*(t)/*p*(W) of J/ Vcmax C1: 0.1 and C2 0.4468.

The variables obtained by the curve A/PAR are presented in the appendix (Figure 4A) and the results are found in figure 4. The LCP, Amax e q showed no significant difference between treatments.

Figure 4. Parameters obtained from the A/PAR curve under 21% oxygen. The treatments used: 0 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S2).

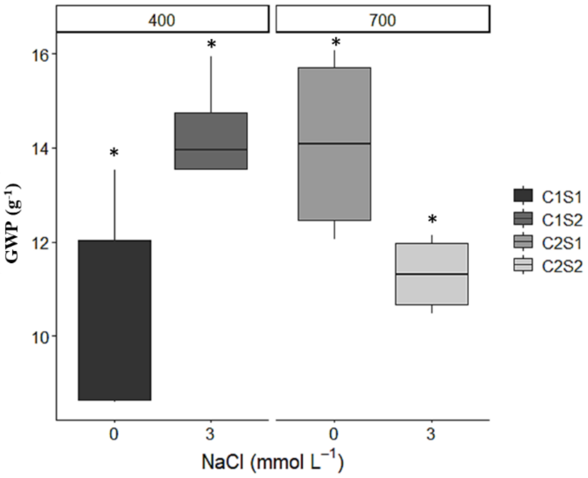


Data shown in Boxplot. Non-significant ns, by the t-student test. Value *p*(t)/*p*(W) of LCP C1: 0.4429 and *p*(t)/*p*(W) C2: 0.3493; Value *p*(t)/*p*(W) of Amax C1: 0.2741 and C2 0.4117; *p*(t)/*p*(W) of q C1: 0.1449 and C2 0.1481.

3.4 Production parameters

Regarding the production parameters, the weight of grains per plant (GWP) is shown in figure 5. There was a significant difference ($p < 0.05$, F-test) in C1, with C1S2 being greater than C1S1 and for C2, C2S1 was greater than C2S2.

Figure 5. Grain weight parameter per plant (GWP). The treatments used: 0 mmol L-1 NaCl and 400 µmol mol^{-1} CO_2 (C1S1); 3 mmol L^{-1} NaCl and 400 µmol mol^{-1} CO_2 (C1S2); 0 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S1) and 3 mmol L^{-1} NaCl and 700 µmol mol^{-1} CO_2 (C2S2).



Data shown in Boxplot. The asterisk indicates a significant difference between the means at 5% significance, by the t-student test. Value *p*(t)/*p*(W) of GWP C1: 0.0146 and *p*(t)/*p*(W) C2: 0.0229.

4. Discussion

The increase in CO_2 concentrations considerably influences the physiology of plants, especially those with C3 metabolism (Wang et al., 2019). Thus, our results contribute to broaden the knowledge about the physiological responses of C3 grasses to the elevation of atmospheric CO_2 and to conditions of low concentration of NaCl in the substrate. Our findings are important, especially in the context of forecasted atmospheric conditions for the coming years, as NaCl can influence nitrogen metabolism, with sodium (Na^+) acting as a cofactor in pyruvate transport, having a direct role in the regeneration of Phosphoenolpyruvate carboxylase (PEPC), which, in turn, provides carbon skeletons necessary for nitrogen assimilation, including ammonium assimilation and amino acid biosynthesis (Sauer & Eikmanns, 2005).

Although there are no studies on the relationship of NaCl with PEPC in rice plants, this influence has already been proven in grasses with C4 metabolism (Johnston et al., 1988; Kronzucker et al., 2013). Our results indicated that there was no change in the leaf N content, but there was an increase in K at the highest concentration of CO_2, and the C2S2 treatment had a greater amount of N than C2S1, demonstrating the importance of the presence of NaCl for assimilation and metabolism of nitrogen under current CO_2 conditions. Unlike the work (Kazemi et al., 2018) which showed a reduction in the content of leaf N and total chlorophyll under conditions of 700 µmol / mol of CO_2 under increasing doses of NaCl (30, 60 and 90 mmol. L-1). One of the justifications is that, in our work, rice plants were not under abiotic stress in relation to the production of reactive oxygen species.

Na^+ can act directly on several pathways exclusive to potassium. This allows the plant to allocate a greater amount of Na^+ in the vacuoles, favoring the flow of free K^+ ions to the cytosol, in order to be used in enzymatic activation and in various metabolic processes. It was possible to notice an increase in the K^+ content under conditions of increased CO_2 concentration (C2), when compared to C1 conditions (Kronzucker et al., 2013; Reddy et al., 2017).

The reduction in the metabolic demand of N under conditions of higher concentrations of CO_2 and presence of NaCl is related to lower photorespiration (Bloom, 2015). There was no change in the Rd values between treatments, demonstrating that the fundamental role in the production of rice grains was maintained, through greater efficiency in the use of photoassimilates, as it increases the carbon balance in the plant. Contrary to the results of (Wang et al., 2019) which showed an increase in Amax and Rd with an increase in CO_2 and temperature, our results were unfavorable for leaf photosynthesis, but showed gains in production, increased CO2 or supply of a low dose of NaCl.

Elevated CO2 increases the amount of substances that associate with the local catalyst of ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco), as found in the present work. Demonstrating that the gains were more in relation to the efficiency of allocation to the product of interest, which are grains, contradicting the work of Dusenge et al (2019) and Long (1991) that showed an increase in the total plant dry mass under high conditions of CO_2.

Under atmospheric conditions with 21% O_2, the J / Vcmax ratio was higher for the C1S2 treatment than for C1S1, however there was no difference in high CO_2. These results are directly related to the conditions imposed on the rice plant in a high CO_2 scenario, the J/Vcmax ratio is intrinsically related to Rubisco biosynthesis, and these results are corroborated by Ainsworth and Rogers (2007) and Wang et al. (2019), working on an external atmospheric CO_2 enrichment system (FACE) in soybean crop and in open top systems using rice crop, respectively.

Rubisco demands about 50% of all the nitrogen produced by the plant. A lower need for this enzyme motivated by greater activity can result in greater availability of nitrogen for other processes, allowing an increase in the efficiency of N use by the plant (Wang et al., 2019). In general, elevated CO_2 promotes the leaf photosynthetic rate of C3 species, but this positive effect diminishes with development (Cai et al., 2018; Chen et al., 2014). Regarding the parameters arising from the photosynthesis modeling through the A/PAR curve, which did not show any significant difference, our results are contrary to those found by Fahad et al. (2017). Demonstrating that the CMG-2085 strain was little influenced by the increase in CO_2.

Regarding chlorophyll fluorescence, the Fv / Fm ratio with NaCl supply decreased, however it did not harm the studied strain, as the results are within the patterns observed for most plant species in the absence of any abiotic stress and / or biotic (Rohacek and Bartak, 1999; Hunt, 2003).

One of the possible explanations for this is meeting the energy demands to supply grain production under high CO_2 conditions (Wang, et al., 2019). The variable GWP increased with the supply of low dose of NaCl under current conditions of CO_2 was evaluated, however there was a decrease under high CO_2, however this is due to the effects of the environment in these conditions that cause the plant to reduce transport via sodium xylem for aerial part and with that diminishing the positive effects of the supply of NaCl under high CO_2. Our results provide important information about the effects of climate change, specifically under high CO_2 conditions on photosynthesis and rice production.

5. Conclusion

It was possible to prove that a low dose of NaCl influences the photosynthetic and grain production processes of an upland rice line.

Declarations section

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of the manuscript, should the article be accepted by the Editor-in-chief upon completion of the refereeing process.

Availability of supporting data

The raw data which generated the presented results is available through request to the corresponding author.

Competing interests

The authors declare no competing interests.

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Authors' contributions

JPRADB, DAV, PERM, FBSB and MATH planned the research. DAV and MATH executed the experiment. DAV, JPP, MATH and JPRADB anlyzed the data. All the authors wrote the manuscript.

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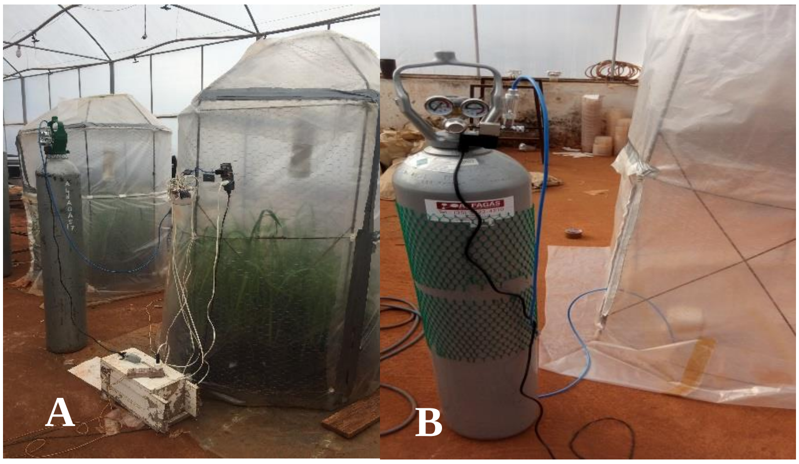
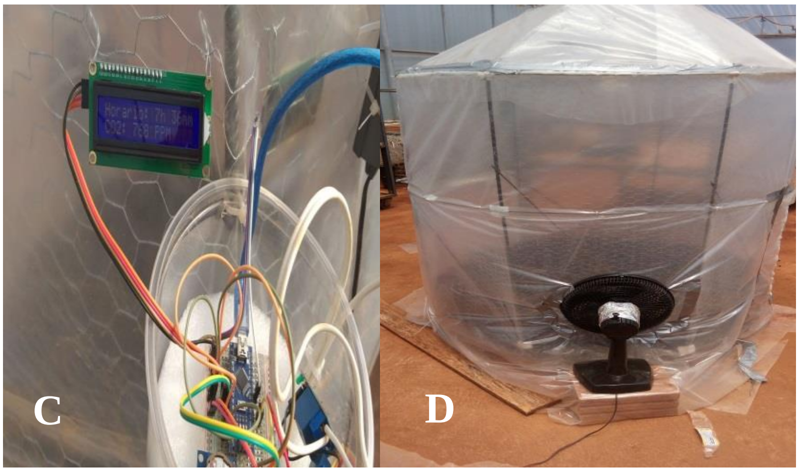
Appendix

Construction of the chamber, automation of the CO_2 supply and air circulation systems

Two open-top chambers with a circular shape were assembled with a structure composed by bars, arches and galvanized steel frame. The base had a diameter of 1.3 m and a circle area of 1.3273 m^2; the top with a diameter of 0.5 m and circle area of 0.1963 m^2; the height was of 1.8 m (Figure 1A).





Source: Vieira et al.(2020).

Figure 1A. Open top chambers (A); demonstration of the automated system with a pressure gauge and solenoid valve for CO_2 (B); CO_2 reading display inside the chamber, Arduino UNO microcontroller and relay (C); Fan (D).

The entire chamber was lined with two layers of transparent 100-micron plastic film for agricultural greenhouses, and the ends of the plastic were sealed with polyethylene tape, cotton fabric and rubber resin adhesive in order to prevent CO_2 leakage. The inner layer of plastic film had several holes with a diameter of 0.05 m and circle area of 0.0078 m^2 aiming to facilitate air circulation, that was provided by a 30 cm table fan with the characteristics of a circulation area of 20 m, power of 50W, voltage of 110V and six propellers.

An automated system (Figures 1B and 1C) was set up to control the amount and distribution of CO_2 inside the open top chamber. The CO_2 amount was read by an infrared CO_2 sensor (MH-Z14), connect to a microcontroller (arduino-uno), which was calibrated with base on another sensor (SBA-5 CO_2 Gas Analyzer brand PP system). When the reading of the CO_2 amount inside the open top chamber was below 700 ppm CO_2, the arduino-uno sent an analog signal to a two-channel electromagnetic switch (relay) to turn on the solenoid valve and if it exceeded the 700 ppm CO_2 range, the solenoid valve was turned off to optimize the system. The valve was coupled to a 25kg CO_2 cylinder and the solenoid valve outlet had a hose connected with an open top chamber to supply CO_2. The hose exit was placed in front of the fan in order to improve the circulation and homogenization of CO_2.

There was also the installation of a fan (Figure 1D) for circulating and homogenizing the CO_2 which was also controlled by an (arduino-UNO) board that sent an analogical signal to a two-channel relay. The fans worked during the day without interruption from 6:00 am to 6:00 pm and at night they were activated every 30 minutes for a period of 5 minutes of operation to maintain air circulation.

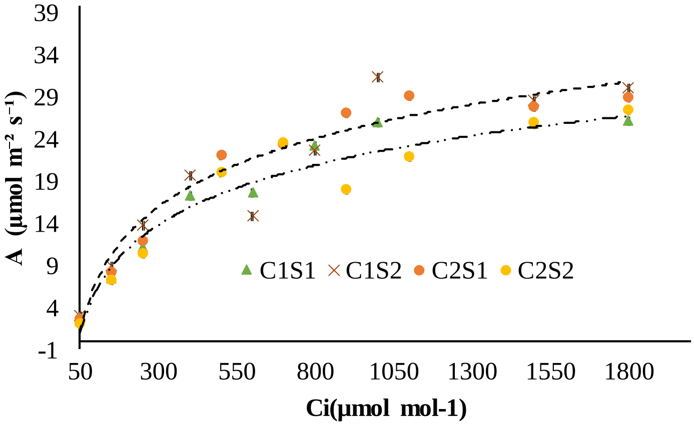


Figura 2A. Net photosynthesis vs intercellular CO_2 concentration (A/Ci), based on averages values, under conditions of 2% O_2 in the air, for the combination of treatments: 400 µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C1S1); 400 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C1S2); 700 µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C2S1); and 700 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C2S2).

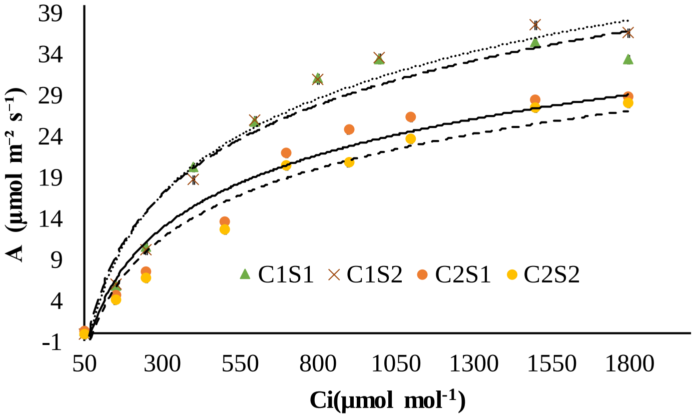


Figura 3A. Net photosynthesis vs intercellular CO_2 concentration (A/Ci), based on averages values, under conditions of 21% O_2 in the air, for the combination of treatments: 400 µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C1S1); 400 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C1S2); 700

µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C2S1); and 700 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C2S2).

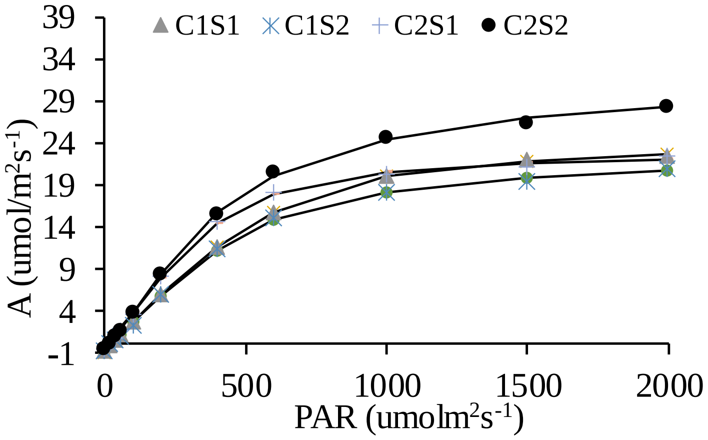


Figura 4A. Net photosynthesis vs photosynthetic active radiation (A/PAR), based on averages values, for the combination of treatments: 400 µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C1S1); 400 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C1S2); 700 µmol mol^{-1} CO_2 and 0 mmol L^{-1} NaCl (C2S1); and 700 µmol mol^{-1} CO_2 and 3 mmol L^{-1} NaCl (C2S2).

Table 1SM. Physicochemical analysis of the substrate used in the experiment

pH(Kcl)	**Ph**	**K**	**P**	**Na**	**Ca**	**Mg**	**Al**	**H+Al**
		-------	Mg/dm^3	-------	-------	cmolc/dm^3	----	-------
6.49	6.2	63.49	14.03	0.01	1.52	0.22	0.05	1.00
SB	**T**	**T**	**V**	**M**	**M.O.**	**P-Rem**	**Zn**	**Fe**
	cmolc/dm^3		---%---		Dag/Kg	Mg/L		
1.90	1.95	2.90	65.61	2.56	0.72	21.79	3.57	59.81
Mn	**Cu**	**B**	**S**	**Argila**	**Silte**	**Areia**		
28.70	18.64	0.13	54.31	35	4	61		

FINAL CONSIDERATIONS

In the first article, our hypothesis that increasing NaCl doses beyond a threshold would affect germination, survival, gas exchange and production was met. Rice lines L6 (CMG 2085), L7 (CMG F6 LAV1-7) and L8 (CMG 1896) were the most tolerant to salt, with CMG 2085 standing out for having the highest grain weight per plant. Of the dosages tested, 3 mmol. L^{-1} of NaCl increased germination and did not affect survival, gas exchange and rice production.

In the second article, our hypothesis that a low dose of NaCl (3 mmol. L-1 of NaCl) would be beneficial for gas exchange was met under 400 µmol mol^{-1} CO_2 conditions, however it was not met at 700 µmol mol^{-1} CO_2 as it did not affect the parameters of photosynthesis modeling. The supply of NaCl under current conditions of CO_2 decreased the N content and that may have contributed to a greater allocation of photoassimilates to the grains, as this treatment had higher GWP than the control treatment. However, at high CO_2 there was no change in the N content with the supply of NaCl, which may have contributed to the performance in GWP reduction, not meeting our hypothesis, as an increase in GWP was expected. Our work is relevant due to the importance of developing cheap technologies and through the selection of rice genotypes and the use of a beneficial dose of NaCl to ensure food security in a future scenario of uncertainties regarding the effects of climate change.