**FEDERAL UNIVERSITY OF PELOTAS**
**Institute of Biology**
**Post-Graduate Program in Plant Physiology**

Thesis

# Metabolic adaptations of soybean plants submitted to recurrent periods of root system hypoxia associated with nitrate application

Darwin Alexis Pomagualli Agualongo

Pelotas, 2021

**UNIVERSIDADE FEDERAL DE PELOTAS**
**Instituto de Biologia**
**Programa de Pós-Graduação em Fisiologia Vegetal**

Tese

# Adaptações metabólicas de plantas de soja submetidas a períodos recorrentes de hipóxia do sistema radicular e associados à aplicação de nitrato

**Darwin Alexis Pomagualli Agualongo**

Pelotas, 2021

Universidade Federal de Pelotas / Sistema de Bibliotecas

Catalogação na Publicação

A281m Agualongo, Darwin Alexis Pomagualli

Metabolic adaptations of soybean plants submitted to recurrent periods of root system hypoxia associated with nitrate application / Darwin Alexis Pomagualli Agualongo ; Luciano do Amarante, orientador ; Ana Claudia Barneche de Oliveira, Denise Colares de Oliveira, coorientadoras. — Pelotas, 2021.

206 f.

Tese (Doutorado) — Programa de Pós-Graduação em Fisiologia Vegetal, Instituto de Biologia, Universidade Federal de Pelotas, 2021.

1. *Glycine max*. 2. Waterlogging. 3. Antioxidant activity. 4. Fermentative metabolism. 5. Priming/stress memory.. I. Amarante, Luciano do, orient. II. Oliveira, Ana Claudia Barneche de, coorient. III. Oliveira, Denise Colares de, coorient. IV. Título.

CDD : 633.34

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

**Darwin Alexis Pomagualli Agualongo**

# Metabolic adaptations of soybean plants submitted to recurrent periods of root system hypoxia associated with nitrate application

**Thesis submitted to the Post-Graduate Program in Plant Physiology, Institute of Biology, Department of Botany, at the Federal University of Pelotas, as partial requirement for obtaining the title of Doctorate in Plant Physiology.**

Advisor: Prof. Dr. Luciano do Amarante

Co-Advisor: Pesq. Dra. Ana Claudia Barneche de Oliveira

Pesq. Dr. Giovani Greigh, de Brito

Prof. Drª. Denise Colares de Oliveira

Pelotas de 2021

Darwin Alexis Pomagualli Agualongo

Metabolic adaptations of soybean plants submitted to recurrent periods of root system hypoxia associated with nitrate application

**Thesis approved as partial requirement for obtaining the degree of Doctor of Plant Physiology, Post-Graduate Program in Plant Physiology, Department of Botany, Institute of Biology, Federal University of Pelotas.**

Date of Defense: 22-01-2021.

Examining committee:

_____

Dr. Luciano, do Amarante

PhD in Plant Biology from the State University of Campinas (UNICAMP).

_____

Dr. Amanda Avila Cardoso

PhD in Plant Physiology from the Federal University of Viçosa (UFV)

_____

Dr. André Luis Thomas

PhD in Plant Biology from the State University of Campinas (UNICAMP)

_____

Dr. Halley Caixeta de Oliveira

PhD in Functional and Molecular Biology from the State University of Campinas (UNICAMP)

_____

Dr. Junior Borella

PhD in Plant Physiology from the Federal University of Pelotas (UFPel).

To my family, Pomagualli - Agualongo

To my mother Flor Hortencia Agualongo

To my brothers and nephews

To my uncles Julio, Alberto, Ana, Aurora, Olga

To Sonia Toapanta

**OFFER AND DEDICATION**

**Acknowledgements**

I thank God and Pachamama, for life and health.

I could write many concrete thanks to the people who during these years (or during all my life as my family) gave me their support and collaboration that was not little (colleagues and friends...).

Even so I would like to express my gratitude to Prof. Dr. Luciano do Amarante, for his understanding when working, for his advice and patience and of course in the way of organizing the group, in this group we work as a team and we all help and support each other

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarship. The Federal University of Pelotas (UFPel) and the Graduate Program in Plant Physiology (PPGFV) for the opportunity to perform the PhD in a public, free and quality institution. To the teachers of the PPGFV for the knowledge passed on and for the contribution in my training.... To colleagues, for the moments of companionship, collaboration and support ... Thank you!

To the researcher. Dr Ana Claudia Barneche de Oliveira, co-supervisor, of EMBRAPA, for the support and help in conducting experiments and moments of teaching and friendship ... To the staff of the 'Celeiro' of ETB for the moments of relaxation and help during the field experiments. Thank you very much!

To my colleagues and friends from the Plant Biochemistry Laboratory for helping in the development of the analyses and for the moments of friendship and understanding: Fabiane, Natália, Kassia, Angelita, João Victor, Rafael, Renan, Shimoia, Cristiane, Tamires, Fernanda, Douglas,

To all the people who, in one way or another, collaborated in the realization of this dream and who encouraged me in the search for the full realization of my ideals THANK YOU!!! from the bottom of my heart. To those who did not, I encourage you to start, because it is never too late to participate in the improvement of society.

"**Education is the most powerful weapon you can use to change the world**."

Nelson Mandela.

## Abstract

POMAGUALLI, Darwin Alexis Agualongo. **Metabolic adaptations of soybean plants, submitted to recurrent periods of hypoxia of the root system and associated with nitrate application.** 2021. 206f. Thesis (Ph.D.) – Post-Graduate Program in Plant Physiology, Institute of Biology, Department of Botany, Federal University of Pelotas, Pelotas, 2021.

The increase in unpredictable rainfall associated with limited soil drainage often causes flooding or waterlogging due to climate change, altering the soil aeration which results in oxygen deficiency for plant roots, and the change from aerobic t o anaerobic respiration, reducing cellular energy status, in addition to oxidative stress and reduced gas exchange. Waterlogging stress has often been transient and recurrent. Plants have the ability to retain information about a past stress signal and possibly result in a modified response to recurrent stress, or a sustained response to future exposure through memory acquisition. Recent studies have shown that nutrition with $NO_3$- increases nitric oxide levels under hypoxia and can reduce fermentative enzymes activities and the production of reactive oxygen species in waterlogged plants. Considering the above, the objectives of this work were: I- to explore the mechanisms of a possible "priming" of a previous waterlogging carried out at the vegetative stage of soybean plants (V3) to alleviate the negative stress effect of a second waterlogging carried out at the reproductive stage (R2) and II- to evaluate if the application of nitrate during stress events (stages V3 and R2) can improve the tolerance of the plant to waterlogging through the "priming capacity" in plants cultivated without the supply of nitrate (nodulated plants). For this purpose, two experiments were conducted with soybean plants [*Glycine max* (L.) Merril genotype BR11-6042] grown in the field, in boxes (1 x 1 x 2 m), containing soil. Experiment I- Treatments: Control (non-waterlogged plants) and waterlogging, in plants at stage V3 and R2, separated into three groups: waterlogging at V3 stage for seven days (V3 group); waterlogging at V3 stage (primary treatment) and later at R2 stage for five days (V3R2 group) and waterlogging only at R2 stage for five days (R2 group). The waterlogging was carried out by keeping a layer of water on the ground. Experiment II- Treatments: Flooding without nitrate supply, V3R2 and R2 groups and flooding with nitrate supply during the flooding, groups V3R2N and R2N, with their respective control (with and without nitrate application). In each box, 25.78g of $KNO_3$ was applied at a time, three days before the flooding and three days after the start of the waterlogging. The waterlogging was performed according to experiment I. They following measurements were carried out during waterlogging and reoxygenation periods: Gas exchange (liquid $CO_2$ assimilation, transpiration and stomatal conductance), photosynthetic pigment concentrations, antioxidant enzyme activities (SOD, APX, CAT, GPOD and DHAR), hydrogen peroxide contents and lipid peroxidation, concentrations of total soluble amino acids (TAA), total soluble sugars (TSS), sucrose in leaves and roots, fermentative enzyme activity (LDH, PDC and ADH) and AlaAT in roots, nitrate content and NR activity in leaves and roots, and yield components. In all variables analyzed, V3R2 plants under hypoxic and reoxygenation condition present memory responses to stress, effectively improving photosynthetic efficiency, antioxidant capacity by reducing oxidative damage, faster recovery from fermentation metabolism, resulting in lower activities of LDH, PDC, ADH and AlaAT enzymes;

availability of TSS, sucrose, TAA and nitrate is favored and yield components are increased compared to R2 plants. Nitrate apparently improves the plants' tolerance to flooding (groups R2 and V3R2) without, however, nullifying the effects obtained by "priming by flooding" conducted at V3 stage. Thus, groups V3R2 and V3R2N showed the main metabolic aspects that were modified as a result of recurrent stress treatments in improving tolerance to hypoxia, providing a better understanding of the processes involved in the "priming effect" in increasing the tolerance of soybean plants to waterlogging.

Keywords: *Glycine max*; waterlogging; fermentative metabolism; antioxidant activity; priming/stress memory.

## Resumo

POMAGUALLI, Darwin Alexis Agualongo. **Adaptações metabólicas de plantas de soja, submetidas a períodos recorrentes de hipóxia do sistema radicular e associados à aplicação de nitrato.** 2021. 206f. Tese (Doutorado em Fisiologia Vegetal) – Programa de Pós-Graduação em Fisiologia Vegetal, Departamento de Botânica, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, 2021.

O aumento de chuvas imprevisíveis associadas à drenagem limitada dos solos, frequentemente causam inundações ou alagamento devido às mudanças climáticas, alterando o estado de aeração do solo que resulta em deficiência de oxigênio para as raízes das plantas, e a mudança da respiração aeróbica para anaeróbica, reduzindo o status energético celular, além de desencadear o estresse oxidativo e a redução das trocas gasosas. O estresse por alagamento tem sido muitas vezes transitório e recorrente. As plantas têm a capacidade de reter informações sobre um sinal de estresse passado e possivelmente resultar em uma resposta modificada ao estresse recorrente, ou uma resposta sustentada para futuras exposições por meio da aquisição de memória. Estudos recentes revelaram que a nutrição com $NO_3^-$ aumenta os níveis de óxido nítrico sob hipóxia, podendo reduzir a atividade das enzimas fermentativas e a produção de espécies reativas de oxigênio em plantas alagadas. Considerando o exposto, os objetivos deste trabalho foram: I- explorar os mecanismos de um possível "priming" de um alagamento prévio realizado no estádio vegetativo de plantas de soja (V3) para aliviar o efeito negativo do estresse de um segundo alagamento realizado na fase reprodutiva (R2) e II- avaliar se a aplicação do nitrato durante os eventos de estresse (estádios V3 e R2) pode melhorar a tolerância da planta ao alagamento através da "capacidade de priming" em plantas cultivadas sem o fornecimento de nitrato (plantas noduladas). Para isso, dois experimentos foram conduzidos com plantas de soja [(*Glycine max* (L.) Merril genótipo BR11-6042] cultivadas a campo, em caixas (1 x 1 x 2 m,) contendo solo. Experimento I- Tratamentos: Controle (plantas não alagadas) e alagamento, em plantas no estádio V3 e R2, separadas em três grupos: alagamento no estádio V3 durante sete dias (grupo V3); alagamento no estádio V3 (tratamento primário) e posteriormente no estádio R2 por cinco dias (grupo V3R2) e alagamento apenas no estádio R2 por cinco dias (grupo R2). O alagamento foi realizado por meio da manutenção de uma lâmina de água sobre o solo. Experimento II- Tratamentos: Alagamento sem fornecimento de N, grupos V3R2 e R2 e alagamento com fornecimento de nitrato durante o alagamento, grupos V3R2N e R2N, com o seu respectivo controle (com e sem aplicação de nitrato). Em cada caixa, foi aplicado por vez, 25,78g de $KNO_3$, três dias antes do alagamento e três dias após o início do alagamento. O alagamento foi realizado conforme experimento I. Foram avaliados: trocas gasosas (assimilação líquida de $CO_2$, transpiração e condutância estomática), concentrações de pigmentos fotossintéticos, atividades de enzimas antioxidantes (SOD, APX, CAT, GPOD e DHAR), teores de peróxido de hidrogênio e peroxidação lipídica, concentrações de aminoácidos solúveis totais (TAA), açúcares solúveis totais (TSS), sacarose em folhas e raízes, atividade de enzimas fermentativas (LDH, PDC e ADH) e AlaAT

em raízes, teor de nitrato e atividade da enzima NR o em folhas e raízes, e componentes de rendimento . Em todas as variáveis analisadas, as plantas V3R2 em condição de hipóxia e reoxigenação apresentam respostas de memória ao estresse, melhorando efetivamente a eficiência fotossintética, a capacidade antioxidante ao reduzir os danos oxidativo, a recuperação mais rápida do metabolismo fermentativo, resultando em menores atividades da enzima LDH, PDC, ADH e AlaAT; a disponibilidade de TSS, sacarose, TAA e nitrato é favorecida e os componentes de rendimento são aumentados em comparação a plantas R2. O nitrato aparentemente melhora a tolerância das plantas ao alagamento (grupos R2 e V3R2) sem, no entanto, anular os efeitos obtidos pelo “priming por alagamento” conduzido no estádio V3. Assim, os grupos V3R2 e V3R2N mostraram os principais aspectos metabólicos que foram modificados como resultado dos tratamentos de estresse recorrente na melhoria da tolerância à hipóxia, fornecendo uma melhor compreensão dos processos envolvidos no “efeito priming” em aumentar a tolerância de plantas de soja ao alagamento.

Palavras-chave: *Glycine max*; alagamento; estádio vegetativo; metabolismo fermentativo; atividade antioxidante; priming/memória ao estresse.

List of figures

**General Introduction**



**Chapter I - Changes in physiological and biochemical mechanisms of hypoxia tolerance in soybean plants submitted to recurrent waterlogging stress.**

## List of Tables

## List of Abbreviations

ADH - alcohol dehydrogenase

Ala-AT - alanine aminotransferase

ANOVA - analysis of variance

APX - ascorbate peroxidase

ATP - adenosine triphosphate

EDTA - ethylenediaminetetraacetic acid

$H_2O_2$ - hydrogen peroxide

HCl - hydrochloric acid

LDH - lactate dehydrogenase

MDA - malondialdehyde

N – nitrogen

$NAD^+$ - nicotinamide adenine dinucleotide

NBT - nitro-tetrazolium blue

$NO_3^-$ - nitrate

$O_2^{\bullet-}$ - superoxide

PDC - pyruvate decarboxylase

PVPP - polyvinylpolypyrrolidone

R - recovery/reoxygenation

RN - recovery/reoxygenation with nitrate

ROS - reactive oxygen species

SOD - superoxide dismutase

SUC - sucrose

TAA - total amino acids

TBA - thiobarbituric acid

TCA - trichloroacetic acid

TSS – total soluble sugars le

W - flooded

WN - flooded with nitrate

## Summary

## 1. General Introduction

Soybean [*Glycine max* (Merr.) L.], is an important leguminous crop widely used for human food, animal feed, biofuel production and many other products due to its high protein and edible oil content, and lower carbohydrate content than other leguminous crops. Global soybean production is approximately 341 million tonnes worldwide (UNITED STATES DEPARTMENT OF AGRICULTURE, 2020).

Abrupt changes in climate conditions are posing a potential threat to biodiversity, increasing the likelihood of extreme episodes of drought and flooding in many regions of the globe (DONAT et al., 2016). Unpredictable and untimely rainfall or poor drainage often causes flooding or submergence by altering the aeration state of the soil, replacing it with water (ZHANG et al., 2019c). Waterlogging is a widespread phenomenon that drastically reduces soybean growth and production in many regions of the world (SOSBAI, 2018; VAN NGUYEN et al., 2017), mainly due to the occurrence of flat topography (COLLAKU; HARRISON, 2002), high water tables and poor drainage of clayey soils (JITSUYAMA, 2017). In the case of southern Brazil, soybean cultivation in lowland regions has expanded as an alternative in rotation with irrigated rice cultivation (AGOSTINETTO et al., 2009; PEDÓ et al., 2015). To address these challenges, significant efforts have been made by researchers to elucidate stress signalling pathways in response to individual stress. While under natural growing conditions, plants are often exposed to more than one stress simultaneously or sequentially, these stresses have synergistic or antagonistic effects on each other due to extensive 'cross talking' in underlying signalling cascades (GUPTA et al., 2016; SHARMA et al., 2013). Its effect can be even greater in highly susceptible crop species; soybean plants, can lose up to 93% of their yield when exposed to soil waterlogging during the vegetative stage (ALAM et al., 2010; BOARD, 2008b). Moreover, it drastically limits growth and yield in many regions of the world (MUSTAFA; KOMATSU, 2014; TOUGOU et al., 2012a; VAN NGUYEN et al., 2017), especially during the vegetative and seed germination stages (GITHIRI et al., 2006; PHUKAN; MISHRA; SHUKLA, 2016).

Soil flooding and waterlogging cause $O_2$ suppression in submerged organs, leading to the triggering of hypoxic stress in plants. Thus, the survival of plant species or the development of tolerance to $O_2$ deficiency depends on a

series of adaptive mechanisms that occur in three stages. First, the plant rapidly induces a series of signal transduction components. Next, metabolic adaptations are made, involving the primary carbon and nitrogen pathways, and finally, depending on the tolerance of the species, there is the development of morphological changes such as aerenchyma and/or adventitious root formation (BAILEY-SERRES; VOESENEK, 2008; GIUNTOLI; PERATA, 2018; LIU; RENNENBERG; KREUZWIESER, 2014; THOMAS; GUERREIRO; SODEK, 2005).

During waterlogging, the low oxygen diffusion rate induces oxygen deficiency of the soil environment, resulting in limited oxygen availability for plant roots and soil microorganisms (BALAKHNINA, 2015) and a shift from aerobic to anaerobic respiration (VOESENEK; BAILEY-SERRES, 2013), which causes cytosol acidification in plant cells, leading to inhibition of aquaporins and consequently limits water uptake by roots and hence hydraulic conductivity (HEMANTARANJAN, 2014; TOURNAIRE-ROUX et al., 2003a). Oxygen deficiency in general induces a rapid reduction in the rate of photosynthesis that is generally considered as a result of reduced stomata opening; decreased leaf chlorophyll content, early leaf senescence and a reduction in leaf area can also contribute to the inhibition of photosynthesis, thus affecting all growth components (ANDRADE et al., 2018; GARCIA et al., 2020). In fact, waterlogging tends to reduce the translocation of the product of photosynthesis from the leaf to the root, as in submerged roots the demand for carbohydrates for respiration would be greatly reduced, the carbohydrate translocation from the leaves to the roots would be minimal or non-existent, resulting in reduced carbon accumulation in the leaves and progressive carbohydrate depletion in the roots (YORDANOVA; CHRISTOV; POPOVA, 2004). . As a consequence plants suffer oxidative stress (HASANUZZAMAN et al., 2012) and decreased energy status (TAMANG; FUKAO, 2015; WANY et al., 2019). Biochemical adaptations to hypoxia include the induction of anaerobic metabolic pathways and protective enzymes for the elimination of phytotoxic by-products (EVANS; GLADISH, 2017), essential for the survival of plants under flooded conditions.

Thus, when the $O_2$ supply is insufficient for aerobic respiration, the fermentative metabolism of the roots is activated by pyruvate accumulated due to glycolysis (BORELLA et al., 2019), and the fermentative enzymes use it as

an electron acceptor for the reoxidation of cytosolic NADH, allowing the continuity of glycolysis and the production of ATP at the substrate level, leading to the production of lactate, by the action of the enzyme lactate dehydrogenase (LDH). This process is transient, since the accumulation of lactate leads to a rapid decrease in intracellular pH and with this, a shift from lactic fermentation to alcoholic fermentation occurs, since the enzymes for these metabolic pathways differ from the ideal pH range for their greatest activity (MORALES-OLMEDO; ORTIZ; SELLÉS, 2015), and under acidic pH conditions LDH is inhibited and the enzyme pyruvate decarboxylase (PDC) is activated. Thus, pyruvate is converted to ethanol through two subsequent reactions catalysed by PDC and alcohol dehydrogenase (ADH) (ZABALZA et al., 2009a; ZHANG et al., 2017). Thus, waterlogging and ultimately anaerobic metabolism potentially result in acute growth inhibition, accumulation of toxic products (such as lactate) and even death in most crops due to energy limitation.

Plants have developed a complex defence system that aims to minimize or suppress the harmful effects of reactive oxygen species (ROS), including enzymatic components such as the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX) and guaiacol peroxidase (GPOD), and non-enzymatic components, e.g. ascorbate (AsA) glutathione (GSH); tocopherols; and carotenoids (BAILEY-SERRES; LEE; BRINTON, 2012; BLOKHINA; FAGERSTEDT, 2010; GILL; TUTEJA, 2010). Under normoxic conditions, the antioxidant defence system provides adequate protection against active oxygen and free radicals. However, under stress situations, the balance between ROS production and elimination may be disturbed and the response becomes moderate or low (GILL; TUTEJA, 2010). The activity of the components of this system varies greatly with the duration of the stress and the genotype of the plant, among others (LORETI; VAN VEEN; PERATA, 2016).

In the course of evolution, plants have evolved a multitude of mechanisms to cope with environmental stresses, adapting to various ranges of biotic and abiotic stresses. They respond to this variability and unpredictability of stresses by adapting genotypic and phenotypic traits, especially when stress occurs regularly (HILKER; SCHMÜLLING, 2019a) because they have 'stress memory' capacity, i.e. priming capacity involving previous exposure to a stressor for better

plant responses to subsequent stress exposure (BRUCE et al., 2007; MARTINEZ-MEDINA et al., 2016), i.e. prior induction modifies a plant for future exposure to stress (CONRATH et al., 2015; HILKER et al., 2016). When there is an induction in the primary state, plants respond to the stress stimulus by activating stress resistance mechanisms, compared to plants in the innate (unaffected) state (LÄMKE; BÄURLE, 2017a), demonstrating that the process of priming (or hardening) by exposure to abiotic stresses can prepare their tolerance to waterlogging (LI et al., 2012; WANG et al., 2016), cold, drought or osmotic stress (BRUCE et al., 2007; MUTAVA et al., 2015b; WALTER et al., 2013), salinity (PARRA et al., 2007) and stress combinations (SUZUKI et al., 2014).

However, in a first stress, the experience can be the organism's main tool for a better response to the subsequent stress (LÄMKE; BÄURLE, 2017a). Plant tolerance to flooding has the plasticity to adjust its physiology resulting in metabolic, morphological and anatomical acclimation or genotypic adaptation to stress, which is advantageous, especially when stress occurs regularly (VOESENEK, 2013). Plants can cope with this variability and unpredictability of stress occurrence by genotypic and phenotypic plasticity (AUGE et al., 2017) that facilitates gas exchange between submerged organs and ambient air, conservation of energy and carbohydrates to prolong survival to flooding and allow growth during recovery when flooding ceases (COLMER; VOESENEK, 2009). Currently, there is a great interest in knowing more about this phenomenon, since the knowledge about the mechanisms involved in memory and its regulation is still limited, and a deepening of this subject would allow us to better understand the systems that plants have to face under different stresses (HILKER et al., 2016; LI; LIU, 2016). Moreover, this phenomenon is of great interest, not only in the agronomic field to "train" plants for future stresses and increase resistance and therefore production, but also in the environmental field to improve the management of water resources in natural ecosystems.

Nitrogen (N) has a major impact on all the above processes due to its importance for plants in the biosynthesis of amino acids and many secondary metabolites; its level in soil varies greatly, while the form of available N has a substantial impact on growth, development and biotic stress (GUPTA et al., 2013; POSSO et al., 2020). An important mechanism of plant survival during hypoxia is the cycle involving non-symbiotic hemoglobin (phytoglobin; Pb) and nitric oxide

(NO) which leads to the formation of methemoglobin and nitrate ($NO_3^-$) (GUPTA, 2020; PUCCIARIELLO; PERATA, 2017a; SINGH; BHATLA, 2019).

It is important to note that plants are able to assimilate inorganic nitrogen in the form of nitrate or ammonium, so that the complexity of NO biosynthesis in plants compared to other eukaryotic organisms is possibly due to the differences between them in nitrogen metabolism (MAIA; MOURA, 2014), and can be processed by both root and leaf cells. Nitrate is used by higher plants in various processes, including absorption, storage in vacuoles, transport through the xylem, reduction and incorporation into organic forms (LASA et al., 2002). Under periods of waterlogging plants to survive activate several mechanisms such as the cycle involving non-symbiotic hemoglobin (phytoglobin; Pb) and nitric oxide (NO) dependent of nitrate (GUPTA et al., 2020; WANY; KUMARI; GUPTA, 2017) (Figure1).

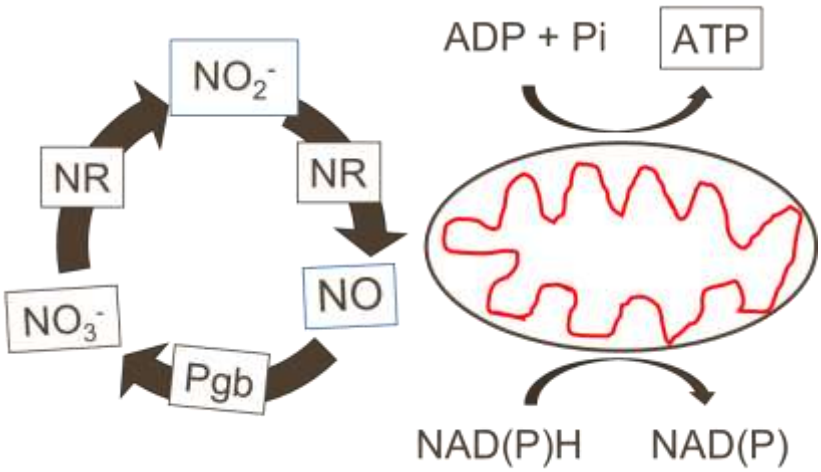


Figure 1. Model describing the effects of hypoxia in the presence of Pb, the effects of hypoxia can be alleviated, resulting in improvement of energetic status, reduced ROS and absence of programmed cell death (PCD).

Non-symbiotic hemoglobins or phytoglobins (Pb) have oxygen and NO transport functions in processes related to cell division and growth, as well as hypoxia stress responses (MANRIQUE-GIL et al., 2020; MIRA; HILL; STASOLLA, 2016; PERAZZOLLI et al., 2004). Previously, it has been shown that $NO_3^-$ levels influence NO production through the production of nitrite, which can be transported to the mitochondria where it can be reduced to NO at complex III and IV sites and by alternative oxidase (GUPTA; IGAMBERDIEV, 2016). Nitrate nutrition modulates alternative pathways that result in reduced ROS production

and oxidative stress and increases ATP levels (DA-SILVA; DO AMARANTE, 2020a; IGAMBERDIEV, 2004). On the other hand, it increases the levels of alanine aminotransferase (Ala-AT) enzyme activity, while decreasing lactate and ethanol levels in soybean plants under hypoxic stress (DA-SILVA; DO AMARANTE, 2020a; POSSO et al., 2020).

Therefore, the search for mechanisms to increase plant tolerance to water excess in soil with natural drainage deficiency has shown increasing importance. It may also be possible to distinguish hypoxia tolerance mechanisms by identifying physiological and biochemical changes resulting from exposure to these environmental conditions, which will contribute to the characterisation and generation of more adapted cultivars, aiming to a better understanding of the hypoxia influence on plant metabolism at different stages of phenological development, thus making the current production model in these areas, largely occupied by irrigated rice monoculture, more efficient.

The objective of this study was to explore the possible stress priming mechanisms of a previous waterlogging performed at the V3 stage to alleviate the negative stress effect of a second waterlogging performed at the R2 stage, favouring the evolution of priming and to evaluate how nitrate application could improve the metabolic mechanisms of plant adaptation to hypoxia favouring priming capacity. The results should help us to better understand some physiological mechanisms and important biochemical events during hypoxia in the waterlogging sensitive genotype BR11-6042 that may help in the exploration of waterlogging stress mitigation practices in soybean crop.

# 2. Chapter I - Changes in physiological and biochemical mechanisms of hypoxia tolerance in soybean plants submitted to recurrent waterlogging stress.

## 2.1. Introduction

The current rapid and continuous changes in climatic conditions increase the frequency of extreme weather, that are abiotic factors which have a huge impact on world agriculture and account for more than a 50% reduction in average potential yields for most major crops (MUTAVA et al., 2015a), negatively affecting their growth and development, and can ultimately result in the death of the plant (VRIET; HENNIG; LALOI, 2015). The differential response of the plant to a single episode of stress or to repeated episodes is what is known as memory. In this context, stress memory can be defined as the ability of organisms to respond better to a particular stress when the plant has already been exposed to it, compared to plants facing stress for the first time (TREWAVAS, 2003, 2005). Another strategy to combat environmental threats is phenotypic plasticity, even if it can be achieved by the memory of an event experienced at a certain stage of life, that is, the ability to retain information about that event, is not necessarily permanent, but can be of short duration or long duration (HILKER; SCHMÜLLING, 2019b). Directly, it is the ability to access past experience and incorporate relevant past information into new responses. However, an adequate and efficient defense response to lasting adverse environmental conditions may require precise reprogramming of genetic expression to rebalance growth, development and survival (BRUCE et al., 2007; FLETA-SORIANO; MUNNÉ-BOSCH, 2016; HILKER et al., 2016). Stress memory, which refers to the changes after exposure to stress that can persist after the stress has stopped, depends largely on the frequency of stress, conditioning an improvement in the stress response and greater tolerance of stress in a subsequent stress event (WALTER et al., 2011, 2013).

Currently, there is a great interest in knowing more about this phenomenon since the knowledge about the mechanisms involved in memory and its regulation is still limited, and a deepening of this subject would allow us to better understand the systems that plants have to face different stresses (HILKER et al., 2016; LI;

LIU, 2016). Moreover, this phenomenon is of great interest, not only in the agronomical field to "train" the plants for future stresses and increase the resistance and therefore the production, but also in the environmental field to improve the management of the water resources in natural ecosystems.

Soybean is an important leguminous crop widely used for human food, animal feed, biofuel production, and many other products owing to its high protein and edible oil content, and fewer carbohydrates than other leguminous crops. Global soybean production is of approximately 341 million tons worldwide (UNITED STATES DEPARTMENT OF AGRICULTURE, 2020). However, waterlogging is a widespread phenomenon drastically reducing the growth and production of soybean in many regions of the world (VAN NGUYEN et al., 2017), mostly due to the occurrence of flat topography (COLLAKU; HARRISON, 2002), high water tables and poor drainage of clay-like soils (JITSUYAMA, 2017). To cope with these challenges, significant efforts have been made by researchers toward elucidating stress signalling pathways in response to individual stresses. Whereas, under natural growth conditions, plants are often exposed to more than one stress, simultaneously or in a sequential manner, these stresses have synergistic or antagonistic effects on each other due to extensive cross talk in underlying signalling cascades (GUPTA et al., 2016; SHARMA et al., 2013). Its effect can be even higher in highly susceptible crop species; plants of soybean [*Glycine max* (L.) Merr.] can lose up to 93 % of its production when exposed to soil waterlogging during early vegetative stages (ALAM et al., 2010; BOARD, 2008a).

Soybean plants are sensitive to waterlogging. One of the most serious problems faced by plants subjected to waterlogging is the energy deficit as a result of inhibition of root respiration caused by the shortage of $O_2$ (VAN DONGEN; LICAUSI, 2015a). However, plants can readily respond to the energy lack induced by hypoxia by activating the lactic and ethanolic fermentative enzymes lead the regeneration of $NAD^+$ that is essential to ATP production at substrate level (GARCIA et al., 2020). Alanine aminotransferase (Ala-AT) activity increases during oxygen deficit (DE SOUSA; SODEK, 2003) and produce alanine, that plays an important role in regulating the glycolytic flux by preventing the excessive accumulation of pyruvate (ZABALZA et al., 2009a) while retaining

carbon and nitrogen resources for the plant (GARCIA et al., 2020; ROCHA et al., 2010a). If the stress is extended, the production of reactive oxygen species increase , which threatens the cells by causing lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, activation of the programmed cell death (PCD) pathway and ultimately cell death (MITTLER; BLUMWALD, 2015; SHARMA et al., 2012). Oxidative stress is essentially a regulated process, and the balance between ROS and antioxidant capacity determines the fate of the plant.

Studies have shown that numerous environmental cues are capable to prime a plant for improved resistance to repeated environmental stress. As in the case of the exposure of a plant to mild abiotic stress can pre pare its resistance to subsequently occurring severe heat, cold, drought, waterlogging, salt shock, high humidity and temperature, or osmotic stress (BATISTA et al., 2016; DO AMARAL et al., 2020; HINCHA; ZUTHER, 2014; SUZUKI et al., 2014; TIAN et al., 2019a; ZHANG et al., 2016; ZHOU et al., 2020). Despite the knowledge about adaptation mechanisms and regulation at the molecular level, understanding the response mechanisms of plants to waterlogging is very limited. The waterlogging can produce effects on physiological processes such as decreased activities of antioxidants and reduced photosynthetic performance of crop plants (TIAN et al., 2019b). Therefore, plants have the ability to retain information about a sign of past stress and result in a modified response to recurrent stress or a sustained response after the sign of priming stress that can be short or long term (LÄMKE; BÄURLE, 2017a). This response is essential for plant survival. The retention of an experienced environmental event can affect an individual's response to future environmental conditions (HILKER et al., 2016; HILKER; SCHMÜLLING, 2019b)

This study attempts to explain this information retention capacity through the biochemical tolerance mechanisms of soybean plants in response to waterlogging stress. We emphasized the ROS metabolism induced by hypoxic stress and the differential regulation of the antioxidant defense system (both enzymatic and non-enzymatic) as well as the anaerobic mechanisms by the activation of the fermentative pathways during waterlogging and recovery. Through these results we inferred that there was a tolerance induction to

waterlogging stress when the recurrent events occur at different developmental stage of soybean plants.

## *2.2.* Material and methods

### *2.2.1.* Plant material and growth conditions

This study was performed with soybean genotype PELBR10-6042 (6042), of undetermined growth, sensible to waterlogging, according to Embrapa Soybean Breeding Program (EMBRAPA SOJA, [s.d.]). The experiment was carried out in the field, at the experimental farm of Embrapa Lowlands Experimental Station, Capão do Leão (31º80'36"S and 52º41'12"W), RS-Brazil from October 2017 to February 2018.

The experiment was carried out in boxes of 1x1.2x1m (36 plants per box) containing soil natural from lowland areas (Typical Hydromorphic Eutrophic Planosol). Soil fertility was amended with 350 kg $ha^{-1}$ of commercial formulation NPK 00-25-25 and pH was elevated to 6.0. Seeds were inoculated on sowing with *Bradyrhizobium japonicum* strain SEMIA 5079 (BIOAGRO) and plants were cultivated under natural light and temperature (18-39ºC) conditions (Figure. 1).

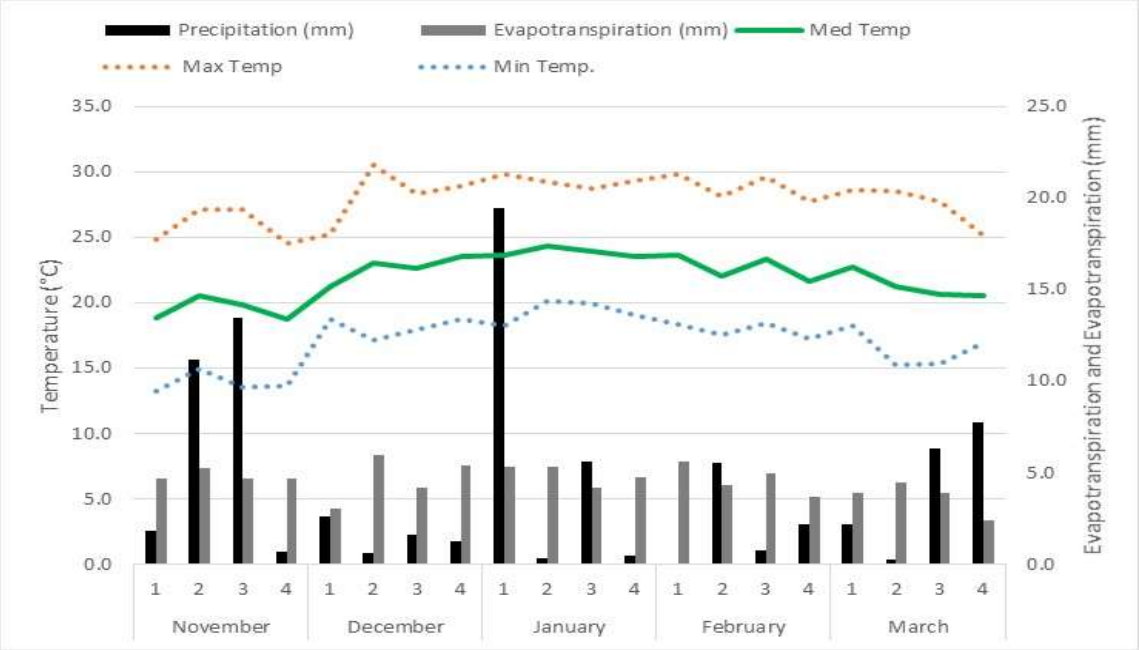


Figure 1. Minimum daily temperature (Min Temp-blue dotted line), medium daily temperature (Med Temp-green line) and maximum daily temperature (Máx Temp-orange dotted expressed in ºC, precipitation (mm) (black bars) and evapotranspiration (mm) (grey bars) for the corresponding experimental period (2017/2018 agricultural year). Source: Agroclimatological Station Pelotas (Capão do Leão), Embrapa Temperate Climate Agricultural Research Center, Pelotas, RS, Brazil. Available at: http://agromet.cpact.embrapa.br/.

Plants were subjected to waterlogging at the V3 and R2 stages (FEHR et al., 1971) in three groups: waterlogged only at V3 stage for seven days (see supplementary material Fig. S1); waterlogged at V3 stage (priming treatment) and subsequently at R2 stage (V3R2) and waterlogged only at R2 stage (R2). Waterlogging was imposed by maintaining a 2-3 cm water layer above the soil during seven and five days at V3 and R2 stages, respectively. Posteriorly the boxes were drained and plants were allowed to recover for additional five days. Control and waterlogged plants from the V3 (were collected at two and seven days of waterlogging (2DW-7DW) and at two and five days of recovery, same the others groups), and group R2 and V3R2 groups were collected at two and five days of waterlogging (2DW and 5DW), and at two and five days of recovery (2DR and 5DR) all only at R2 stage for biochemical analysis. Soil moisture was kept at field capacity during the whole growth period. Disease, weeds, and pests were well controlled in each treatment.

During sampling, roots were carefully washed, and homogenous healthy leaves of the same size and age (first fully expanded trifoliate from the apex) were collected, weighed and immediately stored frozen (-86°C) until biochemical analysis.

*2.2.2.* Hydrogen peroxide and malonyldialdehyde (MDA) levels

The levels of $H_2O_2$ and lipid peroxidation were determined according to VELIKOVA; YORDANOV; EDREVA, (2002). Leaves and roots (0.25 g) were ground into powder in liquid nitrogen and homogenized in 2 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 g for 20 min at 4 ºC. An aliquot of the supernatant was added to the reaction mixture, containing 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide, with a final volume of 200 µL. Reaction absorbance was measured at 390 nm. The levels of $H_2O_2$ were given on a standard curve prepared with known $H_2O_2$ concentrations and the results expressed in µmol $H_2O_2$ $g^{-1}$ of fresh weight. Lipid peroxidation was determined by measuring the amount of MDA, a product of lipid peroxidation that reacts with thiobarbituric acid (TBA) reaction (VELIKOVA; YORDANOV; EDREVA, 2002). Aliquots of the supernatant were added to the reaction mixture, which was composed of 0.5% (w/v) TBA in 10% (w/v) TCA solution, and then incubated at 90 ºC for 20 min. The reaction was

stopped by cooling in an ice-bath for 10 min. Afterwards, the samples were centrifuged at 10,000 g for 5 min and the absorbance was read at 535 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was determined from the molar extinction coefficient (155 $mM^{-1}$ $cm^{-1}$) and the results were expressed as µmol MDA $g^{-1}$ of fresh weight.

*2.2.3.* Enzymatic antioxidant system

Leaves and roots (0.25 g) were ground in liquid nitrogen and homogenized with 10% (w:w) polyvinylpolypyrrolidone (PVPP) in 1.5 mL of extraction buffer (100 mM potassium phosphate buffer, pH 7.8, 0.1 mM EDTA, 10 mM ascorbic acid). The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C and the supernatant was collected and used for ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) activities measurements (BIEMELT; KEETMAN; ALBRECHT, 1998). From the same extract, the total content of soluble proteins was determined (BRADFORD, 1976) for expression of enzymatic activities.

The SOD (EC 1.15.1.1) activity was measured by the ability of enzyme to inhibit the photoreduction of nitroblue tetrazolium NBT at 560 nm (GIANNOPOLITIS; RIES, 1977). An aliquot of enzymatic extract was added to the incubation medium (final volume of 200 µL) containing 50 mM potassium phosphate (pH 7.8), 14 mM methionine, 0.1 mM EDTA, 75 µM of NBT, and 2 µM riboflavin. Then, the reaction medium was illuminated for 10 min with a fluorescent lamp (20 W). The control of the reaction was the reaction medium without enzymatic sample. The results were expressed as U $mg^{-1}$ protein.

The CAT (EC 1.11.1.6) activity was assessed by the decrease in absorbance at 240 nm, monitored by the consumption of $H_2O_2$ (AZEVEDO et al., 1998). The enzyme activity was evaluated by adding aliquots of enzymatic extract in the incubation medium containing 100 mM potassium phosphate buffer (pH 7.0), and 12.5 mM $H_2O_2$. CAT activity was calculated using the molar extinction coefficient (ε) of $H_2O_2$ (36 $mM^{-1}$ $cm^{-1}$). The results were expressed as µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein.

The APX (EC 1.11.1.11) activity was assayed by monitoring of the rate of oxidation of ascorbate (NAKANO; ASADA, 1981). The reaction medium was

composed of 37.5 mM potassium phosphate buffer (pH 7.0), 0.25 mM AsA and 5 mM $H_2O_2$, and enzymatic extract. The APX activity was measured by the decrease in absorbance at 290 nm and estimated using ε of ascorbate (2.8 $mM^{-1}$ $cm^{-1}$). The results were expressed in μmol ascorbate $min^{-1}$ $mg^{-1}$ protein.

*2.2.4.* Photosynthetic pigments

Chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*) and carotenoids were determined as described (WELLBURN, 1994), using dimethyl sulfoxide for pigment extraction from leaves.

*2.2.5.* Fermentative enzymes and Ala-AT

Frozen roots (0.250 g) were ground into powder using an extraction buffer (50 mM Tris-HCl pH 7.5, 1 mM dithiothreitol and 3% (w/w) PVPP). The homogenate was centrifuged at 12,000 × g at 4 °C for 20 min, and an aliquot of the supernatant was desalted using a PD-10 column (GE Healthcare, Buckinghamshire, UK). The eluted protein fraction was used to determine ADH (Alcohol dehydrogenase; EC 1.1.1.1), PDC (Pyruvate decarboxylase; EC 4.1.1.17), LDH (Lactate dehydrogenase; EC 1.1.1.17) and Ala-AT (Alanine aminotransferase; EC 2.6.1.2) activities. The enzymatic assays were performed by monitoring the oxidation of NADH at 340nm at 30 °C.

ADH and PDC activities were assayed following the method proposed by HANSON; JACOBSEN; ZWAR (1984) with some modifications. For ADH, the reaction mix contained 50 mM phosphate buffer (pH 7.0), 0.6 mM NADH and 5.0 mM acetaldehyde. The PDC assay was monitored in a reaction mix containing 50 mM MES buffer (pH 6.0), 0.2 mM NADH, 0.5 mM thiamine pyrophosphate, 1 mM magnesium chloride, 20 mM oxamic acid, 5 units of ADH and 10 mM Na-pyruvate. The LDH assay followed the method proposed by Hanson et al. (1984) with some modifications: the reaction was monitored in a reaction mix containing 50 mM buffer Tris-HCl (pH 7.5), 0.6 mM NADH, 3.0 μM KCN, 0.2 mM 4-methylpyrazole and 10 mM Na-pyruvate. The Ala-AT assay contained 10 mM L-alanine, 5 mM 2-oxoglutarate, 0.6 mM NADH, 50 mM Tris/HCl (pH 7.5) and 5 units of lactate dehydrogenase (GOOD; MUENCH, 1992).

*2.2.6.* Analysis of total phenolic compounds, amino acids, total soluble sugars, sucrose and nitrate

Phenolic compounds, amino acids, total soluble sugars, sucrose and nitrate were extracted from 0.250 g of fresh roots and leaves and 10 mL of MCW solution (methanol: chloroform: water (12:5:3 v/v/v)) solution per gram of plant material (BIELESKI; TURNER, 1966).

The aqueous phase resulting from MCW was used for the analysis of the total phenolic contents according to the method described (JENNINGS, 1981). Briefly, 0.5 mL of the sample was added to 0.5 mL of distilled water and 0.5 mL of 1N Folin–Ciocalteau reagent followed by mixing for 1 min. After 15 min, 5 mL sodium carbonate solution (0.08 g/mL) were added. The mixture was left for 1 h at room temperature in a dark place and the absorbance was measured at 760 nm using a UV/VIS spectrophotometer (SpectraMax M3, Series Multi-Mode Microplate Readers, Molecular Devices. LLC. USA). The phenols concentration of each sample was calculated from the phenic acid standard curve (0 to 200 µg/mL), and the quantification was expressed at µg $g^{-1}$ fresh weight.

The determination of total soluble amino acids was performed according to YEMM; COCKING; RICKETTS (1955), total soluble sugars according to GRAHAM; SMYDZUK (1965), sucrose contents according to VAN HANDEL (1968) and inorganic nitrate solute ($NO_3^-$) levels in the tissues, according to the salicylic acid method (CATALDO et al., 1975).

*2.2.7.* Yield components

For the yield components, study group boxes were left randomly distributed until the cycle was completed and the soybean plants were harvested. Boxes consisted of three rows 1.2 m long with 30 cm between rows. The center 1.2 m of the middle row was harvested for seed yield. A 0.5 m section located near the middle of the center row of each box was used to measure the following traits: seed weight as grams per 100 seeds; pod number per plant; seeds per pod; seed weight per plant; node number per plant; height of plant and insertion height of first pod at maturation. Seed yield in grams (converted to t $ha^{-1}$) was taken both from the entire plot and from the 1.2-m row section.

### *2.2.8.* Statistics analysis

The experiment was a completely random design with soil moisture regimes. Boxes were used, with three rows of plants per box, three plants from each row joined together to form a single replica. There were three replicates for each treatment (2DW, 5DW, 2DR and 5DR). Effects of waterlogging treatment, stages and corresponding interactions were submitted to Shapiro-Wilk to check the normality and to Levene's test to verify homoscedasticity, followed by analysis of variance according the software RStudio (R CORE TEAM, 2020). The means separations were performed using Fisher's protected least significant difference (LSD) at a 0.05 significant level, used to compare among the two waterlogging and the two recovery treatments (e.g. 2DW vs 5DW; 2DR vs 5DR) and Tukey-test ($P<0.05$) was used to compare plants under waterlogging and recovery conditions within each period of treatment, was compared with its corresponding control (e.g. in 2DW: V3R2, R2 vs Control; 2DR:V3R2, R2 vs Control). All yield and productivity data were subjected to unidirectional analysis of variance (ANOVA) to determine significant differences between treatments.

## *2.3.* Results

### *2.3.1.* $H_2O_2$ and. MDA levels

The levels of $H_2O_2$ in leaves of waterlogged plants increased to values higher than control in V3R2 group, but lower than the R2 levels at 5DW, and at this same period in the R2 group, it increased. Under recovery, levels were markedly higher in both groups at 2DR reducing at 5DR to control levels in V3R2 group (Fig 2A). In the roots, the $H_2O_2$ content varied for both groups under waterlogging, at 2DW, both groups presented the same $H_2O_2$ content as the control, continuing the same at 5DW for the V3R2 group, and, decreasing in the R2 group. At 2DR both groups (V3R2 and R2) decreased the peroxide content compared to the control, remaining so throughout the recovery (Fig. 2C).

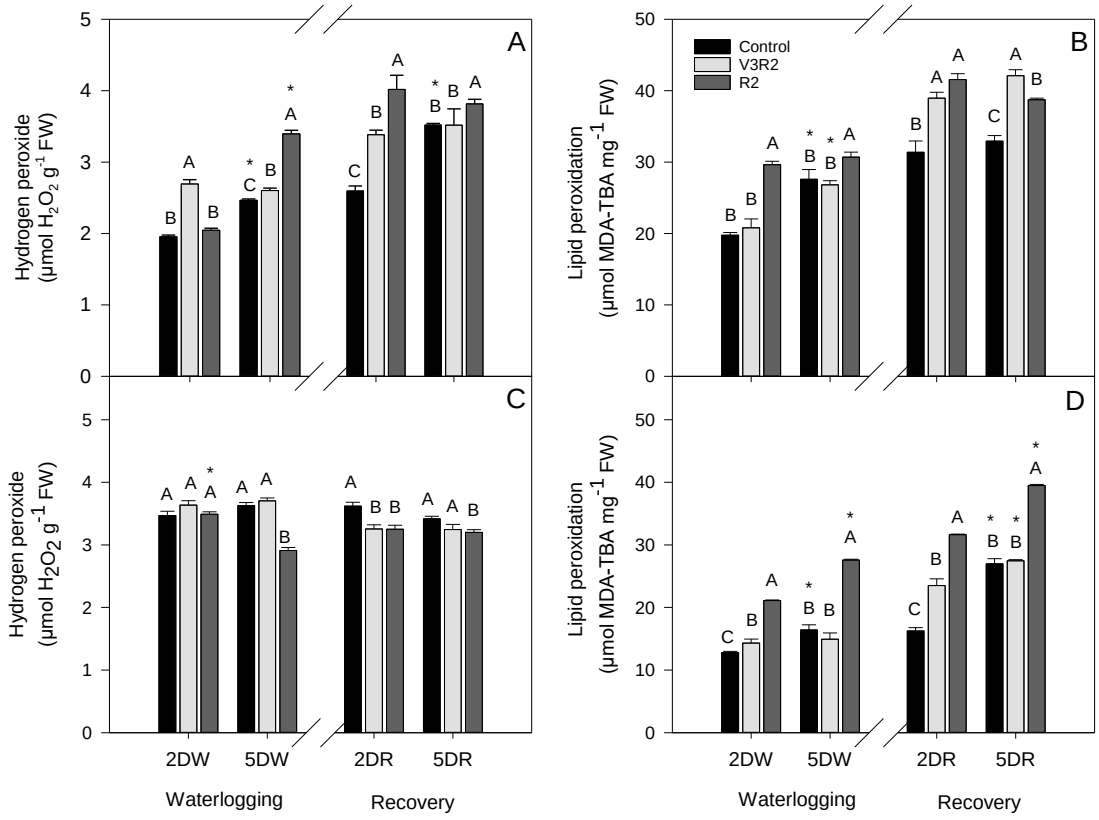


Figure 2. Contents of $H_2O_2$ and lipid peroxidation in leaves (A, B) and roots (C, D) of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (2DW, 5DW) and recovery (2DR, 5DR). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). "Asterisks indicate significant differences among periods of waterlogging or recovery treatments" by t-test ($P \leq 0.05$), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test ($P \leq 0.05$).

The MDA (malonyldialdehyde) content is generally used to assess lipid peroxidation. The MDA levels at 2DW in the leaves of the V3R2 group remained the same as the control, unlike R2 which increased by 1.6 fold. At 5DW, the MDA increased significantly by once fold for the R2 group and in the V3R2 group remained the same as the control. Under post-hypoxia the content increased by 1.2 fold for the V3R2 group, and by 1.3 fold for R2 group at 2DR. The MDA content remained higher by about 1.3 and one fold for the V3R2 and R2 groups respectively, compared to the 5DR control (Fig. 2B). In the roots, MDA content increased at 2DW in both groups, by 1.3 fold for V3R2 and 1.6 fold for R2 compared to the control. At 5DW, MDA contents remained the same as the control in V3R2 group and significantly higher in R2 group (1.6 fold). In the following periods (recovery), the MDA content of the V3R2 group increased 1.8

fold at 2DR and remained at control levels at 5DR while increased significantly in plants of the R2 group, by 1.8 fold at 2DR and 1.4 fold at 5DR (Fig. 2D).

*2.3.2.* Enzymatic antioxidant.

Leaves of V3R2 plants subjected to waterlogging showed the same control SOD activity and lower than R2 group, and during recovery, the activity decreased to lower levels than the control and R2 group of plants. On the other hand, SOD activity in plants subjected to waterlogging only at R2 stage was higher (2 fold and 1,2 folds at 2DW and 5DW) than control during the period of waterlogging and kept similar to control during recovery (Fig. 3A). The SOD activity in roots of waterlogged plants increased markedly in V3R2 group at 2 DW comparing to control and R2 groups, kepting higher than the control at 5 DW, and at lower levels than the R2 group. During the recovery, SOD activity increased in the V3R2 and R2 groups (1.2 and 2 folds, respectively), being higher in the R2 group. At 5DR it increased in both groups, reaching the highest values in R2 group (around 6,6 fold the control) (Fig. 3B).

The CAT activity in the leaves and roots of V3R2 and R2 plants was distinct in a treatment- and period- manner during the experimental period. In the leaves, CAT activity in the V3R2 group and control was similar at the waterlogging periods, whereas its levels increased at two days of recovery and reached again control levels at five days of recovery. Regarding CAT activity of leaves in R2 plants, a significant increase was only observed at five days of waterlogging, reaching higher levels than the control and V3R2 group. On the other hand, CAT activity was reduced to control levels at two days of recovery (Fig 3C).

In the roots of waterlogged soybeans, CAT activity in plants of V3R2 group increased markedly by 23 and 13 folds, compared to control and R2 plants in both waterlogging periods, and in R2 plants increasing by 5 folds at 5DW. During recovery, CAT increased in V3R2 by 15 and 4,8 folds compared to the control and R2 levels respectively, at five days of recovery. However, in plants of the R2 group, CAT activity was higher than control at 2DR and 5DR of recovery increasing respectively by 7 and 3 folds (Fig. 3D)

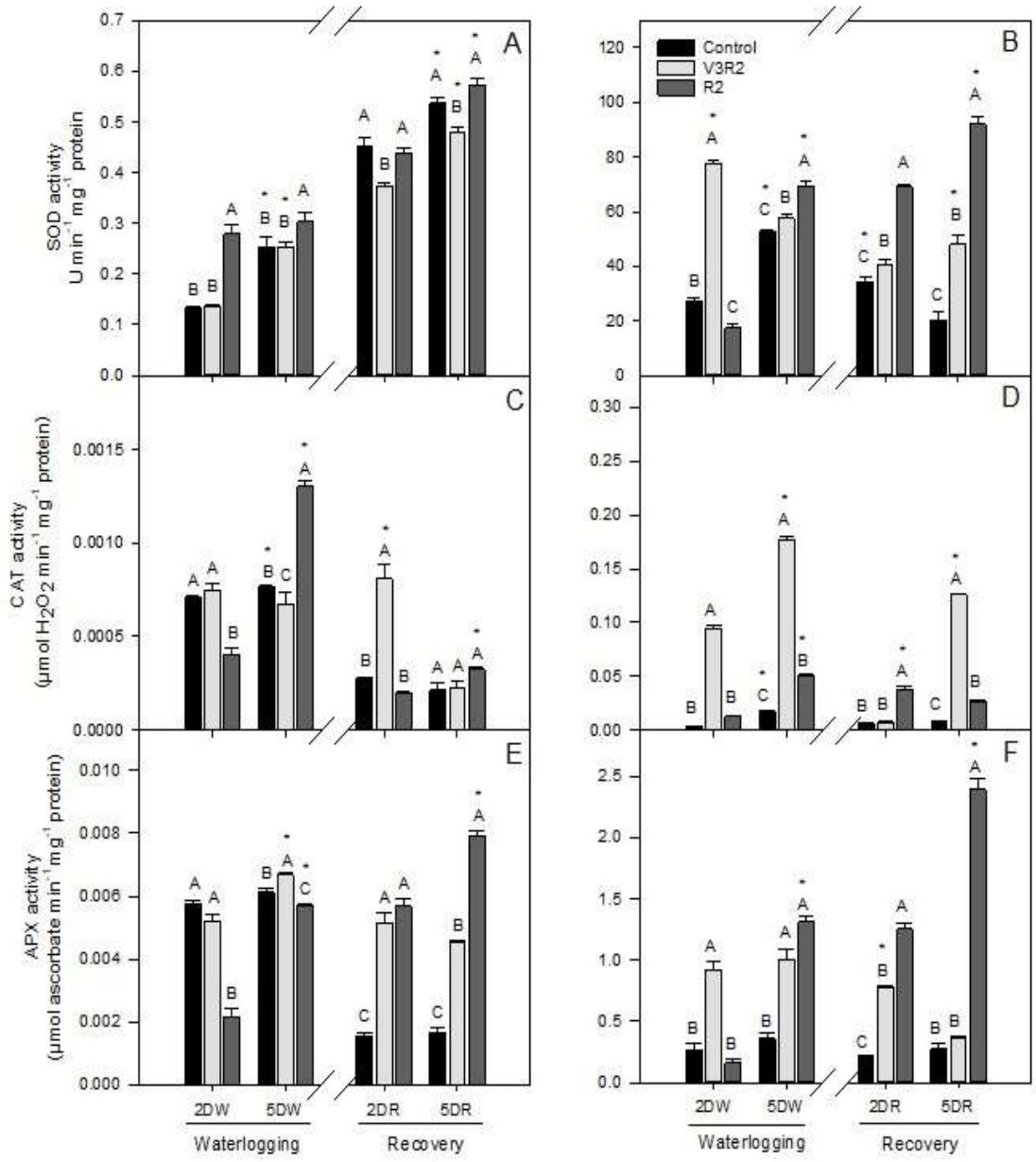


Figure. 3. SOD, CAT and APX activities in leaves (A, C and E) and roots (B, D and F) of soybean genotype PELBR10-6042 at R2 stage under two and five days of waterlogging (2DW, 5DW) and recovery (2DR, 5DR). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test ($P \leq 0.05$), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test ($P \leq 0.05$).

The APX activity in the leaves of the V3R2 group was same as the control plants at 2DW, and increased at 5DW waterlogging periods when compared to the control. In plants of the R2 group under both waterlogging periods, the activity of APX was lower as than the control and V3R2 plants. In both recovery (2DR and 5DR) periods the APX activity increased by 3.3 and 2.6 folds in the V3R2 group, and by 3.6 and 4.7 folds in the R2 group, compared to the

control, respectively (Fig. 3E). Overall, the levels of APX activity in the roots were higher than in leaves. At two and five days of waterlogging, APX activity increased by 3.7 and 2.8 folds in the V3R2 group. The R2 group highly increased 3.7 folds its APX levels at five days of waterlogging, when compared to control. After drainage, APX activity of the V3R2 group was higher than in the control at 2DR and in R2 plants increased in both periods, presenting the highest levels (8.8 folds) after five days of recovery (Fig. 3F).

*2.3.3.* Phenols content

Total phenolic content decreased in leaves of plants under two days of waterlogging, but at five days after the water layer was imposed there was a remarkable increase in the V3R2 group (around 1.5 fold), while in the R2 group it was slightly below control level. At the beginning of recovery, phenols content in the V3R2 group remained higher (around 1.6 fold) than control and R2 groups but decreased at five days of recovery, not reaching control level. On the other hand, in the R2 group phenols content increased at five days after waterlogging differing significantly from the other groups (Fig. 4A). In roots, two days waterlogging have not influenced total phenols content in the V3R2 group but caused a decrease in the R2 group, while at five days of waterlogging the contents in V3R2 group decreased below control levels, unlike the R2 group which reached control levels. Under two days of recovery, phenols content in plants were similar to those in control, but decreased at five days of recovery in both groups (Fig. 4B).

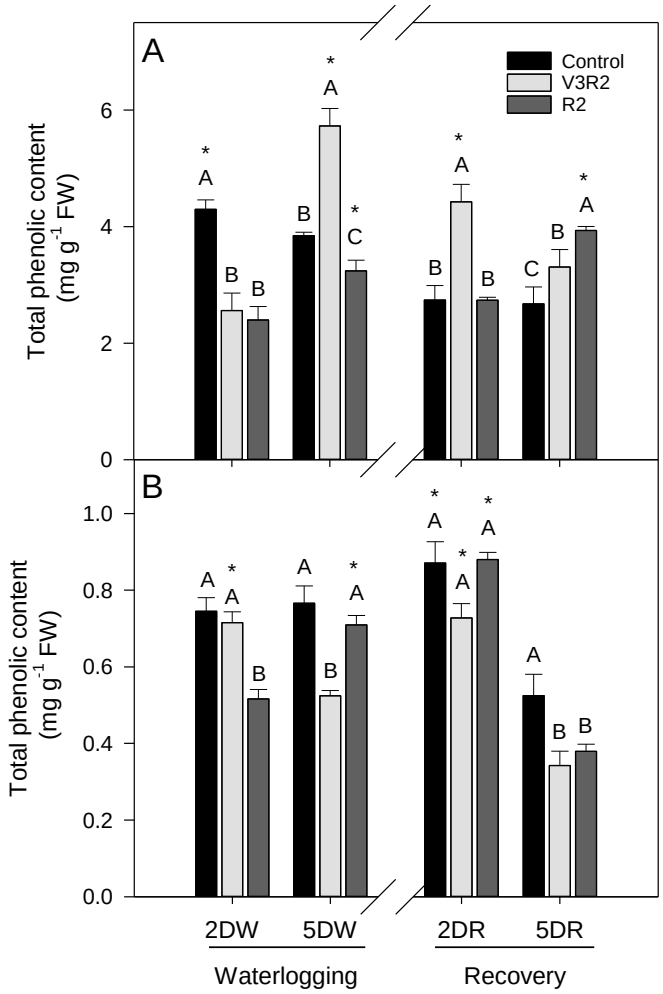


Figure. 4. Total phenols contents in leaves (A), and roots (B) of soybean genotype PELBR10-6042 at R2 stage under two and five days of waterlogging (*2DW, 5DW*) and recovery (*2DR, 5DR*). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test ($P \leq 0.05$), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test ($P \leq 0.05$).

### *2.3.4.* Fermentative enzymes and Ala-AT

Throughout the experiment, the activity of the fermentative enzymes (LDH, ADH and PDC,) and Ala-AT were highly affected. Overall, the activity of these enzymes increased at seven days of waterlogging when plants were at the V3 stage, and after two days of recovery, their activity were still higher than control levels (See supplementary material Fig S2).

The activity of LDH was markedly higher than control plants for R2 group at two days of waterlogging and increased at five days of waterlogging, reaching equivalent values in both groups (V3R2 and R2). At two days of recovery, decreases in LDH activity were observed in V3R2 plants compared to the same

group at five days of waterlogging, while the group of plants R2 showed a great increase at this period (2DR) . In both groups, LDH activity reached control levels at five days of recovery (Fig. 5A).

Increases in ADH activity were observed at five days of waterlogging for groups V3R2 and R2 compared to control and group R2 presented the highest levels (2.3 fold higher than V3R2). At two days of recovery, ADH levels increased compared to control in both groups (V3R2 and R2; approximately 5.4 and 5.8 folds higher) and decreased sharply at five days of recovery, thereby not reaching control levels. Similar to ADH (Fig. 5B), the activity of the enzyme PDC also increased tightly at five days of waterlogging in both groups (V3R2 and R2) and it was maintained elevated at two days of recovery compared to control, but plants of the V3R2 group presented lower PDC activity than those of R2. At five days of recovery, the activity of both groups was similar to control (Fig. 5C).

The Ala-AT activity for V3R2 and R2 groups varied in all evaluated periods. At two days of waterlogging the Ala-AT levels increased 3 fold compared to control in plants of the R2 group, and were even higher at five days of waterlogging (19 folds compared to control). Regarding the V3R2 group, at five days of waterlogging the Ala-AT activity was higher than control plants, but much lower than the R2 group. At two days of recovery Ala-AT activity was higher than the control for both groups (V3R2 and R2), differing from the last recovery period (5DR) where all plants reached pre-waterlogging activity of this enzyme (Fig. 5D).

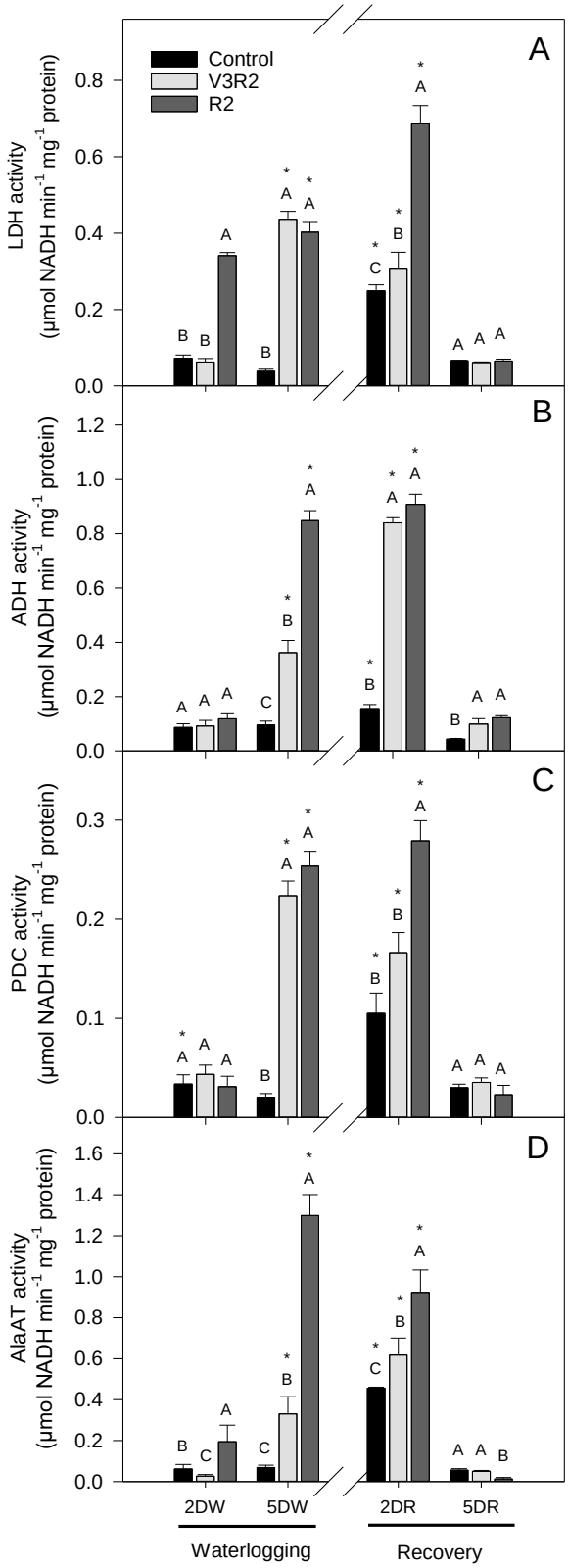


Figure 5. LDH (A), ADH (B), PDC (C) and Ala-AT (D) activities in roots of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (2DW, 5DW) and recovery (2DR, 5DR). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test *(P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test (*P ≤ 0.05*).

*2.3.5.* Analysis of amino acids, total soluble sugars, sucrose and nitrate

The content of total amino acids (TAA) in leaves of waterlogged plants at V3 stage (Sup. Fig. S3A) decreased compared to control since the first waterlogging period (2WD), and did not reach pre-hypoxic values during recovery. In the roots, at seven days of waterlogging, the contents increased compared to control plants and reached control levels at two days of recovery (Sup. Fig. S3B). Regarding the groups V3R2 and R2, at two days of waterlogging the TAA content in leaves decreased in the same manner compared to control, and at five days of waterlogging TAA content increased in the group V3R2 whereas in the group R2 remained lower than control. At two days of recovery, decreases in TAA content were observed for both groups compared to control plants being an expressive reduction in R2 group, and at five days of waterlogging the group V3R2 as well as the group R2 reached control levels (Fig. 6A). In the roots, TAA content increased at two days of waterlogging for R2 group, while V3R2 did not differ from control. At five days of waterlogging, the TAA content reduced in both groups, and this decrease was more pronounced in the group R2. At two days of recovery, all plants reached control levels of TAA content (Fig. 6B).

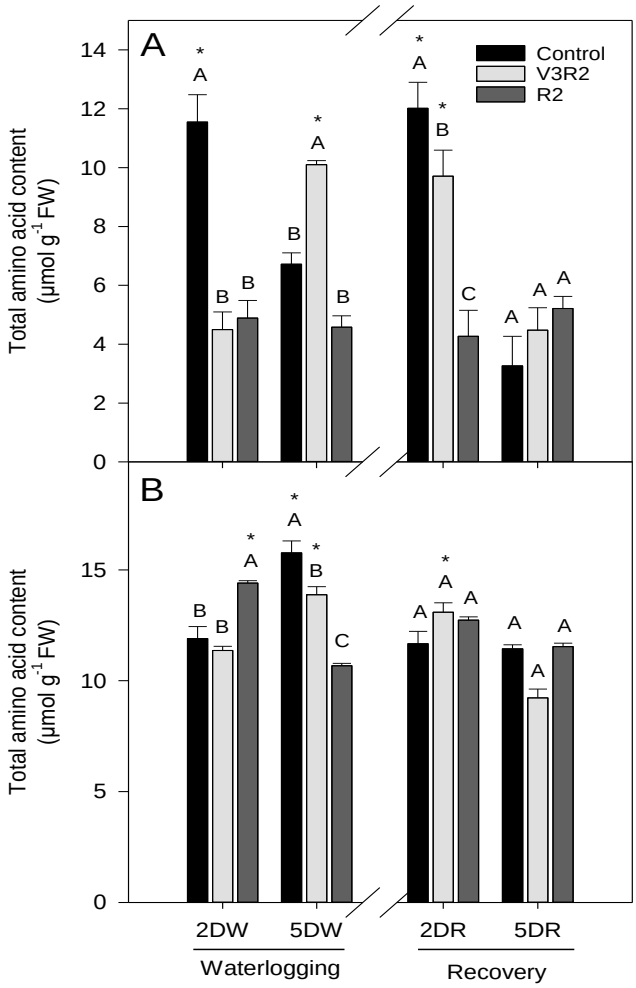


Figure. 6. Total amino acid contents in (A) leaves and roots (B) of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (*2DW, 5DW*) and recovery (*2DR, 5DR*). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test (*P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test *(P ≤ 0.05*).

The total soluble sugars (TSS) content in shoots increased significantly during the waterlogging at the V3 stage since the first assessment period (2DW). This increase was maintained above control at 7DW and during recovery, when it reached the highest levels, being equivalent at 2DR and 5DR. (Sup. Fig. S4A). In roots, the levels rose significantly to 7DW and remained above the control at 2DR, returning to 5DR (Sup. Fig. S4B). In group V3R2 the TSS contents increased in the leaves at 5DW and remained higher than the control at recovery, this response was more expressive for V3R2 group at 2DR being equivalent to R2 plants at 5DR (Fig. 7A). In the roots, TSS levels increased in V3R2 at 2DW relative to the control, and decreased in the R2 group. At 5DW and the first

recovery (2DR), TSS levels were equivalent to the control levels in both plant groups, increasing in the R2 group at 5DR (Fig. 7B).

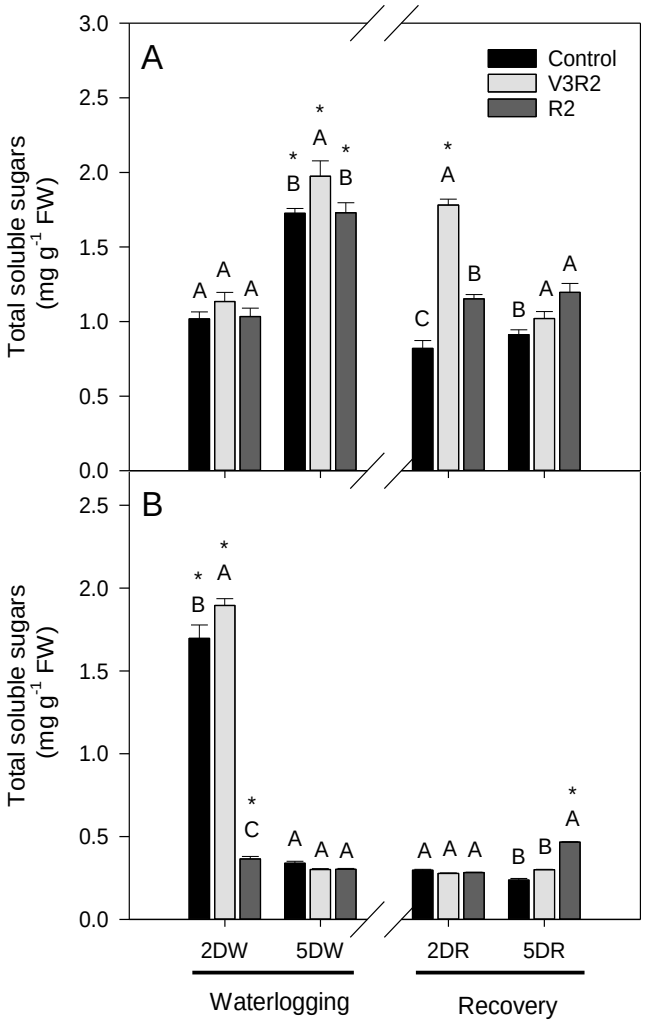


Figure 7. Total soluble sugars content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (*2DW, 5DW*) and recovery (*2DR, 5DR*). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test (*P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test *(P ≤ 0.05*).

The sucrose content in leaves of waterlogged plants at V3 stage increased at two days of waterlogging, decreasing at seven days of waterlogging (Sup. Fig. S5A) and during recovery sucrose content remained stable and higher than control levels. In the roots, the sucrose content in waterlogged plants was higher than control, and at seven days of waterlogging the sucrose content increased when compared to those in the two days of waterlogging. At two days of recovery sucrose contents remained higher than control, and then reached control levels at five days of recovery (Sup. Fig. S5B).

At two days of waterlogging, the sucrose content in leaves decreased in both groups V3R2 and R2, when compared to control, and at five days of waterlogging they did not differ from control levels. However, the sucrose content at the recovery periods (2DR and 5DR) was higher for the V3R2 group than those of control and R2 group (Fig. 8A). In roots, at two days of waterlogging, sucrose content of V3R2 and R2 groups was same as than the control. This trend was different at five days of waterlogging, sucrose content increased in both V3R2 and R2 groups, while V3R2 group surpassed both R2 and control groups. Recovery for two days resulted in increased sucrose content in V3R2 and lower content for the group R2, however, it was allowed to reach control levels after five days of recovery.

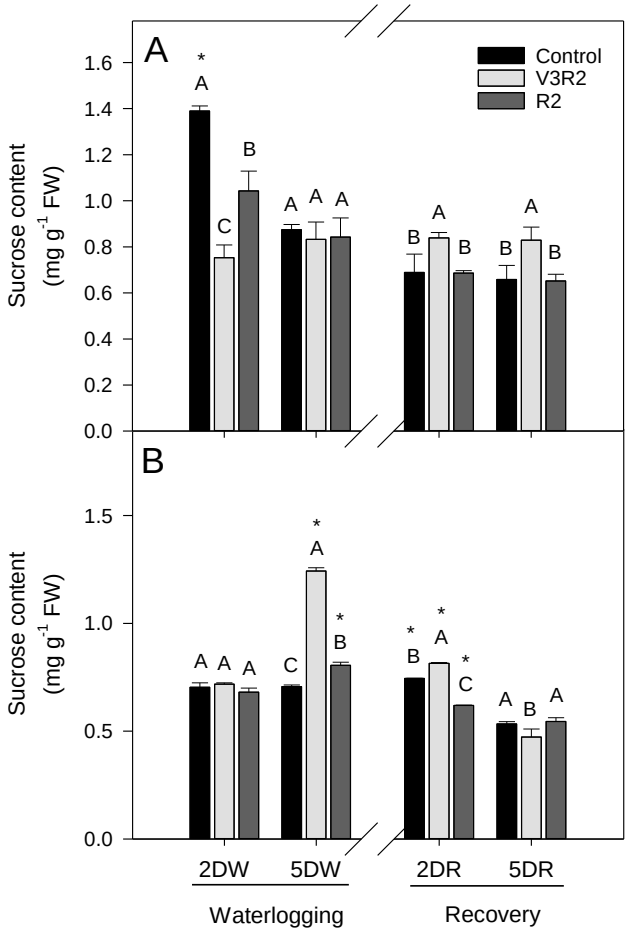


Figure. 8 Sucrose content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (*2DW, 5DW*) and recovery (*2DR, 5DR*). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test (*P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test *(P ≤ 0.05*).

The nitrate levels ($NO_3^-$) in leaves decreased under waterlogging at V3 stage (2WD and 7WD), remaining lower than control over the recovery period (Sup. Fig. S6A).. Waterlogging at the reproductive stage (R2) caused similar decreases in V3R2 and R2 groups at two days of waterlogging, but at five days of waterlogging the nitrate levels of both groups reached control levels. At two days of recovery, V3R2 plants showed a high accumulation of nitrate levels and R2 reached control levels. On the contrary, at five days of recovery, the R2 group surpassed V3R2 and control levels of nitrate (Fig. 9A). In the roots of waterlogged plants (V3 stage) the nitrate concentration decreased at two days of waterlogging, which increased at seven days of waterlogging. Same trend was observed for recovery periods: decrease at 2DW and increase at 5DR, compared to control levels (Sup. Fig. S6B). Waterlogging at the reproductive stage (R2) did not affect nitrate levels at the first period (2DW). At five days of waterlogging, nitrate levels of V3R2 and R2 groups remained stable and higher than control levels. When plants returned to normoxia, both groups sharply decrease, and at the five day of recovery the group V3R2 reached control levels while the group R2 surpassed the rest (Fig 9B).

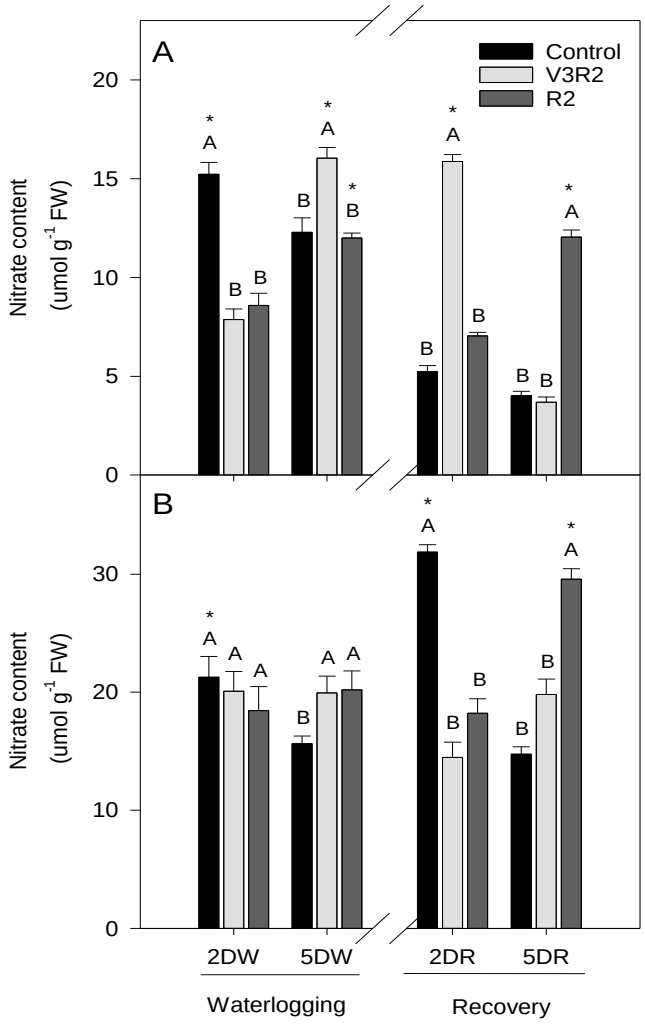


Figure. 9. Nitrate content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at R2 stage, under two and five days of waterlogging (*2DW, 5DW*) and recovery (*2DR, 5DR*). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test (*P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test *(P ≤ 0.05*).

### *2.3.6.* Photosynthetic pigments

The levels of photosynthetic pigments varied during the waterlogging and recovery periods (Fig. 10). At two days of waterlogging, levels of *Chl a* in V3R2 plants decreased below control, while it increased by 1.4 fold in the R2 group compared to the control. However, after five days of waterlogging the *Chl a* levels decreased in the R2 group and increased by 1.2 fold in the V3R2 group, compared to control levels. On day two of recovery, both groups V3R2 and R2 presented levels of *Chl a* below the control and this effect was stronger in the R2 group. At five days of recovery the *Chl a* content of the V3R2 group was similar

to the control plants (Fig.10A) and remained below control in the R2 group. Most of the variation of *Chl b* levels were similar to *Chl a*, except for the V3R2 group, in which *Chl b* levels were similar to control at two days of waterlogging, and at five days of recovery, the *Chl b* levels of the R2 group reached control levels (Fig. 10B).

*Chl a+b* levels in waterlogged plants (two days) increased by 1.4 fold in the R2 group compared to the control, while in the V3R2 group *Chl a+b* levels decreased slightly. However, at five days of waterlogging *Chl a+b* levels increased by 1.4 fold in plants of the V3R2 group, and the R2 levels reduced compared to control levels (by 1.2 fold). During recovery, *Chl a+b* contents in both V3R2 and R2 groups reduced by 1.2 and 1.4 folds, respectively. At five days of recovery, the V3R2 group reached control levels, different from the R2 group which presented lower levels (Fig. 10C).

At two days of waterlogging, carotenoid levels reduced by once fold in the V3R2 group and increased by 1.6 fold in the R2 compared to the control. In the second waterlogging period (5DW) carotenoid levels increased in the V3R2 group (by 1.2 fold) and reduced to less than half of the control in R2. Under recovery, levels of both groups reduced and remained below control. At five days of recovery the stress effect was higher in R2 (about 1.7 fold lower than control) (Fig. 10D).

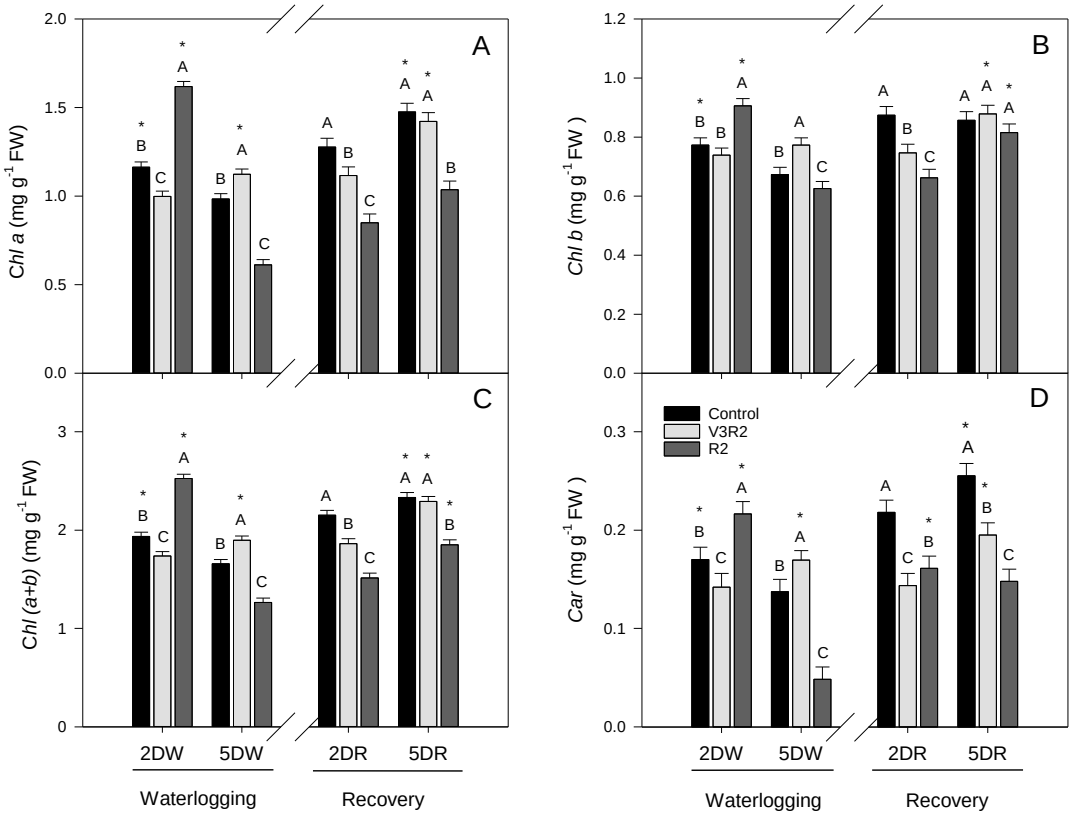


Figure 10. Chlorophyll *a* (*Chl a* – A), chlorophyll *b* (*Chl b* – B), total chlorophyll (*Chl a* + *b* – C), and total carotenoids (*Car* - D) contents in leaves of soybean genotype PEL BR10 - 6042 at R2 stage, under two and five days of waterlogging (2DW, 5DW) and recovery (2DR, 5DR). Plants were submitted to one cycle (at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments by t-test (*P ≤ 0.05*), and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the Tukey's test *(P ≤ 0.05*).

### *2.3.7.* Yield components

Most of the yield components which were assessed here differed in the two groups of plants (V3R2 and R2). The plants in group R2 showed a reduction in grain yield, number of pods/plant, weight of grains per plot, number of nodes per plant, and the first pod insertion height in relation to group V3R2 (Table 1). In the contrary, the group V3R2 reached control values for these components, except for the first pod insertion where this group surpassed control values. On the other hand, the dry weight of 100 grains of both groups (V3R2 and R2) were affected compared to control.

Table 1. Yield components of soybean genotype BR6042 plants at R8 stage after exposure to one cycle (at V3 stage; V3 and at R2 stage; R2) and two cycles (at V3 and R2 stages; V3R2) of waterlogging.

| Variables/ Treatments | Yield Kg/ha | Pods/ plant | Seeds/ pod | Seeds weight/plant | Dry weight of 100 seeds | Seed weight per plot | Nodes number | Firth pod insertion height | Plant height |
|---|---|---|---|---|---|---|---|---|---|
| Control | 2205.07 ab | 79.11 a | 2.05 a | 8.6 a | 20.92 a | 264.61 ab | 25.11 a | 19.04 c | 118.23 a |
| V3 | 2152.51 ab | 58.33 b | 2. 13 a | 7.6 a | 21.17 a | 258. 30 ab | 23.89 ab | 26.64 a | 107.26 b |
| V3R2 | 2305.12 a | 72.44 a | 1.98 a | 7.3 a | 19.42 b | 276.61 a | 24.67 a | 22.77 b | 114.84 a |
| R2 | 1929.47 b | 54.00 b | 2.10 a | 5.1 b | 18.80 b | 231.53 b | 21.44 b | 17.05 c | 113.59 ab |
| CV (%) | 14.75 % | 21.16% | 11.16% | 21.14% | 5.01% | 14.75% | 11.32 | 12.15 | 5.92 |

Means followed by equal letters, lowercase in the columns, do not differ by Tukey test at 5% probability. CV (%), coefficient of variation.

## *2.4.* Discussion

The distinct responses to different types of stress are relatively well studied, but in nature, stress is often habitual or repetitive and responses to recurrent stresses are much less comprehended. However, acclimatization may aid plants to reduce the damage caused by stress and promote their recovery (WALTER et al., 2013). Our results indicate that soybean plants from the V3R2 group, which were subjected to two waterlogging cycles (firstly at the V3 stage and then at R2), are more prone to acclimate to stress regarding those plants which were exposed to waterlogging only at the R2 stage. Therefore, we show here that the tolerance to waterlogging stress in soybean plants may be improved possibly through mechanisms induced by the acclimatization process, and such effect is dependent on the period of stress exposure and developmental stage (see the supplementary material on the plants V3 stage).

Thus, both energetic deficit and the formation of reactive oxygen species (ROS) induced by waterlogging are better utilized in acclimated plants. Possibly by increasing the efficiency of the carbon metabolism and by activating the mechanisms of antioxidant defense, the plant is allowed to “scape” the stress and counteract the adverse effects of such stress. These mechanisms result from subtle transformations in order to achieve tolerance to recurrent stress when "stress memory" persists in (WALTER et al., 2013).

### *2.4.1.* $H_2O_2$ and. MDA levels

Our results demonstrate that the $H_2O_2$ levels in leaves increased in the two groups (V3R2 and R2), both under waterlogging and recovery (Fig. 2A). The increase in $H_2O_2$ concentration during the hypoxia and post-hypoxia in soybean plants was observed as a common effect among genotypes which presented different waterlogging tolerance mechanisms (GARCIA et al., 2020). This response resulted in oxidative stress evidenced by the increases in MDA content, which is related to membranes damage in both groups, essentially for the R2 group. The $H_2O_2$ is a strong oxidant which may initiate localized oxidative damages triggering to disturbance of the metabolic function and to the loss of cellular integrity on the accumulation sites (FOYER et al., 1997). The $H_2O_2$ as well as other ROS such as $OH^{\bullet}$, $^1O_2$ e $O_2^-$ lead to lipid peroxidation and thus increase the membrane permeability, promoting foliar senescence (KHAN;

KHAN, 2017; LIU; GUO; BAI, 2010). It is evident that oxidative damage is more severe under reoxygenation in the leaves of waterlogged plants, which may result in an over-reduction of the photosynthetic electron transport chain (ETC), causing the generation of several types of ROS and, consequently, triggering oxidative stress (AHMED et al., 2002; GILL; TUTEJA, 2010).

The hypoxia resulted in an extensive lipid peroxidation, which has been frequently used as an indicator of oxidative damage in membranes (BIN et al., 2010; KHAN; KHAN, 2017; LI et al., 2011a). Our results indicated that R2 group was more affected by waterlogging considering that lipid peroxidation increased rapidly in leaves of these group in comparison to V3R2 plants (2DW data) which levels were similar to control. At 5W, these differences between both groups were kept, however, under recovery, differently from roots, both groups of plants increased MDA levels in leaves at same levels, increasing slightly in V3R2 at 5DR in comparison to R2 plants. Possibly the differences in MDA of V3R2 and R2 groups indicated that acclimatization process by recurrent waterlogging triggers degrees of tolerance that can be differently expressed according to the plant organ (part of plant) and duration of stress. In the roots, the content of $H_2O_2$ in both groups (V3R2 and R2) tended to reduce below control levels under waterlogging and recovery (Fig. 2C). However, an accumulation of MDA in the roots was observed in a continuous manner throughout hypoxic and recovery periods in the soybean plants from R2 group, i.e. increasing lipid peroxidation, contrasting to V3R2 group wich levels increased slightly only at 2DW and 2DR (Fig 2D). This suggests that the flooding of the root system provoked damage to cellular membranes, that could lead to the indirect inhibition of the photosynthesis by reducing the hydraulic conductivity that limited the stomata opening, and leakage of electrolytes, indicating that V3R2 suffered less oxidative damage and maintained a relatively better membrane stability compared to the R2 group under waterlogging stress (BRAMLEY et al., 2010; JITSUYAMA, 2017).

### *2.4.2.* Enzymatic antioxidant system

Plants have developed various detections and signalling mechanisms to properly perceive and respond to recurrent stress or sustained response after the onset of primary stress (LORETI; STRIKER, 2020; WANG; LIU; JIANG, 2017), including the synthesis of antioxidant enzymes and metabolites for the

detoxification of ROS. The activities of SOD, APX and CAT in the present study, indicated that the groups V3R2 and R2 developed different capacities to sequester $O_2^-$ and $H_2O_2$ in conditions of hypoxia and recovery (Fig. 3). At two days of waterlogging the leaves of the V3R2 group showed a more rapid accumulation of $H_2O_2$, probably leading to a higher APX activity (Fig. 2A, 3) and lower MDA production compared to the R2 group (Fig. 2B). On the other hand, at five days of waterlogging both the maintenance of APX activity (higher than control) and the stability of CAT activity were sufficient to maintain the production of $H_2O_2$ at control levels, but still the accumulation of MDA was observed probably due to the lower SOD activity in this period, which may have contributed for the generation of ROS. During the recovery periods (2DR and 5DR) the activity of SOD lower than control may be related to a greater accumulation of MDA in these periods, even with the accumulation of phenols that possibly contributed to the detoxification of ROS (Fig. 2B, 3A, 5A). Our results were consistent with previous studies which assessed this soybean genotype (GARCIA et al., 2020) and mung bean (BANSAL et al., 2019; KUMAR et al., 2013a).

Our results show that, in contrast to leaves, waterlogging at the V3 stage (V3R2) resulted in plants with a faster ability to activate the roots' antioxidant enzymes (SOD, CAT and APX) in response to waterlogging when compared to the R2 group (waterlogged at the R2 stage). Moreover, higher activities of these enzymes were observed in this organ in V3R2 plants. The increase in the concentration of antioxidant enzymes in plants under hypoxic conditions is a parameter of tolerance to waterlogging stress (JIMÉNEZ S.; MORENO F.; MAGNITSKIY, 2013).

Since the SOD is the enzyme which dismute the superoxide radical and forms $H_2O_2$, it is usually considered the first defense line against the oxidative stress. In this study, the increase in SOD activity in the roots in the V3R2 group compared to R2 suggests that the hypoxic stress in the V3 stage improved the waterlogging tolerance in this group of plants, probably due to a better detoxification ability by scavenging $O_2^-$, conferring a characteristic of tolerance to oxidative stress caused by waterlogging in the R2 stage. Increased SOD activity was associated with a greater protection against oxidative damage associated

with oxidative stress and, therefore, a better ability to tolerate the stress (ANJUM et al., 2017; BOARETTO et al., 2014).

Although there are many enzymes to regulate intracellular $H_2O_2$ levels, CAT and APX are considered the most important (LI et al., 2011b; TANG et al., 2010). Thus, the activity of APX and CAT in both leaves and roots of V3R2 and R2 increased significantly, but these enzymes were more responsive in the roots during waterlogging, following variations in SOD activity. These increases were higher in V3R2 than in R2, especially at 2DW, which suggests that flooding prior to stage R2 (first cycle of flooding at stage V3) provided a better ability to activate the antioxidant mechanisms to act on $H_2O_2$. The increased activity of these enzymes during waterlogging was also able to prevent a higher generation of ROS, resulting in less lipid peroxidation in the roots over the period of hypoxia and during post-hypoxia recovery in the V3R2 group compared to R2 (Fig. 2B, Fig. 2D). According to our results (Fig, 3 and 4) it is suggested that APX is the most important $H_2O_2$ neutralizing enzyme in leaves; and possibly in the roots, due to its activity much higher than that of CAT, contributing significantly for the decomposition of $H_2O_2$ in the roots and, probably, for a greater waterlogging tolerance ((LIN et al., 2004; WANG et al., 2016).

Therefore, according to KHAN; KHAN ( 2017) ( our data suggest that CAT and APX activities, coordinated with SOD activity, play a central protective role in the process of removing $O_2^-$ and $H_2O_2$ and the activities of these enzymes is related , at least in part, with the tolerance to oxidative stress induced by waterlogging in soybean plants.

Non-structural phenolic compounds perform a variety of functions in plants, including acting as antioxidants (COUTINHO et al., 2018; SARKER; OBA, 2018). It is possible that the lower activity of SOD in the V3R3 group at 5DW or even at the beginning of recovery (2DR) in the leaves of soybean plants in the V3R2 group may have been compensated, at least in part, by an increase in non-enzymatic antioxidants, like phenols, helping to remove ROS (Fig. 3; Fig. 5A). In the roots, no changes in the phenol concentrations were observed, remaining at or below the control levels (Fig. 5B). Phenolic compounds are non-enzymatic removers that work together to alleviate cell damage under oxidative stress conditions (ANEE et al., 2019a; PODSĘDEK, 2007), they also act as reducing

agents, metal chelators and simple oxygen extinguishers, considered multifunctional antioxidants (KANCHEVA, 2009).

*2.4.3.* Fermentative enzymes and Ala-AT

In hypoxic and anoxic (0% oxygen) environments caused by waterlogging, plants developed physiological and biochemical mechanisms to defend themselves against stress, such as increasing the soluble sugar content, promoting the activity of fermentative enzymes, and implementing the antioxidant defense system (HOSSAIN; UDDIN, 2011). When plants are under waterlogging, the glycolytic pathway is less efficient in producing total ATP, limited by the rate of oxidation of NADH, compensated by fermentation, and most efficient production (Pasteur effect) to increase ATP production at the substrate level when there is little oxygen. Under these conditions, there is an increase in the ethanolic fermentation involving the activity of the alcohol dehydrogenase enzyme (ADH) and lactic fermentation that depends on the activity of the enzyme lactate dehydrogenase (LDH), both involved in the reoxidation of $NAD^+$, an essential mechanism for the continuity of glycolysis in $O_2$ deficiency conditions (BAILEY-SERRES; VOESENEK, 2008; GIBBS; GREENWAY, 2003; SOUSA; SODEK, 2002a) On the other hand, the increase in the activity of the enzyme alanine aminotransferase (Ala-AT) under hypoxic conditions, seems to be necessary for the accumulation of alanine and to prevent the loss of carbon from pyruvate through ethanol, in addition to allowing an extra synthesis of ATP by a metabolic circuit involving glycolysis and the tricarboxylic acid cycle (TCA) (DE SOUSA; SODEK, 2003; MIYASHITA et al., 2007; ROCHA et al., 2010b). Lactic fermentation occurs through the reduction of pyruvate by the reaction catalysed by the LDH enzyme with the concomitant oxidation of NADH to $NAD^+$. It is activated by the low oxygen content in almost all plant species, producing lactate, which promotes the acidification of the cytoplasm (BANTI et al., 2013; SWEETLOVE et al., 2000).

Overall, the lowest LDH activities were observed in plants of the V3R2 group compared to R2, where the activity was superior to the control, i.e. this group of plants went into hypoxia faster than V3R2 plants, so the LDH induction was from the first waterlogging period (2DW), increasing at 5DW and 2DR, and decreasing to control levels only at 5DR. In this sense, it is possible that the

production of lactate had a greater negative effect on the cytoplasmic pH (BANTI et al., 2013) of R2 plants, promoting a higher acidification of the cytoplasm and toxicity (ROBERTS et al., 1984), than in V3R2 plants, possibly the priming could give an advantage to plants in the V3R2 group, in relation to metabolic adjustment under hypoxia, by inducing $O_2^-$ sparing mechanisms and protection mechanisms. (DIAB; LIMAMI, 2016)

The ethanolic fermentation consists of converting pyruvate to ethanol through coupled reactions: the first is catalyzed by the enzyme pyruvate decarboxylase (PDC) in which the pyruvate is decarboxylated to acetaldehyde and the second by the enzyme alcohol dehydrogenase (ADH), resulting in the conversion of acetaldehyde in ethanol (LICAUSI; PERATA, 2009). PDC activity increased markedly at 5 DW in groups V3R2 and R2 in an equivalent manner, equaling the control at 2DR in group V3R2, demonstrating a faster recovery compared to group R2, which maintained a high activity during this period. The activation of the PDC enzyme against the waterlogging condition and the restoration of activity in pre-hypoxic conditions is a characteristic that can differentiate the tolerance among soybean genotypes (BORELLA et al., 2014; GARCIA et al., 2020). The reduction in cell pH caused by the accumulation of lactate, due to the activation of LDH, possibly favored the catalytic activity of the PDC, which is responsible for the conversion of pyruvate to acetaldehyde, substrate for the production of ethanol under flooding conditions (GOOD; CROSBY, 1989) thereby leading to a similar response from the ADH enzyme to groups R2 and V3R2 (Fig. 6A).

The role of ethanolic fermentation is to allow the production of ATP through the glycolytic pathway by recycling $NAD^+$ through the action of two key enzymes, PDC and ADH (LORETI; PERATA, 2020). In this study, we observed an abrupt increase in ADH activity (Fig.6A) at 5DW in the waterlogged plants, especially in the R2 group, remaining high at 2DR, and not reaching control levels at 5DR in both groups. This demonstrate that the hypoxic condition must have been maintained even during the recovery period, possibly due to the poor drainage of the substrate (floodplain soil). The high activity of ADH under hypoxia allows the production of ATP, even if in a limited way, a condition that can improve the growth and survival of plants under oxygen deficiency (ISMOND et al., 2003;

TESNIERE et al., 2006). The enzymes ADH and PDC are responsible for the production of ethanol, the main fermentation product in tissues of higher plants that determine tolerance to oxygen deficiency (BORELLA et al., 2014). In plants of the V3R2 group, the lower activity of the fermentative enzymes under hypoxia, especially ADH, may have been the result of a gene regulation initiated at the V3 stage, involving those genes that are responsible for the perception of oxygen deprivation and that exhibit an enzymatic cascade similar to that of other species under hypoxia (KOMATSU et al., 2009). Thus, we suggest that the V3R2 group did not need, at least in the first periods of hypoxia, to increase the synthesis of fermentative enzymes in the same proportion as the R2 group, possibly the priming effect, could give an advantage to this plant group, in relation to metabolic adjustment under hypoxia.

The amino acids alanine and γ-aminobutyric acid (GABA) are products of anaerobic metabolism (BAILEY-SERRES; VOESENEK, 2008). Under waterlogging, alanine is produced by the transference of an amino group from glutamate to pyruvate, with the generation of α-ketoglutarate as a co-product (ROCHA et al., 2010a), mediated by alanine aminotransferase enzyme (Ala-AT), highly activated in roots under hypoxia (DE SOUSA; SODEK, 2003; MIYASHITA et al., 2007; RICOULT; CLIQUET; LIMAMI, 2005). Ala-AT activity (Fig. 6D) increased from the first days of waterlogging (2DW) to the first days of recovery (2DR) in R2 plants. In the V3R2 group, increases in the activity occurred at 5DW and later followed the same pattern as R2, but with lower activity values. The activity of Ala-AT followed the variations of the LDH enzyme probably as a tolerance mechanism, since alanine metabolism can prevent the accumulation of pyruvate, facilitating the continuous operation of glycolysis during waterlogging (BORELLA et al., 2017; ROCHA et al., 2010a). It is also known that aminotransferases located in chloroplasts play an important role in the synthesis of amino acids.

*2.4.4.* Nitrate, amino acids, total soluble sugars and sucrose

Plants assimilate most of the nitrate absorbed by their roots into nitrogenous organic compounds and in the case of soybeans, the roots may be responsible for up to 30% of the total nitrate assimilation in the plant (CRAFTS-BRANDNER; HARPER, 1982), which under anaerobic conditions can be

seriously restricted (BRANDÃO; SODEK, 2009). The nitrate concentration in the leaves decreased dramatically after hypoxia and recovery in the V3 stage (Sup. Fig. 9A), and in 2DW plants during hypoxia, but in the V3R2 group it increased at 5DW. Also in the latter group, the levels increased to 2DR, exceeding the levels of control, suggesting a greater transport to the aerial part due to the normoxic condition (Fig. 9A). The $NO_3^-$ is easily transported to the leaves for reduction and assimilation (CRAWFORD; FORDE, 2002). However, it is important to note that the nitrate reduction is active and has a high energy requirement since nitrate must be reduced to nitrite and then to $NH_4^+$ in the plastid through the NR activity, which is inhibited by $O_2$ deficiency and the availability of ATP is minor (BRANDÃO; SODEK, 2009; CRAWFORD; FORDE, 2002)

The levels of nitrate in the roots (Fig. 9B) were maintained in concentrations similar to the control at 2DW, increasing at 5DW, indicating that despite the lower availability of energy, there was absorption and probably an accumulation of nitrate due, in some extent, to the inhibition of NR triggered by hypoxia (BOTREL; KAISER, 1997; BRANDÃO; SODEK, 2009); and also, the scarcity of ATP for the assimilation into ammonium (LIMAMI; DIAB; LOTHIER, 2014). In fact, nitrate absorption is active (occurs against an electrochemical gradient) (DECHORGNAT et al., 2011). Although its absorption and assimilation in soybean plants under hypoxia conditions has restrictions in the roots, transport and assimilation in the leaves (OLIVEIRA; FRESCHI; SODEK, 2013) may have contributed to its increase at 5DW in these organs (Fig. 9A). At 2DR, the decrease in nitrate content is possibly associated with its transport to the aerial part and also its assimilation into amino acids in the roots (Fig. 9A, Fig. 6B).

The concentrations of total soluble amino acids (TSAA) in the leaves of plants at the V3 stage remained below control throughout the periods of waterlogging and recovery, indicating a drastic effect of hypoxic treatment on nitrogen metabolism in this group of plants. In contrast, in the roots concentrations remained stable or above control, which may be related to the redirection of assimilated nitrogen from the aerial part to the roots (Fig. 6). Similarly, in the groups V3R2 and R2 there was a decrease of TSAA content at 2DW, suggesting a possible targeting of the amino acids produced from the shoot to the root system or even a proteolysis increase in the roots (REGGIANI et al.,

1988; SOUSA; SODEK, 2002b) to support the hypoxic metabolism of this organ. In fact, even under hypoxia of the root system of soybean plants in a medium containing $^{15}NO_3^-$, it was possible to observe the presence of $^{15}N$-amino acids transported to the roots via phloem (OLIVEIRA; FRESCHI; SODEK, 2013).

The concentration of TSAA increased in the V3R2 group, probably due to the nitrate absorbed and directed to the aerial part, resulting in higher levels also in the roots in relation to the R2 group (Fig. 6; Fig. 9A). During recovery, TSAA concentrations decreased initially (at 2DR, less drastic effect in V3R2) probably due to the directing of amino acids to the roots for reparation and post-hypoxia growth, leading to the restoration of amino acid levels in this organ (Fig. 6), period that coincides with the increase in the concentration of nitrate in the leaves and decrease in the roots (Fig. 9). Under conditions of low oxygen concentrations, the transition from aerobic metabolism to fermentative metabolism requires a constant supply of carbohydrates. In fact, maintaining adequate levels of easily fermentable sugars in hypoxic or anoxic roots is one of the main adaptive mechanisms to an oxygen-poor environment (SAIRAM et al., 2009). The concentrations of total soluble sugars in the leaves of plants in stage V3 increased as a common response to waterlogging, possibly supply the demand for carbohydrates in the roots that increased after five days of waterlogging and especially in the initial post-recovery period (2DR). The same response was observed in sucrose levels: this disaccharide played a central role among soluble carbohydrates for metabolic adjustments during waterlogging and recovery (Fig. 7; Fig. 8). In fact, maintaining adequate levels of easily fermentable sugars in hypoxic roots is one of the main mechanisms of adaptation to an oxygen-poor environment (BANTI et al., 2013).

Regarding the levels of total soluble sugars in the leaves of the V3R2 and R2 groups, there was a slightly increase in the waterlogging period, and a considerable increase for the V3R2 group at 2DR, possibly resulting in a better osmotic adjustment in these plants, which may result in better parameters of gas exchange compared to the R2 group during recovery (Fig. 7A). In addition to these aspects, the V3R2 group was able to maintain total soluble sugar levels throughout the waterlogging (Fig. 7B).

At 2WD, the decrease of sucrose content in leaves is related to the maintenance of levels equivalent or superior to the control in the roots. In the longer period of hypoxic stress (5DW) the metabolic adjustments in the leaves allowed both groups of plants to maintain the concentrations of sucrose at the control levels and a considerable increase in the roots of the plants of the group V3R2. Two days of recovery resulted in higher levels of sucrose in the group V3R2 compared to R2 and control groups were also maintained in the leaves during recovery (2DR and 5DR), which allowed the return to control levels of sucrose at 2DR in the group V3R2 (Fig. 8).

Plants of the R2 group behaved as more susceptible to hypoxia because there was a decrease in the demand for carbohydrates to repair stress and resumed growth. Indeed, this maintenance of adequate carbohydrate availability after prolonged anoxia or hypoxia is considered a direct consequence of a well-modulated fermentative metabolic activity, which is a strategy for some tolerant species (BERTRAND et al., 2003). The plants in the R2 group showed characteristics of greater susceptibility to hypoxia considering the decrease in the levels of carbohydrates, such as total soluble sugars and sucrose, probably due to the higher consumption of ATP by the cells to maintain the metabolism, whereas the V3R2 plants showed characteristics of species with greater tolerance, considering the increase in the levels of these carbohydrates. We believe that this response is related to the stress imposed in the V3 stage, which allowed a better adjustment of the cellular metabolism in the V3R2 plants in response to the second waterlogging cycle in the R2 stage, revealing that the stimulus was perceived and involved the plant in a sequence of processes that led to the final response (DEMONGEOT; HASGUI; THELLIER, 2019; HILKER; SCHMÜLLING, 2019b). This may be related to the increased activity of the enzymes of carbohydrate metabolism such as sucrose synthase (SS) (BORELLA et al., 2014; KENNEDY; RUMPHO; FOX, 1992). which is considered a key enzyme responsible for the production of glucose and fructose from sucrose under oxygen deprivation, the main source of energy under hypoxia (KATO-NOGUCHI, 2006; KUMUTHA et al., 2008); then, sucrose is transported through phloem to other plant organs (in this case to the roots) supplying energy or carbohydrates (SPRINGER et al., 1986), Thus, it may explain the capacity of

the V3R2 plants to ferment the sugars available for a proper metabolic function, which may support the plants to tolerate a waterlogged environment.

*2.4.5.* Photosynthetic pigments

In this study, a varied time-dependent response of leaf photosynthetic pigments was observed. Decreases in chlorophyll and carotenoids due to waterlogging stress was observed in several cultures (KUMAR et al., 2013b; SOUZA et al., 2004; WEI et al., 2013; YIU et al., 2008), which have evolved to help protect the plant from environmental pressures (VAN AMERONGEN; CHMELIOV, 2020). Carotenoid pigments protect photosynthetic structures, such as the photosynthetic systems I and II, by neutralizing the triplete *Chl* excited by dissipating energy excess, and inhibiting oxidative damage through the fixing of singlet oxygen (LOGAN; ADAMS; DEMMIG-ADAMS, 2007; PETERMAN et al., 1995). Carotenoid concentrations are determined by genetic, biochemical and physiological attributes of a plant species, as well as by environmental factors such as fertility, light and temperature (KOPSELL et al., 2013) or by stress factors such as drought (SINGH; RAJA REDDY, 2011) and waterlogging (AVOLA et al., 2008).

The waterlogged 6042 soybean plants completely changed the concentration of *Chl* and *Car*. At the end of the hypoxia period, the V3R2 group showed higher concentrations of *Chl a, Chl b* and *Car* compared to the control, in contrast to the R2 group in which the levels of these pigments decreased. The decreases in the V3R2 plants in recovery were also smaller than those of the R2 group. Waterlogging resulted in visible yellowing of the 6042 soybean leaves (data not showed) of the R2 group, followed by the V3R2, caused by the decrease in the concentration of chlorophylls and carotenoids, which is related to nitrogen deficiency. Impaired nitrogen fixation by waterlogging in the soybean plant is inhibited almost immediately due to the decrease in $O_2$ supply (AMARANTE; LIMA; SODEK, 2006). Other studies performed with wheat (COLLAKU; HARRISON, 2002; TAN et al., 2008), mung beans (KUMAR et al., 2013b; POSSO et al., 2018), pepino (BARICKMAN; SIMPSON; SAMS, 2019), and soybean (GARCIA et al., 2020) ound that waterlogging reduces as *Chl* and *Car* concentrations.

In the current study, the V3R2 group developed a stress response to waterlogging by increasing Car concentrations. On the other hand, in the R2 group the decrease in the content of Car may have contributed to the decrease in the concentration of chlorophylls, since they are accessory pigments in the capture of light and perform energy transfer in the photochemical processes, playing an important role in photoprotection of chlorophylls. In this sense, the decrease of these pigments may have contributed to a decrease in the photosynthetic capacity of leaves the R2 group when compared to V3R2 (Fig.11A, B, C and D; 12A) in response to waterlogging, accelerating the senescence process. This resulted in negative photosynthetic properties (VANDOORNE et al., 2014), reduced chlorophyll content, and a reduced photosynthetic capacity in leaves (IRFAN et al., 2010).

*2.4.6.* Yield and yield components

The effects of the waterlogging stress of soybean plants on the performance of production and yield components showed that waterlogging in the stage V3 for seven days improved the yield and its components when these plants were subjected to waterlogging in a second cycle (stage R2) for five days (V3R2) compared to plants that were waterlogged plants only in the R2 stage (Table 1). This suggest that the tight relation between the number of pods per plant and the number of grains per pod has a positive correspondence with the grain yield, while the mass of 100 grains has a negative correspondence (GONÇALVES et al., 2003). However, we believe that the number of grains per legume is a genetic characteristic of the plant itself, we did not observe any difference between treatments, therefore it did not influence the yield.

This relationship could highlight the tolerance of soybean under waterlogging in V3R2 plants that showed better results in terms of yield components compared to R2 plants. These results can be justified by the action of the physiological conditioning, favouring the tolerance in plants under waterlogging. In this case, an initial stress provokes the physiological conditioning and favours greater responses to future stresses that the plant may suffer (BRUCE et al., 2007). This adjustment, commonly translated by genetic and biochemical changes induced by a first exposure to stress, increases resistance to a subsequent adverse condition (SILVA et al., 2016).

Plants of the V3 and R2 groups showed a reduction in the number of pods per plant (SULLIVAN et al., 2001), in accordance to the reduction in the height of the plants that were exposed to waterlogging for seven days (V3), corresponding to other studies (RHINE et al., 2010; SCHÖFFEL et al., 2001; TEWARI; ARORA, 2016a). The plant responses of yield and productivity such as yield (Kg/ha), pods per plant, dry weight of seeds per plant, dry weight of 100 seeds, weight of seeds per box, number of nodes, first pod insertion height, and height of the plant in the V3R2 plants were much better compared to R2, as distinct from to the seeds per pod, which did not differ between treatments

## *2.5.* Conclusions

We demonstrated that, when plants are exposed to hypoxia and recovery at the R2 stage there is a difference in the metabolism of stress priming soybean plants under waterlogging at the V3 stage (V3R2 group) compared to those which were not priming (group R2). Several metabolic factors were improved such as (i) reduced ROS production, (ii) the antioxidant capacity for scavenging ROS, (iii) fermentative metabolism induction and recovery, resulting in lower LDH, PDC, ADH and Ala-AT enzyme activities, (iv) the higher availability of TSS, sucrose, TAA, and nitrate, (v) the yield components, suggesting that the initial stress (stage V3) caused by physiological conditioning substantially favors the yield compared to unconditioned plants despite being smaller than control plants.

Overall, the results show that responses to stress may confer partial tolerance to waterlogging events. These responses may potentially allow for recovery after the occurrence of a waterlogging event. Thus, a deeper and broader understanding of how the effects of stress in the early stage of development in soybean plants may influence the tolerance responses to stress events in more advanced stages of development is necessary, considering the molecular, proteomic and metabolic aspects involved in acclimatization and duration of its effects on plants.

## *2.6.* References


AGOSTINETTO, D. et al. Performance of transgenic soybean cultivars and weed control in function of application times and glyphosate formulations. **Planta Daninha**, v. 27, n. 4, p. 739–746, 2009.

AHMED, S. et al. Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. **Plant Science**, v. 163, n. 1, p. 117–123, 2002.

ALAM, I. et al. Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage. **Journal of Biosciences**, v. 35, n. 1, p. 49–62, mar. 2010.

AMARANTE, L. DO; LIMA, J. D.; SODEK, L. Growth and stress conditions cause similar changes in xylem amino acids for different legume species. **Environmental and Experimental Botany**, v. 58, n. 1–3, p. 123–129, 2006.

AMARANTE, L.; SODEK, L. Waterlogging effect on xylem sap glutamine of nodulated soybean. **BIOLOGIA PLANTARUM**, v. 50, n. 3, p. 405–410, 2006.

ANDRADE, C. A. et al. Hydrogen peroxide promotes the tolerance of soybeans to waterlogging. **Scientia Horticulturae**, v. 232, p. 40–45, 17 fev. 2018.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019a.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019b.

ANJUM, S. A. et al. Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. **Frontiers in Plant Science**, v. 08, 6 fev. 2017.

ARBONA, V. et al. Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. **Physiologia Plantarum**, v. 132, n. 4, p. 452–466, abr. 2008.

AROCA, R.; PORCEL, R.; RUIZ-LOZANO, J. M. Regulation of root water uptake under abiotic stress conditions. **Journal of Experimental Botany**, v. 63, n. 1, p. 43–57, 1 jan. 2012.

ASHRAF, M. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (Panicum antidotale Retz.) under salinity or waterlogging. **Plant Science**, v. 165, n. 1, p. 69–75, 1 jul. 2003.

AUGE, G. A. et al. Adjusting phenotypes via within- and across-generational plasticity. **New Phytologist**, v. 216, n. 2, p. 343–349, 2017.

AVOLA, G. et al. Gas exchange and photosynthetic water use efficiency in response to light, CO2 concentration and temperature in Vicia faba. **Journal of Plant Physiology**, v. 165, n. 8, p. 796–804, 26 maio 2008.

AZEVEDO, R. A. et al. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. **Physiologia Plantarum**, v.

104, n. 2, p. 280–292, out. 1998.

AZIZIAN, A.; SEPASKHAH, A. R. Maize response to water, salinity and nitrogen levels: Physiological growth parameters and gas exchange. **International Journal of Plant Production**, v. 8, n. 1, p. 131–162, 2013.

BAILEY-SERRES, J. et al. Making sense of low oxygen sensing. **Trends in Plant Science**, v. 17, n. 3, p. 129–138, mar. 2012.

BAILEY-SERRES, J.; LEE, S. C.; BRINTON, E. Waterproofing Crops: Effective Flooding Survival Strategies. **Plant Physiology**, v. 160, n. 4, p. 1698–1709, dez. 2012.

BAILEY-SERRES, J.; VOESENEK, L. A. C. J. Flooding Stress: Acclimations and Genetic Diversity. **Annual Review of Plant Biology**, v. 59, n. 1, p. 313–339, jun. 2008.

BALAKHNINA, T. I. Plant Responses to Soil Flooding. In: **Stress Responses in Plants**. Cham: Springer International Publishing, 2015. p. 115–142.

BANSAL, R. et al. Waterlogging tolerance in black gram [Vigna mungo (L.) Hepper] is associated with chlorophyll content and membrane integrity. **Indian Journal of Biochemistry & Biophysics**, v. 56, p. 81–85, 2019.

BANSAL, R.; SRIVASTAVA, J. P. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. **Russian Journal of Plant Physiology**, v. 62, n. 3, p. 322–327, 29 maio 2015.

BANTI, V. et al. Low Oxygen Response Mechanisms in Green Organisms. **International Journal of Molecular Sciences**, v. 14, n. 3, p. 4734–4761, 27 fev. 2013.

BARICKMAN, T. C.; SIMPSON, C. R.; SAMS, C. E. Waterlogging Causes Early Modification in the Physiological Performance, Carotenoids, Chlorophylls, Proline, and Soluble Sugars of Cucumber Plants. **Plants**, v. 8, n. 6, p. 160, 8 jun. 2019.

BATISTA, T. B. et al. Condicionamento fisiológico e stress sob alta umidade e temperatura na qualidade fisiológica de sementes de Brachiaria brizantha cv. MG-5. **Acta Scientiarum - Agronomy**, v. 38, n. 1, p. 123–127, 1 jan. 2016.

BAUER, G. Increasing the endogenous NO level causes catalase inactivation and reactivation of intercellular apoptosis signaling specifically in tumor cells. **Redox Biology**, v. 6, p. 353–371, 1 dez. 2015.

BAXTER, A.; MITTLER, R.; SUZUKI, N. ROS as key players in plant stress signalling. **Journal of Experimental Botany**, v. 65, n. 5, p. 1229–1240, 1 mar. 2014.

BERTRAND, A. et al. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. **Journal of Experimental Botany**, v. 54, n. 388, p. 1721–1730, 2003.

BEUTLER, A. N. et al. Soil hydric excess and soybean yield and development in Brazil. **Australian Journal of Crop Science**, v. 8, n. 10, p. 1461–1466, 2014.

BIELESKI, R. L.; TURNER, N. A. Separation and estimation of amino acids in

crude plant extracts by thin-layer electrophoresis and chromatography. **Analytical Biochemistry**, v. 17, n. 2, p. 278–293, 1 nov. 1966.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. **Plant Physiology**, v. 116, n. 2, p. 651–658, 1998.

BIN, T. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, maio 2010.

BLOKHINA, O. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. **Annals of Botany**, v. 91, n. 2, p. 179–194, 1 jan. 2003.

BLOKHINA, O.; FAGERSTEDT, K. V. Oxidative metabolism, ROS and NO under oxygen deprivation. **Plant Physiology and Biochemistry**, v. 48, n. 5, p. 359–373, maio 2010.

BOARD, J. E. Waterlogging effects on plant nutrient concentrations in soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, maio 2008a.

BOARD, J. E. Waterlogging Effects on Plant Nutrient Concentrations in Soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, 13 maio 2008b.

BOARETTO, L. F. et al. Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. **Plant Physiology and Biochemistry**, v. 74, p. 165–175, jan. 2014.

BONIFACIO, A. et al. Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. **Plant, Cell and Environment**, v. 34, n. 10, p. 1705–1722, 2011.

BORELLA, J. et al. Waterlogging-induced changes in fermentative metabolism in roots and nodules of soybean genotypes. **Scientia Agricola**, v. 71, n. 6, p. 499–508, dez. 2014.

BORELLA, J. et al. Hypoxia-driven changes in glycolytic and tricarboxylic acid cycle metabolites of two nodulated soybean genotypes. **Environmental and Experimental Botany**, v. 133, p. 118–127, 1 jan. 2017.

BORELLA, J. et al. Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. **Scientia Agricola**, v. 76, n. 1, p. 51–62, 1 fev. 2019.

BOTREL, A.; KAISER, W. M. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. **Planta**, v. 201, n. 4, p. 496–501, 9 abr. 1997.

BOTREL, A.; MAGNE, C.; KAISER, W. M. Nitrate reduction, nitrite reduction and ammonium assimilation in barley roots in response to anoxia. **Plant Physiology and Biochemistry**, v. 34, n. 5, p. 645–652, 1996.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 7 maio 1976.

BRAMLEY, H. et al. The contrasting influence of short-term hypoxia on the hydraulic properties of cells and roots of wheat and lupin. **Functional Plant Biology**, v. 37, n. 3, p. 183, 2010.

BRANDÃO, A. D.; SODEK, L. Nitrate uptake and metabolism by roots of soybean plants under oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 21, n. 1, p. 13–23, 2009.

BRITTO, D. T.; KRONZUCKER, H. J. NH4+ toxicity in higher plants: a critical review. **Journal of Plant Physiology**, v. 159, n. 6, p. 567–584, jan. 2002.

BRUCE, T. J. A. et al. Stressful “memories” of plants: Evidence and possible mechanisms. **Plant Science**, v. 173, n. 6, p. 603–608, 1 dez. 2007.

BUI, L. T. et al. Conservation of ethanol fermentation and its regulation in land plants. **Journal of Experimental Botany**, v. 70, n. 6, p. 1815–1827, 27 mar. 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, 1 nov. 1991.

CAPONE, R.; TIWARI, B. S.; LEVINE, A. Rapid transmission of oxidative and nitrosative stress signals from roots to shoots in Arabidopsis. **Plant Physiology and Biochemistry**, v. 42, n. 5, p. 425–428, 1 maio 2004.

CATALDO, D. A. et al. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis**, v. 6, n. 1, p. 71–80, 1 jan. 1975.

CAVALCANTI, F. R. et al. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. **New Phytologist**, v. 163, n. 3, p. 563–571, set. 2004.

CENTRITTO, M. et al. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. **Journal of Experimental Botany**, v. 60, n. 8, p. 2325–2339, 1 maio 2009.

CHAMIZO-AMPUDIA, A. et al. Nitrate Reductase Regulates Plant Nitric Oxide Homeostasis. **Trends in Plant Science**, v. 22, n. 2, p. 163–174, 1 fev. 2017.

CHO, J. W.; YAMAKAWA, T. Effects on growth and seed yield of small seed soybean cultivars of flooding conditions in paddy field. **Journal of the Faculty of Agriculture, Kyushu University**, v. 51, n. 2, p. 189–193, 2006.

COLLAKU, A.; HARRISON, S. A. Losses in Wheat Due to Waterlogging. **Crop Science**, v. 42, n. 2, p. 444–450, 1 mar. 2002.

COLMER, T. D.; VOESENEK, L. A. C. J. C. J. Flooding tolerance: suites of plant traits in variable environments. **Functional Plant Biology**, v. 36, n. 8, p. 665, 2009.

CONRATH, U. et al. Priming for Enhanced Defense. **Annual Review of Phytopathology**, v. 53, n. 1, p. 97–119, 4 ago. 2015.

CORPAS, F. J.; RÍO, L. A. DEL; PALMA, J. M. Impact of Nitric Oxide (NO) on the

ROS Metabolism of Peroxisomes. **Plants**, v. 8, n. 2, p. 37, 10 fev. 2019.

COSKUN, D.; BRITTO, D. T.; KRONZUCKER, H. J. The nitrogen-potassium intersection: membranes, metabolism, and mechanism. **Plant, Cell & Environment**, v. 40, n. 10, p. 2029–2041, 1 out. 2017.

COUTINHO, I. D. et al. Flooded soybean metabolomic analysis reveals important primary and secondary metabolites involved in the hypoxia stress response and tolerance. **Environmental and Experimental Botany**, v. 153, p. 176–187, set. 2018.

CRAFTS-BRANDNER, S. J.; HARPER, J. E. Nitrate Reduction by Roots of Soybean (Glycine max [L.] Merr.) Seedlings. **Plant Physiology**, v. 69, n. 6, 1982.

CRAWFORD, N. M. Nitrate: nutrient and signal for plant growth. **The Plant Cell**, v. 7, n. 7, p. 859–868, jul. 1995.

CRAWFORD, N. M.; FORDE, B. G. Molecular and Developmental Biology of Inorganic Nitrogen Nutrition. **The Arabidopsis Book**, v. 1, p. e0011, jan. 2002.

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, 1 ago. 2020a.

DA-SILVA, C. J.; DO AMARANTE, L. Time-course biochemical analyses of soybean plants during waterlogging and reoxygenation. **Environmental and Experimental Botany**, v. 180, p. 104242, 1 dez. 2020b.

DA-SILVA, C. J.; MODOLO, L. V. Hydrogen sulfide: a new endogenous player in an old mechanism of plant tolerance to high salinity. **Acta Botanica Brasilica**, v. 32, n. 1, p. 150–160, 19 out. 2017.

DA ROCHA, T. S. M. et al. Performance of soybean in hydromorphic and nonhydromorphic soil under irrigated or rainfed conditions. **Pesquisa Agropecuaria Brasileira**, v. 52, n. 5, p. 293–302, 1 maio 2017.

DE AZEVEDO NETO, A. D. et al. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. **Environmental and Experimental Botany**, v. 56, n. 1, p. 87–94, maio 2006.

DE OLIVEIRA, W. J. et al. Leaf gas exchange in cowpea and CO2 efflux in soil irrigated with saline water. **Revista Brasileira de Engenharia Agricola e Ambiental**, v. 21, n. 1, p. 32–37, 2017.

DE SOUSA, C. A. F.; SODEK, L. Alanine metabolism and alanine aminotransferase activity in soybean (Glycine max) during hypoxia of the root system and subsequent return to normoxia. **Environmental and Experimental Botany**, v. 50, n. 1, p. 1–8, 1 ago. 2003.

DECHORGNAT, J. et al. From the soil to the seeds: the long journey of nitrate in plants. **Journal of Experimental Botany**, v. 62, n. 4, p. 1349–1359, 1 fev. 2011.

DEMONGEOT, J.; HASGUI, H.; THELLIER, M. Memory in plants: Boolean modeling of the learning and store/recall memory functions in response to environmental stimuli. **Journal of Theoretical Biology**, v. 467, p. 123–133, 21

abr. 2019.

DIAB, H.; LIMAMI, A. Reconfiguration of N Metabolism upon Hypoxia Stress and Recovery: Roles of Alanine Aminotransferase (AlaAT) and Glutamate Dehydrogenase (GDH). **Plants**, v. 5, n. 2, p. 25, 31 maio 2016.

DIETZ, K.-J.; MITTLER, R.; NOCTOR, G. Recent Progress in Understanding the Role of Reactive Oxygen Species in Plant Cell Signaling. **Plant Physiology**, v. 171, n. 3, 2016.

DO AMARAL, M. N. et al. Long-term transcriptional memory in rice plants submitted to salt shock. **Planta**, v. 251, n. 6, p. 111, 1 jun. 2020.

DONAT, M. G. et al. More extreme precipitation in the world's dry and wet regions. **Nature Climate Change**, v. 6, n. 5, p. 508–513, 7 maio 2016.

EIGENBROD, F. et al. Vulnerability of ecosystems to climate change moderated by habitat intactness. **Global Change Biology**, v. 21, n. 1, p. 275–286, 1 jan. 2015.

EMBRAPA SOJA. **Soja - Portal Embrapa**. Disponível em: <https://www.embrapa.br/soja/cultivos/soja1>. Acesso em: 21 jul. 2020.

EVANS, D. E.; GLADISH, D. K. Plant Responses to Waterlogging. In: **Encyclopedia of Applied Plant Sciences**. Second Edi ed. [s.l.] Elsevier, 2017. v. 1p. 36–39.

FARNESE, F. S. et al. When Bad Guys Become Good Ones: The Key Role of Reactive Oxygen Species and Nitric Oxide in the Plant Responses to Abiotic Stress. **Frontiers in Plant Science**, v. 7, n. APR2016, 12 abr. 2016.

FEHR, W. R.; AND C. E, C. "Stages of soybean development" (1977). Special Report. 80. p. 1–12, 1977.

FEHR, W. R. et al. Stage of Development Descriptions for Soybeans, Glycine Max (L.) Merrill 1. **Crop Science**, v. 11, n. 6, p. 929–931, nov. 1971.

FLETA-SORIANO, E.; MUNNÉ-BOSCH, S. Stress Memory and the Inevitable Effects of Drought: A Physiological Perspective. **Frontiers in Plant Science**, v. 7, n. February, p. 1–6, 15 fev. 2016.

FORDE, B. G.; CLARKSON, D. T. Nitrate and Ammonium Nutrition of Plants: Physiological and Molecular Perspectives. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 30p. 1–90.

FOYER, C. H. et al. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. **Physiologia Plantarum**, v. 100, n. 2, p. 241–254, jun. 1997.

FUKAO, T. et al. Submergence and Waterlogging Stress in Plants: A Review Highlighting Research Opportunities and Understudied Aspects. **Frontiers in Plant Science**, v. 10, p. 340, 22 mar. 2019.

FURTADO, G. DE F. et al. Alterações fisiológicas em feijão-caupi irrigado com água salina e adubação nitrogenada. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 8, n. 3, p. 175–181, 25 out. 2013.

GARCIA, N. et al. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. **Environmental and Experimental Botany**, v. 172, p. 103975, 1 abr. 2020.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, v. 59, n. 2, p. 309–14, 1 fev. 1977.

GIBBS, J.; GREENWAY, H. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. **Functional Plant Biology**, v. 30, n. 1, p. 1, 2003.

GILL, P. K. et al. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. **BULG. J. PLANT PHYSIOL.**, v. 27, n. 2, p. 72–84, 2001.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909–930, dez. 2010.

GITHIRI, S. M. et al. QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. **Plant Breeding**, v. 125, n. 6, p. 613–618, 1 dez. 2006.

GIUNTOLI, B.; PERATA, P. Group VII Ethylene Response Factors in Arabidopsis: Regulation and Physiological Roles. **Plant Physiology**, v. 176, n. 2, 2018.

GONÇALVES, M. et al. Correlations and path analysis of common bean grain yield and its primary components. **Cropp Breeding and Applied Biotechnology**, v. 3, n. 3, p. 217–222, 30 set. 2003.

GOOD, A. G.; CROSBY, W. L. Anaerobic Induction of Alanine Aminotransferase in Barley Root Tissue. **Plant Physiology**, v. 90, n. 4, p. 1305–1309, 1 ago. 1989.

GOOD, A. G.; MUENCH, D. G. Purification and Characterization of an Anaerobically Induced Alanine Aminotransferase from Barley Roots. **Plant Physiology**, v. 99, n. 4, p. 1520–1525, 1 ago. 1992.

GRAHAM, D.; SMYDZUK, J. Use of anthrone in the quantitative determination of hexose phosphates. **Analytical Biochemistry**, v. 11, n. 2, p. 246–255, 1 maio 1965.

GUPTA, K. J. et al. The form of nitrogen nutrition affects resistance against Pseudomonas syringae pv. phaseolicola in tobacco. **Journal of Experimental Botany**, v. 64, n. 2, p. 553–568, jan. 2013.

GUPTA, K. J. et al. Nitric Oxide Is Required for Homeostasis of Oxygen and Reactive Oxygen Species in Barley Roots under Aerobic Conditions. **Molecular Plant**, v. 7, n. 4, p. 747–750, abr. 2014.

GUPTA, K. J. et al. The role of nitrite and nitric oxide under low oxygen conditions in plants. **New Phytologist**, v. 225, n. 3, p. 1143–1151, 11 fev. 2020.

GUPTA, K. J. **Nitrogen Metabolism in Plants**. Springer S ed. New York, NY: Springer New York, 2020. v. 2057

GUPTA, K. J.; IGAMBERDIEV, A. U. Reactive Nitrogen Species in Mitochondria and Their Implications in Plant Energy Status and Hypoxic Stress Tolerance.

**Frontiers in Plant Science**, v. 7, n. MAR2016, p. 369, 24 mar. 2016.

GUPTA, K. J.; KAISER, W. M. Production and scavenging of nitric oxide by barley root mitochondria. **Plant and Cell Physiology**, v. 51, n. 4, p. 576–584, abr. 2010.

GUPTA, K. J.; ZABALZA, A.; VAN DONGEN, J. T. Regulation of respiration when the oxygen availability changes. **Physiologia Plantarum**, v. 137, n. 4, p. 383–391, 2009.

GUPTA, P. et al. Signaling cross talk between biotic and abiotic stress responses in soybean. In: **Abiotic and Biotic Stresses in Soybean Production**. [s.l.] Elsevier, 2016. v. 1p. 27–52.

HANCOCK, J. T.; NEILL, S. J. Nitric oxide: Its generation and interactions with other reactive signaling compounds. **Plants**, v. 8, n. 2, 1 fev. 2019.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984a.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984b.

HARTMAN, S.; SASIDHARAN, R.; VOESENEK, L. A. C. J. **The role of ethylene in metabolic acclimations to low oxygenNew Phytologist**, 2019.

HASANUZZAMAN, M. et al. Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor. In: **Crop Stress and its Management: Perspectives and Strategies**. Dordrecht: Springer Netherlands, 2012. v. 9789400722p. 261–315.

HEMANTARANJAN, A. Flooding: Abiotic Constraint Limiting Vegetable Productivity. **Advances in Plants & Agriculture Research**, v. 1, n. 3, 23 jul. 2014.

HILKER, M. et al. Priming and memory of stress responses in organisms lacking a nervous system. **Biological Reviews**, v. 91, n. 4, p. 1118–1133, nov. 2016.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, mar. 2019a.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, 1 mar. 2019b.

HINCHA, D. K.; ZUTHER, E. Introduction: Plant cold acclimation and freezing tolerance. **Methods in Molecular Biology**, v. 1166, p. 1–6, 2014.

HOLZMEISTER, C. et al. Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. **Journal of Experimental Botany**, v. 66, n. 3, p. 989–999, 1 fev. 2015.

HOSSAIN, A.; UDDIN, S. N. Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. **Australian Journal of Crop Science**, v. 5, n. 9 SPEC. ISSUE, p. 1094–1101, 2011.

HSU, F.-C. et al. Insights into Hypoxic Systemic Responses Based on Analyses

of Transcriptional Regulation in Arabidopsis. **PLoS ONE**, v. 6, n. 12, p. e28888, 15 dez. 2011.

IGAMBERDIEV, A. U. et al. NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. **Planta**, v. 219, n. 1, p. 95–102, 22 maio 2004.

IGAMBERDIEV, A. U. Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. **Journal of Experimental Botany**, v. 55, n. 408, p. 2473–2482, 24 set. 2004.

IGAMBERDIEV, A. U. et al. The Haemoglobin/Nitric Oxide Cycle: Involvement in Flooding Stress and Effects on Hormone Signalling. **Annals of Botany**, v. 96, n. 4, p. 557–564, 1 set. 2005.

IGAMBERDIEV, A. U.; HILL, R. D. Plant mitochondrial function during anaerobiosis. **Annals of Botany**, v. 103, n. 2, p. 259–268, jan. 2009.

IQBAL, N.; NAZAR, R. **Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies**. New Delhi: Springer India, 2016.

IRFAN, M. et al. Physiological and biochemical changes in plants under waterlogging. **Protoplasma**, v. 241, n. 1–4, p. 3–17, 12 maio 2010.

ISMOND, K. P. et al. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. **Plant Physiology**, v. 132, n. 3, p. 1292–1302, 1 jul. 2003.

JENNINGS, A. C. The determination of dihydroxy phenolic compounds in extracts of plant tissues. **Analytical Biochemistry**, v. 118, n. 2, p. 396–398, dez. 1981.

JIMÉNEZ S., J. D. LA C.; MORENO F., L. P.; MAGNITSKIY, S. Plant responses to stress due to flooding. A review. **Revista Colombiana de Ciencias Hortícolas**, v. 6, n. 1, p. 96–109, 4 fev. 2013.

JITSUYAMA, Y. Hypoxia-Responsive Root Hydraulic Conductivity Influences Soybean Cultivar-Specific Waterlogging Tolerance. **American Journal of Plant Sciences**, v. 08, n. 04, p. 770–790, 3 mar. 2017.

JUSTINO, G. C.; SODEK, L. Recovery of nitrogen fixation after short-term flooding of the nodulated root system of soybean. **Journal of Plant Physiology**, v. 170, n. 3, p. 235–241, fev. 2013.

KANCHEVA, V. D. Phenolic antioxidants – radical-scavenging and chain-breaking activity: A comparative study*. **European Journal of Lipid Science and Technology**, v. 111, n. 11, p. 1072–1089, nov. 2009.

KATO-NOGUCHI, H. Pyruvate metabolism in rice coleoptiles under anaerobiosis. **Plant Growth Regulation**, v. 50, n. 1, p. 41–46, 23 nov. 2006.

KENNEDY, R. A.; RUMPHO, M. E.; FOX, T. C. Anaerobic metabolism in plants. **Plant Physiology**, v. 100, n. 1, p. 1–6, 1 set. 1992.

KEUNEN, E. et al. A mutant of the Arabidopsis thaliana LIPOXYGENASE1 gene shows altered signalling and oxidative stress related responses after cadmium exposure. **Plant Physiology and Biochemistry**, v. 63, p. 272–280, fev. 2013.

KHAN, M. I. R.; KHAN, N. A. **Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress**. Singapore: Springer Singapore, 2017.

KINOSHITA, T.; SEKI, M. Epigenetic Memory for Stress Response and Adaptation in Plants. **Plant and Cell Physiology**, v. 55, n. 11, p. 1859–1863, nov. 2014.

KISHOREKUMAR, R. et al. An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants. In: **Methods in Molecular Biology**. [s.l.] Humana Press Inc., 2020. v. 2057p. 1–13.

KOCH, K. E. Carbohydrate-modulated gene expression in plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 47, n. 1, p. 509–540, 1996.

KOLB, R. M.; JOLY, C. A. Flooding tolerance of Tabebuia cassinoides: Metabolic, morphological and growth responses. **Flora - Morphology, Distribution, Functional Ecology of Plants**, v. 204, n. 7, p. 528–535, 1 jan. 2009.

KOMATSU, S. et al. A comprehensive analysis of the soybean genes and proteins expressed under flooding stress using transcriptome and proteome techniques. **Journal of Proteome Research**, v. 8, n. 10, p. 4766–4778, 2009.

KOMATSU, S. et al. Identification of flooding stress responsible cascades in root and hypocotyl of soybean using proteome analysis. **Amino Acids**, v. 38, n. 3, p. 729–738, 31 mar. 2010.

KOMATSU, S.; HIRAGA, S.; YANAGAWA, Y. Proteomics Techniques for the Development of Flood Tolerant Crops. **Journal of Proteome Research**, v. 11, n. 1, p. 68–78, 15 jan. 2012.

KOMATSU, S.; NANJO, Y.; NISHIMURA, M. Proteomic analysis of the flooding tolerance mechanism in mutant soybean. **Journal of Proteomics**, v. 79, p. 231–250, fev. 2013.

KOMATSU, S.; SAKATA, K.; NANJO, Y. 'Omics' techniques and their use to identify how soybean responds to flooding. **Journal of Analytical Science and Technology**, v. 6, n. 1, p. 9, 4 dez. 2015.

KONNERUP, D. et al. Waterlogging tolerance, tissue nitrogen and oxygen transport in the forage legume Melilotus siculus: a comparison of nodulated and nitrate-fed plants. **Annals of Botany**, v. 121, p. 699–709, 2018.

KOPSELL, D. E. et al. Ratio of calcium to magnesium influences biomass, elemental accumulations, and pigment concentrations in kale. **Journal of Plant Nutrition**, v. 36, n. 14, p. 2154–2165, 6 dez. 2013.

KREUZWIESER, J.; RENNENBERG, H. Molecular and physiological responses of trees to waterlogging stress. **Plant, Cell & Environment**, v. 37, n. 10, p. n/a-n/a, 1 maio 2014.

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, 30 abr. 2013a.

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, 30 abr. 2013b.

KUMUTHA, D. et al. Effect of waterlogging on carbohydrate metabolism in pigeon pea (Cajanus cajan L.): Upregulation of sucrose synthase and alcohol dehydrogenase. **Plant Science**, v. 175, n. 5, p. 706–716, 2008.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017a.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017b.

LASA, B. et al. Role of glutamate dehydrogenase and phosphoenolpyruvate carboxylase activity in ammonium nutrition tolerance in roots. **Plant Physiology and Biochemistry**, v. 40, n. 11, p. 969–976, 1 nov. 2002.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011a.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011b.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011c.

LI, X. et al. Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. **Plant and Soil**, v. 352, n. 1–2, p. 377–387, 14 mar. 2012.

LI, X.; LIU, F. Drought Stress Memory and Drought Stress Tolerance in Plants: Biochemical and Molecular Basis. In: **Drought Stress Tolerance in Plants, Vol 1**. Cham: Springer International Publishing, 2016. p. 17–44.

LICAUSI, F.; PERATA, P. Low Oxygen Signaling and Tolerance in Plants. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 50p. 139–198.

LIMAMI, A. M.; DIAB, H.; LOTHIER, J. Nitrogen metabolism in plants under low oxygen stress. **Planta**, v. 239, n. 3, p. 531–541, 27 mar. 2014.

LIN, K.-H. R. et al. Study of the root antioxidative system of tomatoes and eggplants under waterlogged conditions. **Plant Science**, v. 167, n. 2, p. 355–365, ago. 2004.

LINKEMER, G.; BOARD, J. E.; MUSGRAVE, M. E. Waterlogging effects on growth and yield components in late-planted soybean. **Crop Science**, v. 38, n. 6, p. 1576–1584, 1998.

LIU, B.; RENNENBERG, H.; KREUZWIESER, J. Hypoxia induces stem and leaf nitric oxide (NO) emission from poplar seedlings. **Planta**, v. 241, n. 3, p. 579–

589, 2014.

LIU, Z. J.; GUO, Y. K.; BAI, J. G. Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. **Journal of Plant Growth Regulation**, v. 29, n. 2, p. 171–183, 23 jun. 2010.

LOGAN, B. A.; ADAMS, W. W.; DEMMIG-ADAMS, B. Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. **Functional Plant Biology**, v. 34, n. 9, p. 853, 24 set. 2007.

LORETI, E.; PERATA, P. The Many Facets of Hypoxia in Plants. **Plants**, v. 9, n. 6, p. 745, 12 jun. 2020.

LORETI, E.; STRIKER, G. G. Plant Responses to Hypoxia: Signaling and Adaptation. **Plants**, v. 9, n. 12, p. 1704, 3 dez. 2020.

LORETI, E.; VAN VEEN, H.; PERATA, P. **Plant responses to flooding stressCurrent Opinion in Plant Biology**Elsevier Ltd, , 1 out. 2016.

MAIA, L. B.; MOURA, J. J. G. How Biology Handles Nitrite. **Chemical Reviews**, v. 114, n. 10, p. 5273–5357, 28 maio 2014.

MANRIQUE-GIL, I. et al. Nitric oxide function during oxygen deprivation in physiological and stress processes. **Journal of Experimental Botany**, 25 out. 2020.

MARTINEZ-MEDINA, A. et al. Recognizing Plant Defense Priming. **Trends in Plant Science**, v. 21, n. 10, p. 818–822, out. 2016.

MAUREL, C.; VERDOUCQ, L.; RODRIGUES, O. Aquaporins and plant transpiration. **Plant Cell and Environment**, v. 39, n. 11, p. 2580–2587, 2016.

MELZER, J. M.; KLEINHOFS, A.; WARNER, R. L. Nitrate reductase regulation: Effects of nitrate and light on nitrate reductase mRNA accumulation. **MGG Molecular & General Genetics**, v. 217, n. 2–3, p. 341–346, jun. 1989.

MEN, S. et al. Effects of supplemental nitrogen application on physiological characteristics, dry matter and nitrogen accumulation of winter rapeseed (Brassica napus L.) under waterlogging stress. **Scientific Reports**, v. 10, n. 1, p. 10201, 23 dez. 2020.

MILLER, A. J. et al. Amino acids and nitrate as signals for the regulation of nitrogen acquisition. **Journal of Experimental Botany**, v. 59, n. 1, p. 111–119, 18 dez. 2007.

MIRA, M.; HILL, R. D.; STASOLLA, C. Regulation of programmed cell death by phytoglobins. **Journal of Experimental Botany**, v. 67, n. 20, p. 5901–5908, 1 out. 2016.

MITTLER, R. ROS Are Good. **Trends in Plant Science**, v. 22, n. 1, p. 11–19, 1 jan. 2017.

MITTLER, R.; BLUMWALD, E. The roles of ROS and ABA in systemic acquired acclimation. **Plant Cell**, v. 27, n. 1, p. 64–70, 2015.

MIYASHITA, Y. et al. Alanine aminotransferase catalyses the breakdown of

alanine after hypoxia in Arabidopsis thaliana. **The Plant Journal**, v. 49, n. 6, p. 1108–1121, 22 fev. 2007.

MODOLO, L. V. et al. Nitrite as the major source of nitric oxide production by Arabidopsis thaliana in response to Pseudomonas syringae. **FEBS Letters**, v. 579, n. 17, p. 3814–3820, 4 jul. 2005.

MOHN, M.; THAQI, B.; FISCHER-SCHRADER, K. Isoform-Specific NO Synthesis by Arabidopsis thaliana Nitrate Reductase. **Plants**, v. 8, n. 3, p. 67, 16 mar. 2019.

MORALES-OLMEDO, M.; ORTIZ, M.; SELLÉS, G. Effects of transient soil waterlogging and its importance for rootstock selection. **Chilean journal of agricultural research**, v. 75, p. 45–56, 1 ago. 2015.

MORARD, P. et al. Nitrate uptake and nitrite release by tomato roots in response to anoxia. **Journal of Plant Physiology**, v. 161, n. 7, p. 855–865, jul. 2004.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, dez. 2008a.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, 15 dez. 2008b.

MUSTAFA, G.; KOMATSU, S. Quantitative proteomics reveals the effect of protein glycosylation in soybean root under flooding stress. **Frontiers in Plant Science**, v. 5, n. NOV, p. 627, 18 nov. 2014.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 2015a.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 1 jan. 2015b.

NAKANO, Y.; ASADA, K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. **Plant and Cell Physiology**, v. 22, n. May, p. 867–880, 1981.

NEILL, S. J.; DESIKAN, R.; HANCOCK, J. T. Nitric oxide signalling in plants. **New Phytologist**, v. 159, n. 1, p. 11–35, 1 jul. 2003.

NUNES MENOLLI LANZA, L.; FERREIRA LANZA, D. C.; SODEK, L. Utilization of 15NO3- by nodulated soybean plants under conditions of root hypoxia. **Physiology and Molecular Biology of Plants**, v. 20, n. 3, p. 287–293, 2014.

OLIVEIRA, H. C.; FRESCHI, L.; SODEK, L. Nitrogen metabolism and translocation in soybean plants subjected to root oxygen deficiency. **Plant Physiology and Biochemistry**, v. 66, p. 141–149, maio 2013.

OLIVEIRA, H. C.; SODEK, L. Effect of oxygen deficiency on nitrogen assimilation and amino acid metabolism of soybean root segments. **Amino Acids**, v. 44, n. 2, p. 743–755, 19 fev. 2013.

OOSTERHUIS, D. M. et al. Physiological responses of two soybean [Glycine max (L.) Merr] cultivars to short-term flooding. **Environmental and Experimental Botany**, v. 30, n. 1, p. 85–92, 1 jan. 1990.

PARRA, M. et al. Increasing plant vigour and tomato fruit yield under salinity by inducing plant adaptation at the earliest seedling stage. **Environmental and Experimental Botany**, v. 60, n. 1, p. 77–85, 1 maio 2007.

PEDÓ, T. et al. Physiological attributes, growth and expression of vigor in soybean seeds under soil waterlogging. **African Journal of Agricultural Research**, v. 10, n. 39, p. 3791–3797, 2015.

PERAZZOLLI, M. et al. Arabidopsis Nonsymbiotic Hemoglobin AHb1 Modulates Nitric Oxide Bioactivity. **The Plant Cell**, v. 16, n. 10, p. 2785–2794, 1 out. 2004.

PETERMAN, E. J. et al. Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. **Biophysical Journal**, v. 69, n. 6, p. 2670–2678, 1995.

PHUKAN, U. J.; MISHRA, S.; SHUKLA, R. K. Waterlogging and submergence stress: affects and acclimation. **Critical Reviews in Biotechnology**, v. 36, n. 5, p. 956–966, 2 set. 2016.

PLANCHET, E. et al. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. **The Plant Journal**, v. 41, n. 5, p. 732–743, 2 fev. 2005.

PODSĘDEK, A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. **LWT - Food Science and Technology**, v. 40, n. 1, p. 1–11, 1 jan. 2007.

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. 212, 1 dez. 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. 117, 18 jul. 2020.

PUCCIARIELLO, C. et al. Plant responses to flooding. **Frontiers in Plant Science**, v. 5, n. MAY, p. 226, 23 maio 2014.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. **Plant, Cell & Environment**, v. 40, n. 4, p. 473–482, 13 abr. 2017a.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. . 13 abr. 2017 b, p. 473–482.

PUYANG, X. et al. Antioxidant responses to waterlogging stress and subsequent recovery in two Kentucky bluegrass (Poa pratensis L.) cultivars. **Acta Physiologiae Plantarum**, v. 37, n. 10, p. 197, 8 out. 2015.

R CORE TEAM. **A Language and Environment for Statistical ComputingR Foundation for Statistical Computing**Vienna, AustriaR Foundation for

Statistical Computing, , 2020. Disponível em: <https://www.r-project.org/>. Acesso em: 27 dez. 2020

RAHANTANIAINA, M.-S. et al. Glutathione oxidation in response to intracellular H 2 O 2 : Key but overlapping roles for dehydroascorbate reductases. **Plant Signaling & Behavior**, v. 12, n. 8, p. e1356531, 3 ago. 2017.

REDDY, A. S. N. et al. Coping with Stresses: Roles of Calcium- and Calcium/Calmodulin-Regulated Gene Expression. **The Plant Cell**, v. 23, n. 6, p. 2010–2032, 1 jun. 2011.

REGGIANI, R. et al. Accumulation and Interconversion of Amino Acids in Rice Roots under Anoxia. **Plant and Cell Physiology**, v. 29, n. 6, p. 981–987, 1 set. 1988.

REGGIANI, R.; BERTINI, F.; MATTANA, M. Incorporation of nitrate nitrogen in rice seedlings transferred to anaerobic conditions. **Amino Acids**, v. 13, n. 2, p. 183–188, jun. 1997.

RHINE, M. D. et al. Yield and nutritional responses to waterlogging of soybean cultivars. **Irrigation Science**, v. 28, n. 2, p. 135–142, 8 jan. 2010.

RICOULT, C.; CLIQUET, J.-B.; LIMAMI, A. M. Stimulation of alanine amino transferase (AlaAT) gene expression and alanine accumulation in embryo axis of the model legume Medicago truncatula contribute to anoxia stress tolerance. **Physiologia Plantarum**, v. 123, n. 1, p. 30–39, 1 jan. 2005.

ROBERTS, J. K. et al. Mechanisms of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. **Proceedings of the National Academy of Sciences of the United States of America**, v. 81, n. 11, p. 3379–3383, 1 jun. 1984.

ROCHA, M. et al. Glycolysis and the Tricarboxylic Acid Cycle Are Linked by Alanine Aminotransferase during Hypoxia Induced by Waterlogging of Lotus japonicus. **Plant Physiology**, v. 152, n. 3, p. 1501–1513, mar. 2010a.

ROCHA, M. et al. Analysis of alanine aminotransferase in various organs of soybean (Glycine max) and in dependence of different nitrogen fertilisers during hypoxic stress. **Amino Acids**, v. 39, n. 4, p. 1043–1053, 23 out. 2010b.

ROLLAND, F.; BAENA-GONZALEZ, E.; SHEEN, J. SUGAR SENSING AND SIGNALING IN PLANTS: Conserved and Novel Mechanisms. **Annual Review of Plant Biology**, v. 57, n. 1, p. 675–709, jun. 2006.

SAHA, R. et al. Physiological and biochemical changes in waterlog tolerant sesame genotypes. **SAARC Journal of Agriculture**, v. 14, n. 2, p. 31–45, 23 jan. 2017.

SAIRAM, R. K. et al. Physiology and biochemistry of waterlogging tolerance in plants. **Biologia plantarum**, v. 52, n. 3, p. 401–412, 1 set. 2008.

SAIRAM, R. K. et al. Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (Vigna radiata). **Journal of Plant Physiology**, v. 166, n. 6, p. 602–616, 1 abr. 2009.

SARKER, U.; OBA, S. Augmentation of leaf color parameters, pigments,

vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. **Scientific Reports**, v. 8, n. 1, 1 dez. 2018.

SASIDHARAN, R.; VOESENEK, L. A. C. J. Ethylene-Mediated Acclimations to Flooding Stress 1. v. 169, n. September, p. 3–12, 2015.

SCHÖFFEL, E. R. et al. EXCESSO HÍDRICO SOBRE OS COMPONENTES DO RENDIMENTO DA CULTURA DA SOJA. **Ciência Rural**, v. 31, n. 1, p. 7–12, 2001.

SHARMA, P. et al. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. **Journal of Botany**, v. 2012, p. 1–26, 24 abr. 2012.

SHARMA, R. et al. Recent Advances in Dissecting Stress-Regulatory Crosstalk in Rice. **Molecular Plant**, v. 6, n. 2, p. 250–260, 1 mar. 2013.

SILVA, T. A. DA et al. Condicionamento fisiológico de sementes de soja, componentes de produção e produtividade. **Ciência Rural**, v. 46, n. 2, p. 227–232, fev. 2016.

SINGH, N.; BHATLA, S. C. Hemoglobin as a probe for estimation of nitric oxide emission from plant tissues. **Plant Methods**, v. 15, n. 1, p. 39, 23 dez. 2019.

SINGH, S. K.; RAJA REDDY, K. Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (Vigna unguiculata [L.] Walp.) under drought. **Journal of Photochemistry and Photobiology B: Biology**, v. 105, n. 1, p. 40–50, 5 out. 2011.

SOARES, M. M. et al. Estresse hídrico e salino em sementes de soja classificadas em diferentes tamanhos. **Pesquisa Agropecuaria Tropical**, 2015.

SOJKA, R.; SCOTT, H. Aeration Measurement. In: **Encyclopedia of Soil Science, Second Edition**. [s.l.] CRC Press, 2005.

SOSBAI, S. S.-B. D. A. I. **ARROZ IRRIGADO: Recomendações Técnicas da Pesquisa para o Sul do Brasil**. XXXII REUNIÃO TÉCNICA DA CULTURA DO ARROZ IRRIGADO. **Anais**...Farroupilha- RS- Brasil: 2018Disponível em: <http://www.sosbai.com.br/>. Acesso em: 15 abr. 2020

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002a.

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002b.

SOUZA, R. P. et al. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. **Environmental and Experimental Botany**, v. 51, n. 1, p. 45–56, fev. 2004.

SOUZA, S. C. R.; MAZZAFERA, P.; SODEK, L. Flooding of the root system in soybean: biochemical and molecular aspects of N metabolism in the nodule

during stress and recovery. **Amino Acids**, v. 48, n. 5, p. 1285–1295, 29 maio 2016.

SPRINGER, B. et al. The Shrunken gene on chromosome 9 of Zea mays L is expressed in various plant tissues and encodes an anaerobic protein. **MGG Molecular & General Genetics**, v. 205, n. 3, p. 461–468, dez. 1986.

STOIMENOVA, M. et al. The role of nitrate reduction in the anoxic metabolism of roots II. Anoxic metabolism of tobacco roots with or without nitrate reductase activity. **Plant and Soil**, v. 253, n. 1, p. 155–167, jun. 2003.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, 6 jun. 2007a.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, jul. 2007b.

SULLIVAN, M. et al. Evaluating On-Farm Flooding Impacts on Soybean. **Crop Science**, v. 41, n. 1, p. 93–100, 1 jan. 2001.

SUZUKI, N. et al. Abiotic and biotic stress combinations. **New Phytologist**, 2014.

SWEETLOVE, L. J. et al. Lactate metabolism in potato tubers deficient in lactate dehydrogenase activity. **Plant, Cell & Environment**, v. 23, n. 8, p. 873–881, 25 ago. 2000.

TAIZ, L.; ZEIGER, E. **Plant Physiology. 3rd edn.** [s.l: s.n.]. v. 3

TAKAHAMA, U. Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: Physiological significance of the oxidation reactions. **Phytochemistry Reviews**, v. 3, n. 1–2, p. 207–219, jan. 2004.

TAMANG, B.; FUKAO, T. Plant Adaptation to Multiple Stresses during Submergence and Following Desubmergence. **International Journal of Molecular Sciences**, v. 16, n. 12, p. 30164–30180, 17 dez. 2015.

TAN, W. et al. Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. **Photosynthetica**, v. 46, n. 1, p. 21–27, mar. 2008.

TANG, B. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, 1 maio 2010.

TESNIERE, C. et al. Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. **Journal of Experimental Botany**, v. 57, n. 1, p. 91–99, 2006.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016a. v. 2p. 23–40.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016b. v. 2p. 23–40.

TEWARI, S.; MISHRA, A. Flooding Stress in Plants and Approaches to Overcome. In: **Plant Metabolites and Regulation Under Environmental Stress**. [s.l.] Elsevier, 2018. p. 355–366.

THOMAS, A. L.; GUERREIRO, S. M. C.; SODEK, L. Aerenchyma Formation and Recovery from Hypoxia of the Flooded Root System of Nodulated Soybean. **Annals of Botany**, v. 96, n. 7, p. 1191–1198, 1 dez. 2005.

THOMAS, A. L.; SODEK, L. Amino acid and ureide transport in the xylem of symbiotic soybean plants during short-term flooding of the root system in the presence of different sources of nitrogen. **Brazilian Journal of Plant Physiology**, v. 18, n. 2, p. 333–339, jun. 2006.

THOMASHOW, M. F. Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. **Plant Physiology**, v. 154, n. 2, p. 571–577, 2010.

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, 1 jun. 2019a.

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, 1 jun. 2019b.

TIMILSINA, A. et al. Potential Pathway of Nitrous Oxide Formation in Plants. **Frontiers in Plant Science**, v. 11, 31 jul. 2020.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012a.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012b.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003a.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003b.

TREWAVAS, A. Aspects of Plant Intelligence. **Annals of Botany**, v. 92, n. 1, p. 1–20, 9 maio 2003.

TREWAVAS, A. Green plants as intelligent organisms. **Trends in Plant Science**, v. 10, n. 9, p. 413–419, 1 set. 2005.

TRIPATHI, B. N.; MÜLLER, M. **Stress Responses in Plants**. Cham: Springer International Publishing, 2015.

UDVARDI, M.; POOLE, P. S. Transport and Metabolism in Legume-Rhizobia

Symbioses. **Annual Review of Plant Biology**, v. 64, n. 1, p. 781–805, 29 abr. 2013.

UNGER, P. W.; KASPAR, T. C. Soil Compaction and Root Growth: A Review. **Agronomy Journal**, v. 86, n. 5, p. 759–766, 1 set. 1994.

UNITED STATES DEPARTMENT OF AGRICULTURE. **World Agricultural Production**. [s.l: s.n.].

VAN AMERONGEN, H.; CHMELIOV, J. Instantaneous switching between different modes of non-photochemical quenching in plants. Consequences for increasing biomass production. **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, v. 1861, n. 4, p. 148119, 1 abr. 2020.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015a.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015b.

VAN HANDEL, E. Direct microdetermination of sucrose. **Analytical Biochemistry**, v. 22, n. 2, p. 280–283, 1 fev. 1968.

VAN NGUYEN, L. et al. Mapping quantitative trait loci for root development under hypoxia conditions in soybean (Glycine max L. Merr.). **Theoretical and Applied Genetics**, v. 130, n. 4, p. 743–755, 1 abr. 2017.

VANDOORNE, B. et al. Long term intermittent flooding stress affects plant growth and inulin synthesis of Cichorium intybus (var. sativum). **Plant and Soil**, v. 376, n. 1, p. 291–305, 29 nov. 2014.

VELIKOVA, V.; YORDANOV, I.; EDREVA, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. **Plant Science**, v. 151, n. 1, p. 59–66, 2002.

VITOR, S. C.; SODEK, L. Products of anaerobic metabolism in waterlogged roots of soybean are exported in the xylem. **Plant Science**, v. 284, p. 82–90, 1 jul. 2019.

VOESENEK, L. Flooding tolerance : O 2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flooding tolerance: O2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flood adaptive traits and processes: an overview. **New Phytologist**, v. 206, n. 1, p. 57–73, 7 abr. 2015.

VRIET, C.; HENNIG, L.; LALOI, C. Stress-induced chromatin changes in plants: of memories, metabolites and crop improvement. **Cellular and Molecular Life Sciences**, v. 72, n. 7, p. 1261–1273, 13 abr. 2015.

WALTER, J. et al. Do plants remember drought? Hints towards a drought-memory in grasses. **Environmental and Experimental Botany**, v. 71, n. 1, p. 34–40, 1 abr. 2011.

WALTER, J. et al. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. **Environmental and Experimental Botany**, v. 94, p. 3–8, 1 out. 2013.

WANG, X. et al. Physiological and proteomic mechanisms of waterlogging priming improves tolerance to waterlogging stress in wheat ( Triticum aestivum L.). **Environmental and Experimental Botany**, v. 132, p. 175–182, dez. 2016.

WANG, X.; LIU, F.; JIANG, D. Priming: A promising strategy for crop production in response to future climate. **Journal of Integrative Agriculture**, v. 16, n. 12, p. 2709–2716, dez. 2017.

WANY, A. et al. Nitrate nutrition influences multiple factors in order to increase energy efficiency under hypoxia in Arabidopsis. **Annals of Botany**, v. 123, n. 4, p. 691–705, 14 mar. 2019.

WANY, A.; FOYER, C. H.; GUPTA, K. J. Nitrate, NO and ROS Signaling in Stem Cell Homeostasis. **Trends in Plant Science**, v. 23, n. 12, p. 1041–1044, 1 dez. 2018.

WANY, A.; KUMARI, A.; GUPTA, K. J. Nitric oxide is essential for the development of aerenchyma in wheat roots under hypoxic stress. **Plant Cell and Environment**, v. 40, n. 12, p. 3002–3017, 1 dez. 2017.

WEI, W. et al. Morpho-anatomical and physiological responses to waterlogging of sesame (Sesamum indicum L.). **Plant Science**, v. 208, p. 102–111, 1 jul. 2013.

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, n. 3, p. 307–313, 1994.

YEMM, E. W.; COCKING, E. C.; RICKETTS, R. E. The determination of amino-acids with ninhydrin. **The Analyst**, v. 80, n. 948, p. 209–214, 1 jan. 1955.

YETISIR, H.; MEHMET, E. C. Some physiological and growth responses of watermelon [ C itrullus lanatus ( Thunb .) Matsum . and Nakai ] grafted onto Lagenaria siceraria to flooding. **Environmental and Experimental Botany**, v. 58, p. 1–8, 2006.

YIU, J.-C. et al. Changes in antioxidant properties and their relationship to paclobutrazol- induced flooding tolerance in Welsh onion. **Journal of the Science of Food and Agriculture**, v. 88, n. 7, p. 1222–1230, maio 2008.

YORDANOVA, R. Y.; CHRISTOV, K. N.; POPOVA, L. P. Antioxidative enzymes in barley plants subjected to soil flooding. **Environmental and Experimental Botany**, v. 51, n. 2, p. 93–101, 2004.

YU, F. et al. Comparative proteomic analysis revealing the complex network associated with waterlogging stress in maize ( *Zea mays* L.) seedling root cells. **PROTEOMICS**, v. 15, n. 1, p. 135–147, 1 jan. 2015.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, fev. 2009a.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, 1 fev. 2009b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, p. 290–298, 2019a.

ZHANG, J. et al. Sedum mexicanum 'Gold Mound' exhibits better adaptive characters in contrast to S. spurium 'Coccineum' when subjugated to sustained waterlogging stress. **Acta Horticulturae**, v. 1263, n. 1263, p. 141–148, nov. 2019b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, n. 2, p. 290–298, 14 dez. 2019c.

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, 2017.

ZHANG, X. et al. Physiological and transcriptional analyses of induced post-anthesis thermo-tolerance by heat-shock pretreatment on germinating seeds of winter wheat. **Environmental and Experimental Botany**, v. 131, p. 181–189, nov. 2016.

ZHOU, W. et al. Plant waterlogging/flooding stress responses: From seed germination to maturation. **Plant Physiology and Biochemistry**, v. 148, p. 228–236, 1 mar. 2020.

*2.7.* . Appendix A. Supplementary data

Figure S1. Soybean plants (cv. PELBR11-6042 RR) stage V3 and R2, before the waterlogging treatment.

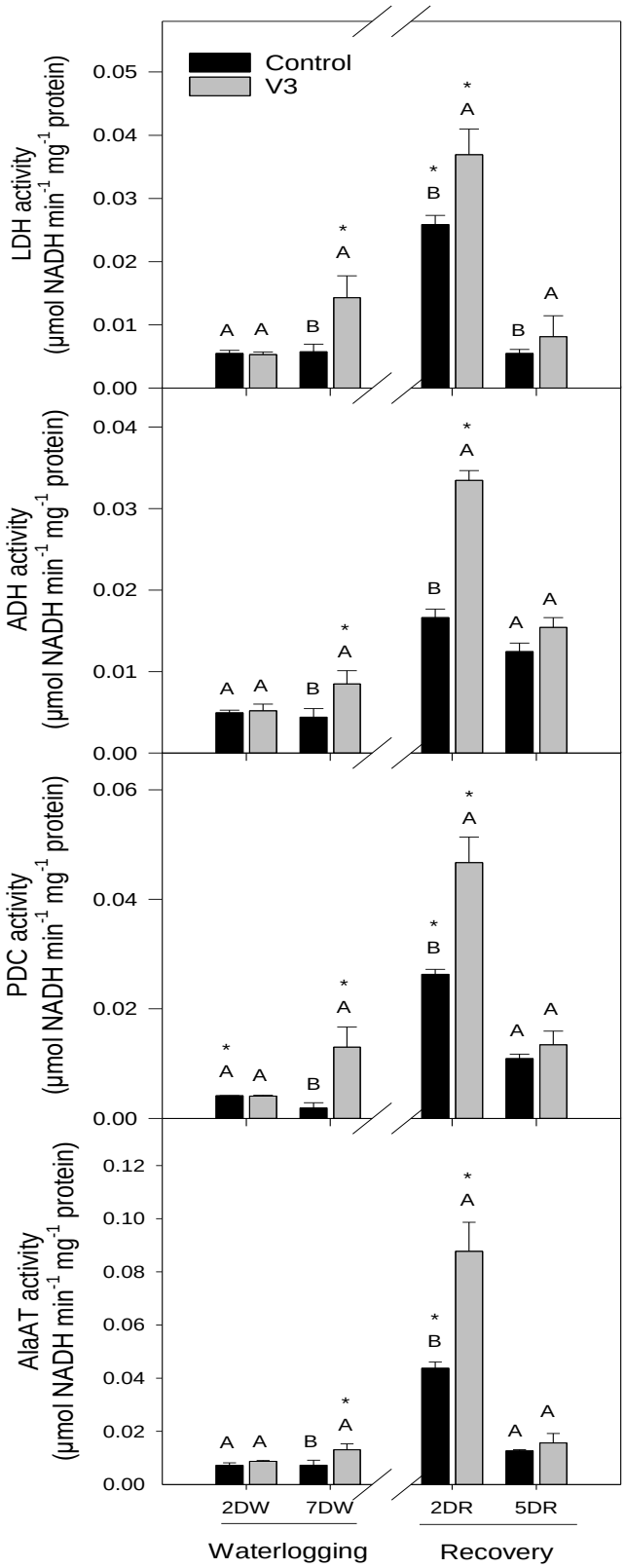


Figure S2. LDH, ADH, PDC and Ala-AT activities in roots of soybean genotype PELBR10-6042 at V3 stage, under two and seven days of waterlogging *(2DW, 7DW*) and recovery *(2DR, 5DR)*. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments, and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the by t-test ($P \leq 0.05$).

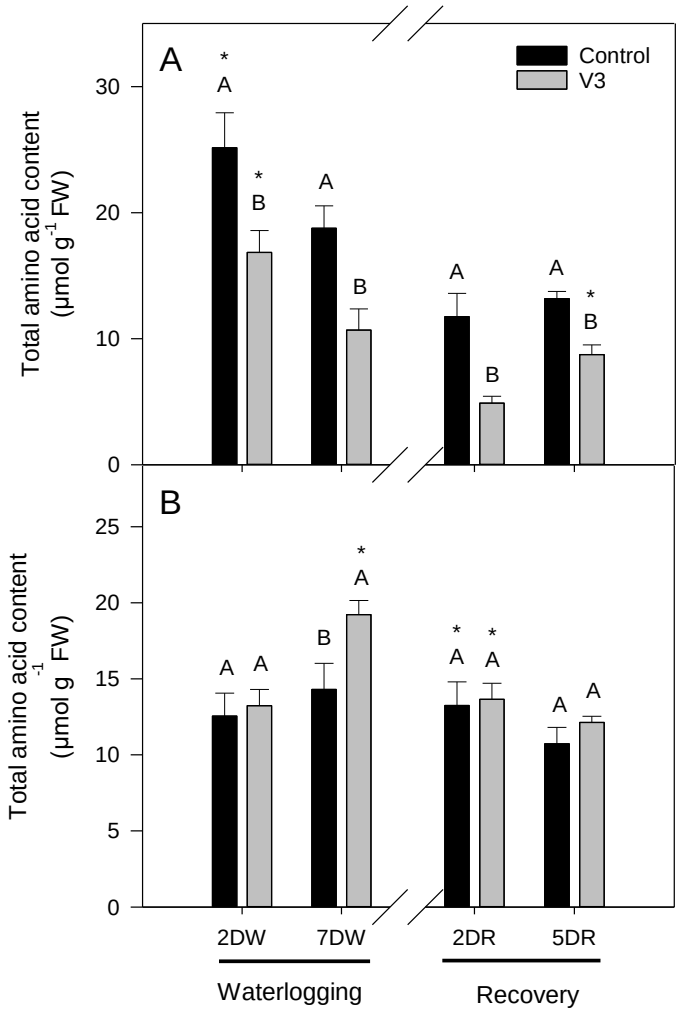


Figure S3. Total amino acid contents in (A) leaves and roots (B) of soybean genotype PELBR10-6042 at V3 stage, under two and seven days of waterlogging *(2DW, 7DW*) and recovery *(2DR, 5DR)*. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments, and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the t-test ($P \leq 0.05$).

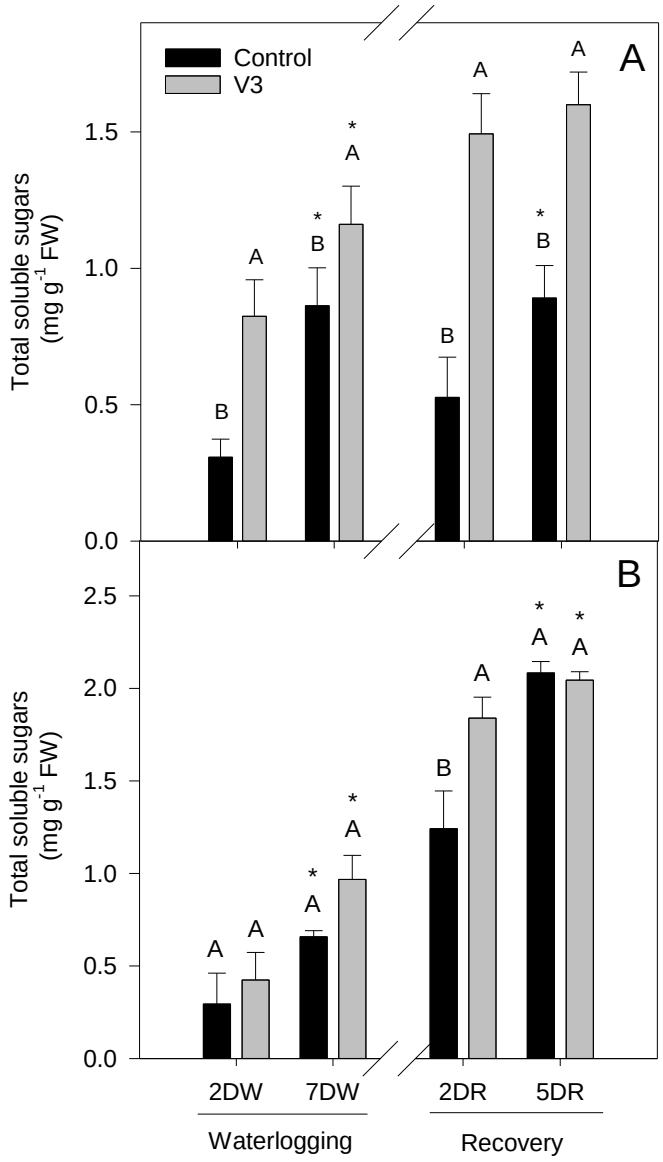


Figure S4. Total soluble sugars content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at V3 stage, under two and seven days of waterlogging *(2DW, 7DW*) and recovery *(2DR, 5DR)*. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments, and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the t-test ($P \leq 0.05$).

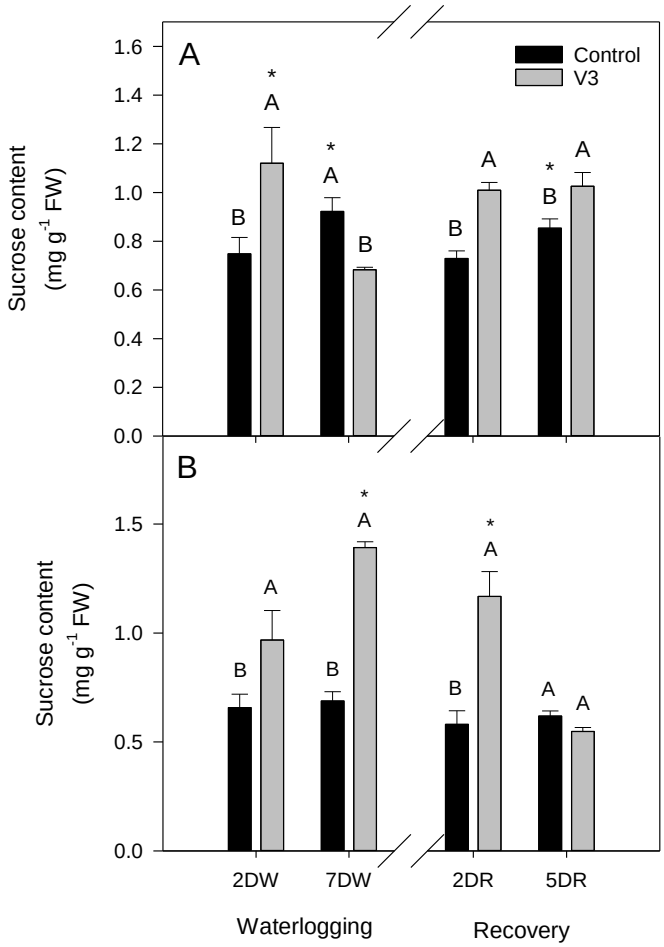


Figure S5. Sucrose content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at V3 stage, under two and seven days of waterlogging *(2DW, 7DW*) and recovery *(2DR, 5DR)*. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments, and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the t-test ($P \leq 0.05$).

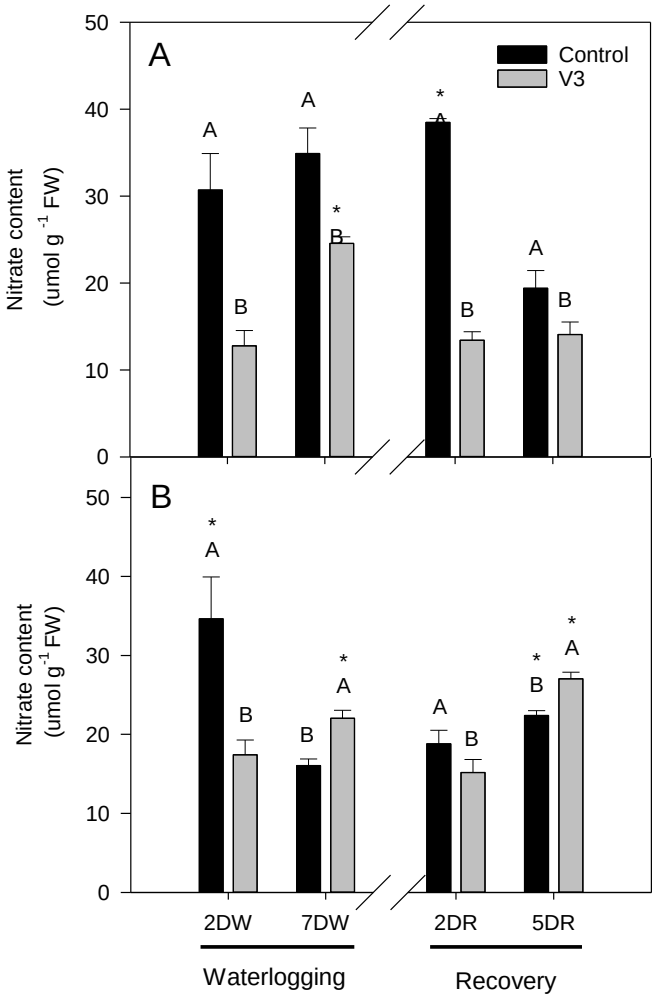


Figure S6. Nitrate content in leaves (A) and roots (B) of soybean genotype PELBR10-6042 at V3 stage, under two and seven days of waterlogging *(2DW, 7DW*) and recovery *(2DR, 5DR)*. The bars represent mean ± SE (n=3). Asterisks indicate significant differences among periods of waterlogging or recovery treatments, and distinct letters indicate significant difference for the same period, between control and waterlogged plant or recovery plant by the t-test ($P \leq 0.05$).

3. Chapter II - Metabolic responses to waterlogging stress memory in soybean roots: application of nitrate enhances the metabolic mechanisms of plant adaptation to hypoxia favouring the priming capacity.

## *3.1.* Introduction

Abrupt changes in climate conditions are posing a potential threat to biodiversity increasing the likelihood of extreme episodes of drought and soil waterlogging in many regions of the globe (DONAT et al., 2016). Unpredictable and untimely rainfall or poor drainage often causes flooding or waterlogging by altering the state of soil aeration, replacing it with water (ZHANG et al., 2019a). Limiting water and air movement in the soil, induces an oxygen deficiency (BALAKHNINA, 2015) and carbon dioxide ($CO_2$) accumulation (MORALES-OLMEDO; ORTIZ; SELLÉS, 2015; YETISIR; MEHMET, 2006) as well as changes in the soils chemical properties (UNGER; KASPAR, 1994) will result in reduced nutrient availability for plants. The composition of other soil gases, such as $CO_2$, ethylene, methane, etc., can affect the response to a given oxygen concentration (SOJKA; SCOTT, 2005); and the change from aerobic to anaerobic respiration (VOESENEK; BAILEY-SERRES, 2013) that causes cytosol acidification in plant cells which leads to inhibition of aquaporines and consequently limits water absorption by roots and hydraulic conductivity (HEMANTARANJAN, 2014). As a consequence, plants suffer oxidative stress (HASANUZZAMAN et al., 2012), decreased energy status (TAMANG; FUKAO, 2015; WANY et al., 2019), inhibition in growth rates and photosynthesis (ANDRADE et al., 2018; GARCIA et al., 2020). Biochemical adaptations to hypoxia include induction of anaerobic metabolism pathways and protective enzymes for the elimination of phytotoxic by-products (EVANS; GLADISH, 2017), essential for plant survival under waterlogged conditions. The loss of productivity in several crops that suffer from waterlogging varies between 15% and 80%, depending on the plant species, soil type and stress duration (HEMANTARANJAN, 2014; TEWARI; ARORA, 2016a). The production and grain yields are affected by waterlogging stress in soybean crops, especially during vegetative and seed germination stages (GITHIRI et al., 2006). For soybean is estimated up to a 25% reduction in soybean crop yield due to waterlogging injuries in all of the world (MUSTAFA; KOMATSU, 2014), however, soybean

yields can be reduced by 17–43% by the waterlogging at the vegetative stage (FEHR, W. R.; AND C. E, 1977; OOSTERHUIS et al., 1990), and about 67% when occurs between R1 and R5 (LINKEMER; BOARD; MUSGRAVE, 1998).

It is important to note that nitrate corresponds to the dominant nitrogen source available for plants and in conventionally prepared soils, may be processed by both root and leaf cells. It is used by higher plants in various processes, including absorption, vacuole storage, xylem transport, reduction and incorporation into organic forms (LASA et al., 2002). Under periods of waterlogging conditions plants to survive develop mechanisms such as the cycle involving non-symbiotic hemoglobin (phytoglobin; Pb) and nitric oxide (NO) from nitrate supply (GUPTA, 2020; PUCCIARIELLO; PERATA, 2017b), where nitrate is converted to nitrite by a nitrate reductase (NR) activity even under hypoxia (BOTREL; MAGNE; KAISER, 1996; TIMILSINA et al., 2020) and the nitrite can be used as an alternative substrate to produce NO under hypoxia (PLANCHET et al., 2005). Moreover the inhibition of the following enzyme, nitrite reductase, could limit nitrogen assimilation during $O_2$ deficiency, resulting in a release of $NO_2^-$ to the external medium (MORARD et al., 2004). Previously, it was shown that $NO_3^-$ levels influence NO production through nitrite production, which can be transported to the mitochondria where it can be reduced to NO at sites of complexes III and IV and by alternative oxidase (DA-SILVA; DO AMARANTE, 2020a; GUPTA et al., 2020; GUPTA; KAISER, 2010). Nutrition with $NO_3^-$ modulates alternative pathways that result in reduction reactive oxygen species (ROS) production and oxidative stress; increases in ATP levels, increases in Ala-AT enzyme activity, and decreases in the levels of lactate and ethanol in soybean plants under waterlogging stress (DA-SILVA; DO AMARANTE, 2020a; TIMILSINA et al., 2020).

In the course of evolution, plants have developed a multitude of mechanisms to deal with environmental stresses by adapting to various ranges of biotic and abiotic stressors. They respond to this variability and unpredictability of stresses by adapting genotypic and phenotypic characteristics, especially when stress occurs regularly (HILKER; SCHMÜLLING, 2019a) because they have a capacity for "stress memory", *i.e.,* capacity to prepare (priming) involving prior exposure to a stress factor for better plant responses to subsequent stress

exposure (BRUCE et al., 2007; MARTINEZ-MEDINA et al., 2016; WANG et al., 2016), *i.e*. induction modifies a plant for future exposure to stress (CONRATH et al., 2015; HILKER et al., 2016). Once there is an induction in the primary state, the plant responds to stress stimulus by activating resistance mechanisms to stress, compared to a plant in the innate (unaffected) state (LÄMKE; BÄURLE, 2017a). It has been demonstrated that the process of preparation (or hardening) by exposure to abiotic stresses can pre-prepare its resistance to waterlogging (LI et al., 2011c; WANG et al., 2016), cold, drought or osmotic stress (BRUCE et al., 2007; MUTAVA et al., 2015b; WALTER et al., 2013), salinity (PARRA et al., 2007) and stress combinations (SUZUKI et al., 2014).

Soybean (*Glycine max* L. Merr.) is an economically important dicot crop because its seeds contain an abundance of oil and proteins. This species is vulnerable to various abiotic pressures, including waterlogging, that becomes a limiting factor for growth and yield, considering that plants are generally sensitive to waterlogging (BORELLA et al., 2017; TEWARI; ARORA, 2016a). Soybean production and yield are particularly affected by waterlogging stress (VAN NGUYEN et al., 2017), that is frequent when cultivation occurs in flood-prone areas, such as soils from Southern Brazil that are hydromorphic, with deficient natural drainage, due to flat terrain, soil profile with shallow surface layer, and almost impermeable subsurface layer (DA ROCHA et al., 2017). Currently, it is used for rice cultivation and can be used to increase food production in a rotation of crops with soybean, improving soil conditions, by providing nitrogen (when grown associated with rhizobia), breaking pests and diseases and contributing to reduce weed cycles (GARCIA et al., 2020).

The benefical effect of nitrate under hypoxic or anoxic conditions is clear and a better understanding of the priming effect of waterlogging is needed. Moreover, most studies on priming memory and nitrate respiration concern root tips or relatively young plant segments under controlled conditions. Our previous research suggested that priming mechanisms in plants under waterlogging (V3R2 group) compared to those that were not primed (R2 group), several metabolic factors were enhanced, and that priming occurs independently of nitrate. In the present work, we have been explored whether or not nitrate enhances priming of a previous waterlogging. The results could contribute to a

better understanding of the main physiological mechanisms and biochemical events (such as fermentation pathways, ROS content and antioxidant activity, nitrate reduction and accumulation of amino acids and carbohydrates) in the genotype BR11-6042, which may be relevant for the selection of tolerant plants to transient soil waterlogging and lead to explore practices to mitigate hypoxia stress.

## *3.2.* Material and methods

### *3.2.1.* Plant growth conditions and experimental design

The experiment was performed with soybean plants genotype PELBR11-6042 RR, (indeterminate growth, sensitive to waterlogging according to Embrapa Soybean Breeding Program), allowing us to easily investigate biochemical changes from hypoxia and seeking a better understanding of stress memory. Seeds were inoculated with *Bradyrhizobium japonicum* strain SEMIA 5079 (BIOAGRO) and sown in boxes of 1 x1.2 x 1m (36 plants per box) containing soil from lowland areas (Typical Hydromorphic Eutrophic Planosol). Soil fertility was corrected with 350 kg $ha^{-1}$ of commercial formulation NPK 00-25-25 and pH was corrected to 6.0.

After germination, plants were cultivated under natural condition (Sup. Fig. 1). The waterlogging was imposed on soybean plants at V3 and R2 stage of growth (Fig. S1; for stage definitions, see Fehr et al. 1971). The cultivated plants were divided into N-free waterlogging and N-waterlogged plants, with their respective control. In N waterlogged plants, nitrogen was applied (25.78g $KNO_3$ diluted in 250ml of water per box) three days before waterlogging and three days after waterlogging has started. $NO_3^-$ and $K^+$ are synergistically associated in plants. While $NO_3^-$ stimulates the uptake and accumulation of $K^+$, the $K^+$ contributes to the activation of enzymes involved in $NO_3^-$ assimilation and protein synthesis, in contrast to antagonistic relationship with $NH_4^+$, which arises from competition for uptake sites between ions sharing the same electrical charge and similar ionic radius ($NH_4^+$=0.143 nm, $K^+$=0.133 nm) (BRITTO; KRONZUCKER, 2002; COSKUN; BRITTO; KRONZUCKER, 2017), The waterlogged plants were separated in four groups, two of them without nitrate supply during waterlogging: V3R2 group (soybean plants initially waterlogged at V3 stage for seven days and posteriorly for five days at R2 stage) and R2 group (waterlogged only at R2 stage

for five days). The other two groups were supplied with nitrate prior and during waterlogging period: V3R2N group (waterlogged as V3R2 group associated to N supply) and R2N (waterlogged only at R2 stage associated with N supply). Waterlogging was performed by keeping the water at 2-3 cm above the soil surface, through a water valve to control the water flow during the stress period. Posteriorly, the boxes were drained and the plants were allowed to recover for five days.

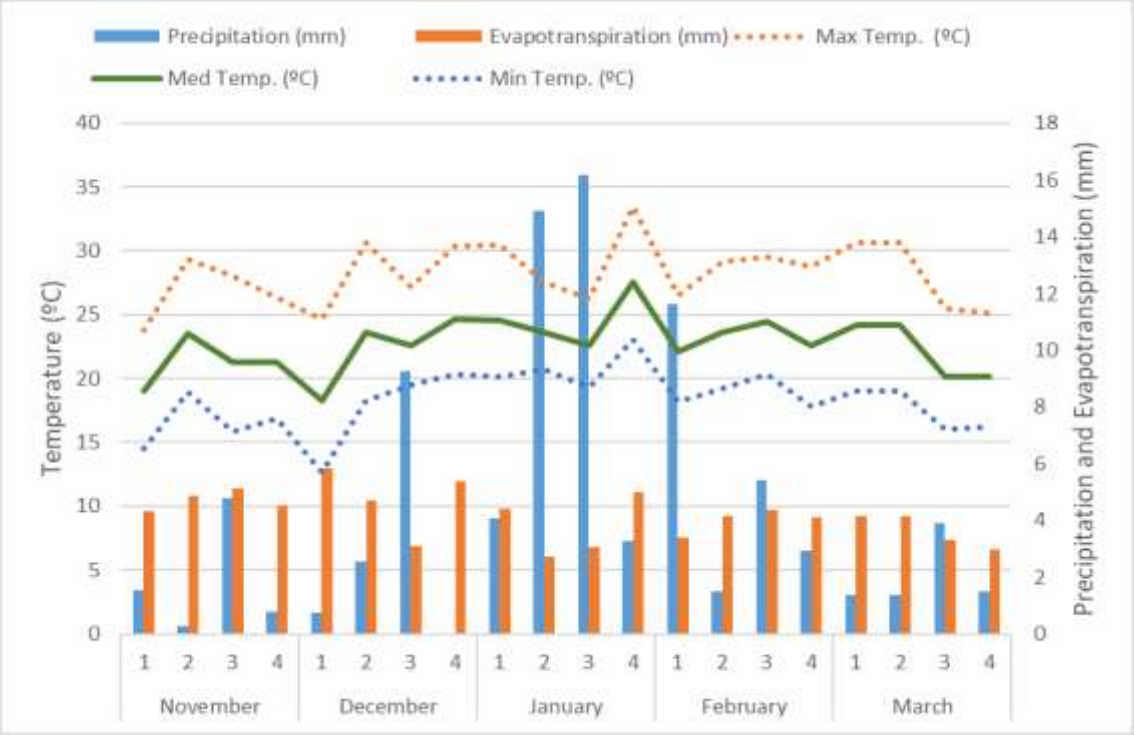


Figure 1. Minimum daily temperature (Min Temp), medium daily temperature (Med Temp) and maximum daily temperature (Max Temp) (lines) expressed in ºC, precipitation (blue bars) and evapotranspiration (orange bars) in mm, for the corresponding experimental period (2018/2019 agricultural year). Source: Agroclimatological Station Pelotas (Capão do Leão), Embrapa Temperate Climate Agricultural Research Center, Pelotas, RS, Brazil. Available at: http://agromet.cpact.embrapa.br/.

Three groups of plants were carried out as controls: a control group without $N\text{-}NO_3^-$ supply (Control) and two with $N\text{-}NO_3^-$ supply, one during periods of waterlogging at V3 and R2 stages (Control V3R2N) and the other, supplied with N only at R2 stage (Control R2N). In these groups, soil moisture was kept at field capacity during the whole growth period.

The effect of nitrate in enhancement of priming by intermittent stress and the role in the tolerance of plants to waterlogging in root system was investigated by sampling roots segments from treatments at two and five days of waterlogging

stress and five days of recovery for analysis of levels of nitrate, nitrate reductase activity, antioxidant enzyme activities, hydrogen peroxide and lipid peroxidation, fermentative enzymes activity, amino acids, total soluble sugars and sucrose concentration. Samples of roots from three plants were collected, carefully cleaned, weighed and immediately stored at -86°C for subsequent biochemical analysis.

*3.2.2.* Nitrate, amino acids, total soluble sugars and sucrose contents

All these metabolites were extracted according to BIELESKI; TURNER (1966). Briefly, frozen roots (0.250 g) were ground into a powder with liquid nitrogen, and the metabolites extracted for 24 h with 10 mL of a methanol:chloroform:water (12/5/3; v/v/v) solution. After centrifugation at 600 × *g* for 10 min, one volume of chloroform and 1.5 vol. of water were added to four volumes of the supernatant. The aqueous phase was collected after 24 h and used for the analysis and used for quantification of nitrate (CATALDO et al., 1975)total soluble amino acids (YEMM; COCKING; RICKETTS, 1955), total soluble sugars (GRAHAM; SMYDZUK, 1965) and the contents of sucrose (VAN HANDEL 1968).

*3.2.3.* Nitrate reductase activity

The activity nitrate reductase (NR; EC 1.6.6.1) was evaluated according to Modolo et al. (2005), with modifications. Roots (0.5 g) were ground in the presence of 1 mL of 50 mM MOPS buffer (pH 7.5) containing 1 mM phenylmethanesulfonyl fluoride. Homogenate was centrifuged at 13,000 × g at 4 °C for 10 min and the supernatant collected for analysis. The supernatant was incubated for 30 min with an equal volume of 50 mM MOPS (pH 7.5) containing 1 mM EDTA, 10 mM $KNO_3$, and 1 mM NADH. The reaction was stopped by adding 1 M zinc acetate followed by centrifugation at 10,000 × g for 5 min. The supernatant was then homogenized with Griess reagent containing 0.5% (w/v) sulphanilamide prepared in 1.25% (v/v) $H_3PO_4$ and 0.5% (w/v) naphthyl ethylene-diamine dihydrochloride. The final supernatant was analyzed at 540 nm for determining the levels of nitrite. A standard curve was prepared using $NaNO_2$.

*3.2.4.* Antioxidant enzyme activity

Antioxidant enzymes were extracted according to Giannopolitis and Ries (1977). Frozen roots (0.25 g) were ground in liquid nitrogen and homogenized with 10% (w:w) polyvinylpolypyrrolidone (PVPP) in 1.5 mL of extraction buffer (100 mM phosphate buffer pH 7.8 containing 0,1 mM EDTA, 10 mM ascorbic acid). The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C and the supernatant was collected for further analysis of SOD (Superoxide dismutase), CAT (Catalase), APX (Ascorbate peroxidise) and GPOD (guaicol peroxidise). DHAR (Dehydroascorbate reductase) was extracted with 1 ml of 50 mM MES/KOH buffer (pH 6.0), containing 40 mM KCl, 2 mM $CaCl_2$, and 1 mM L-ascorbic acid (AsA) (MURSHED; LOPEZ-LAURI; SALLANON, 2008b). All enzyme extraction procedures were performed at 4$^o$ C.

The SOD (EC 1.15.1.1) activity was measured by the ability of enzyme to inhibit the photorreduction of nitroblue tetrazolium by 50% at 560 nm (GIANNOPOLITIS; RIES, 1977). The CAT (EC 1.11.1.6) activity was assessed by the decrease in absorbance at 240 nm, monitored by the consumption of $H_2O_2$. Specific activity was calculated using the molar extinction coefficient ε =36 $mM^{-1}$ $cm^{-1}$) (DE AZEVEDO NETO et al., 2006) The APX (EC 1.11.1.11) activity was assayed by monitoring of the rate of oxidation of ascorbate at 290 nm (NAKANO; ASADA, 1981). Specific activity was calculated using the molar extinction coefficient ε = 2.8 $mM^{-1}$ $cm^{-1}$. The GPOD (EC 1.11.1.7) activity was assayed by monitoring the tetraguaiacol production at 470 nm. The DHAR (EC 1.8.5.1) activity was measured by the addition of DHA (freshly prepared) and the increase in the absorbance at 265 nm was monitored. Specific activity was calculated using the molar extinction coefficient ε =14 $mM^{-1}$ $cm^{-1}$ (MURSHED; LOPEZ-LAURI; SALLANON, 2008b). The protein levels were estimated by Bradford's assay using a BSA (bovine serum albumin) standard curve (BRADFORD, 1976).

*3.2.5.* Hydrogen peroxide and lipid peroxidation levels

The levels of $H_2O_2$ were determined according to Velikova et al. (2002). Roots (0.250 g) were ground into powder in liquid nitrogen and homogenized in 0.1% (w/v) trichloroacetic acid and centrifuged at 13,000 × g at 4 °C for 20 min. Aliquots of 300 µL of supernatant were added to the reaction medium containing 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide. Samples were

incubated at 30 °C for 30 min, and the absorbance was determined at 390 nm. The $H_2O_2$ concentrations were estimated based on a calibration curve. Lipid peroxidation was determined by measuring the levels of malonyldialdehyde (MDA) according to the method of Cakmak and Horst (1991). Aliquots of the supernatant were added to the reaction medium containing 0.5% (w/v) thiobarbituric acid (TBA) and 10% (w/v) trichloroacetic acid (TCA), and then incubated at 90 ºC. After 20 min, the reaction was stopped in an ice bath for 10 min. The absorbance were measured at 535 nm and 600 nm and the concentration of MDA-reactive TBA was calculated using the molar extinction coefficient (Ɛ) of 155 $mM^{-1}$ $cm^{-1}$.

*3.2.6.* Activity of fermentative pathway enzymes and alanine aminotransferase

Frozen roots (0.250 *g*) were ground into a powder and extracted with 50 mM Tris-HCl (pH 7.5), containing 1 mM dithiothreitol and 3 % (w/w) PVPP. Plant extract homogenate was centrifuged at 12,000 × g at 4 °C for 20 min, and an aliquot of the supernatant was desalted using a PD-10 column (BORELLA et al., 2014). The desalted root samples were collected for further analysis of LDH (Lactate dehydrogenase; EC 1.1.1.17), PDC (Pyruvate decarboxylase; EC 4.1.1.17), ADH (Alcohol dehydrogenase EC 1.1.1.1), and Ala-AT (Alanine aminotransferase; EC 2.6.1.2). All the enzymatic assays were performed by monitoring the oxidation of NADH at 340 nm. The LDH assay was monitored in pyruvate → lactate direction in a reaction mix containing 50 mM buffer Tris-HCl (pH 7.5), 0.6 mM NADH, 3.0 µM KCN, 0.2 mM 4-methylpyrazole and 10 mM Na-pyruvate to initiate the reaction (HANSON; JACOBSEN; ZWAR, 1984b). PDC and ADH activity were evaluate as described by Hanson et al. (1984). The reaction medium to determine PDC activity was composed by 50 mM MES buffer (pH 6.0), 0.2 mM NADH, 0.5 mM thiamine pyrophosphate, 1 mM magnesium chloride, 20 mM oxamic acid, 5 units of ADH, and 10 mM Na-pyruvate. ADH activity was determinate using 50 mM phosphate buffer (pH 7.0), 0.6 mM NADH, and 5.0 mM acetaldehyde. The Ala-AT activity was evaluated according to Sousa and Sodek (2003) using 10 mM L-alanine, 5 mM 2-oxoglutarate, 0.6 mM NADH, 50 mM Tris-HCl (pH 7.5), and 5 units of LDH as described by Good and Muench (1992).

### *3.2.7.* Experimental design and statistical analysis

The experimental design was completely randomized consisting of four repetitions for each treatment and the experimental unit was considered three plants per box. Data were analyzed by Shapiro-Wilk for normality test and by Levene's test to verify homoscedasticity, followed by ANOVA analysis. The post hoc comparisons were tested using a Tukey (e.g., 2W vs 2WN; 5W vs 5WN; 5R vs 5RN) or t-test (e.g., 2W vs 5W; 2WN vs 5WN) at the significance level of $p < 0.05$ with RStudio software (R CORE TEAM, 2020).

## *3.3.* Results

### *3.3.1.* Nitrate concentration and NR activity

In this study, the $NO_3^-$ level in the first two days of waterlogging did not differ between treatments that did not receive nitrate (2W), but the supply of $K_2NO_3$ the ion content remained similar in controls, increasing significantly in roots in R2N waterlogged plants and decreasing in group V3R2N relative to their respective controls (Fig. 2). At five days after waterlogging (5W) the plants in group R2 showed a decrease in $NO_3^-$ levels compared to the control and V3R2. In the groups that received nitrate there was a decrease in the ion concentration of the V3R2N control and a sharp increase in the R2 group resulting in surpassing the nitrate concentrations in the waterlogged plants. Thus, in the recovery phase $NO_3^-$ levels decreased in plants V3R2 and increased in plants V3R2N.

NR enzyme activity at two days of waterlogging increased in waterlogged plant groups at stages V3 and R2, regardless of nitrate supply (V3R3 and V3R2N), equivalent to the levels of controls and R2 groups at five days of stress (Fig. 2). At five days of recovery, the group that received nitrate only at stage R2, showed higher levels of NR activity than the other treatments (Fig. 2).

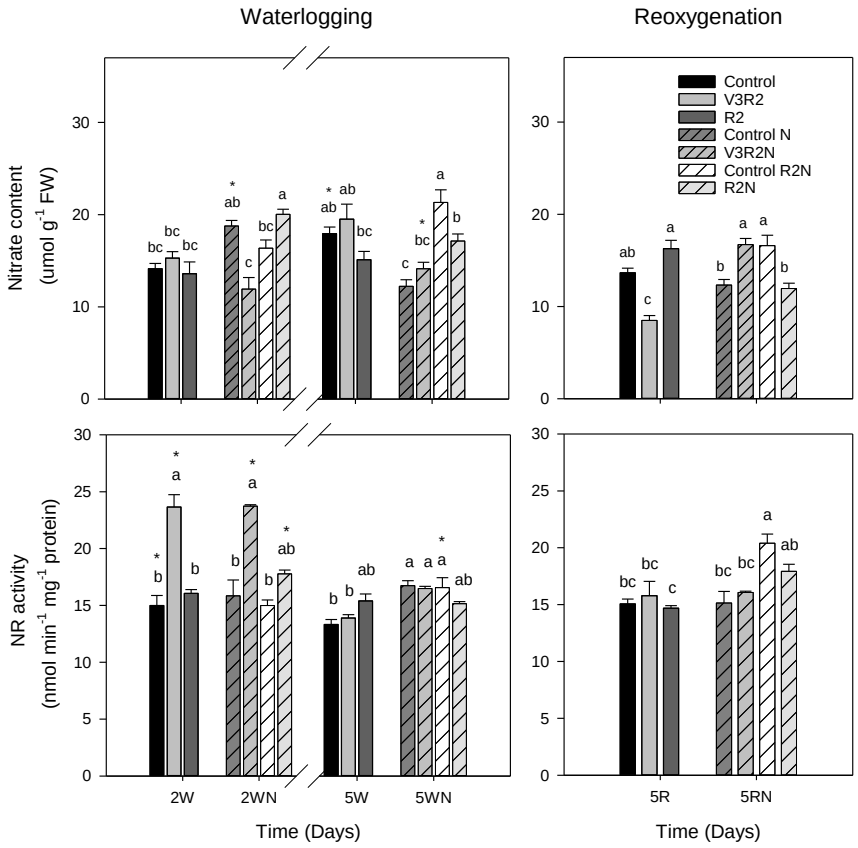


Figure 2. Levels of nitrate and nitrate reductase (NR) activity in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

### *3.3.2.* Hydrogen peroxide and lipid peroxidation levels

Waterlogging resulted in increased levels of $H_2O_2$, and MDA, and the application of nitrate resulted in waterlogged plants with lower levels of ROS and MDA when compared to plants grown without nitrate supply (Fig. 3).

The $H_2O_2$ content in plants in group V3R2 initially increased 1.2 folds at 2W and at 5W it decreased reaching values below the controls (with and without nitrate addition), a more pronounced effect on the R2 group. At 5RN the ROS levels in group V3R2 were equivalent in both nitrate and nitrate free plants, reaching values lower than N or nitrate free controls. In the case of plants in group R2, the contents were higher than in group V3R2 and control without nitrate addition and equivalent to controls with nitrate addition (Fig. 3).

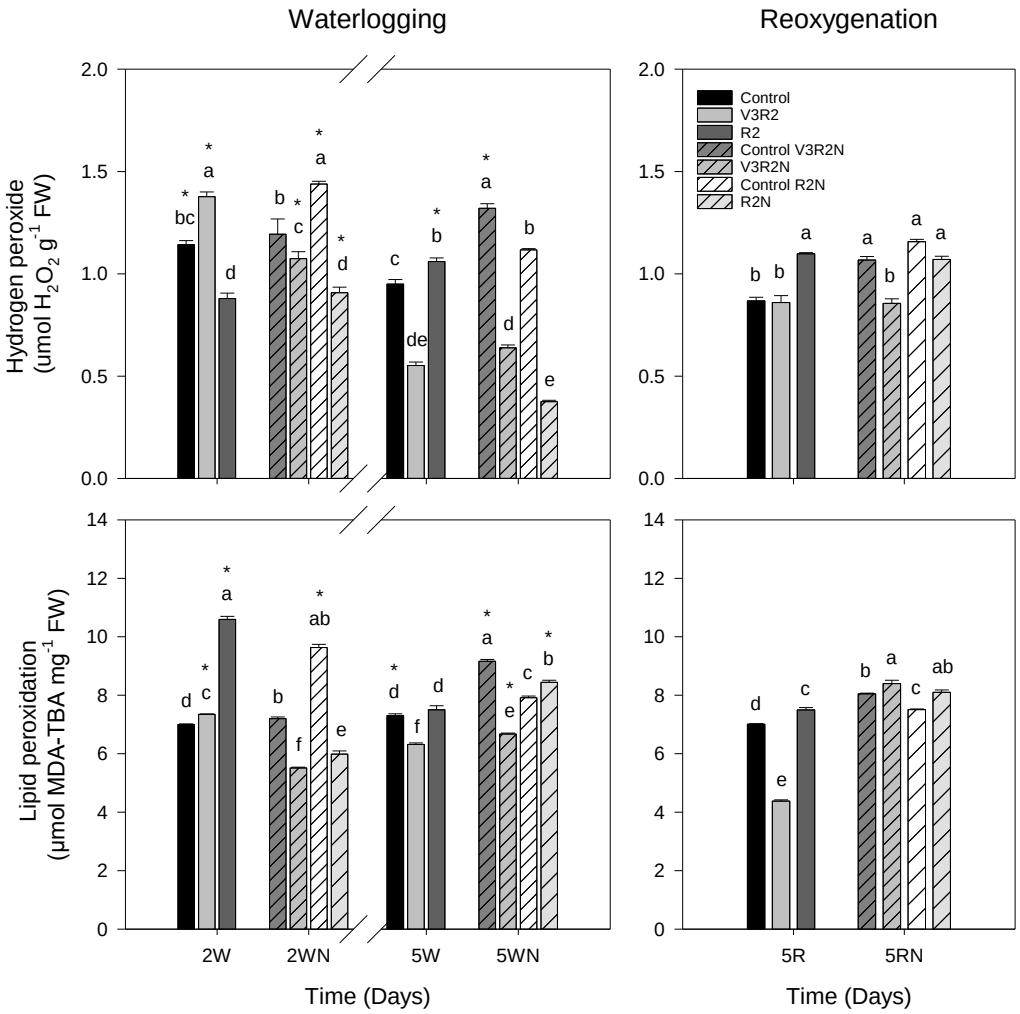


Figure 3. Levels of $H_2O_2$ and lipid peroxidation in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

When plants were subjected to flood stress, oxidative damage resulted in different levels of membrane lipid peroxidation between treatments, measured as MDA content (Fig. 3). At V3R2 the MDA content initially increased at 2W in relation to the N-free control, decreasing at 5W. In the reoxygenation stage it decreased 1.6 folds in relation to the nitrate-free control. In R2 plants the amount of MDA increased 1.5 folds at 2W and at 5W it decreased at the control level (without nitrate). However, in re-oxygenation the MDA content increased by one. In contrast to the addition of nitrate, in plants V3R2N during waterlogging the MDA content decreased 1.8 folds at 2WN and 1.5 folds at 5WN in relation to the V3R2N control. In both waterlogging periods the MDA contents in this group were

also lower than the nitrate-free and R2N control. In re-oxygenation, MDA levels increased above the control (V3R2N) and 1.2 folds in relation to the nitrate-free control. In R2N plants MDA content decreased 1.8 folds at 2WN, increasing slightly at 5WN compared to the control (R2N), and in recovery at 5RN, increased about 1.1 folds compared to the control.

*3.3.3.* Antioxidant enzyme activity

The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOD), ascorbate peroxidase (APX) and dehydroascobate reductase (DHAR) of plant groups V3R2 and R2 were varied in plants under waterlogging with and without the supply of $NO_3$- during waterlogging, compared to unstressed plants (Figs. 4, 5).

SOD activity increased in waterlogged plants, reaching higher values in group V3R2 than R2 at 2W. At 5W activities remained above control, but at equivalent levels in both groups. On the other hand, SOD activity increased in controls with nitrate supply at 2W, and caused a decrease in V3R2N relative to nitrate controls. In the R2N group SOD activity was higher, but lower than in the R2N control. At 5W SOD activity increased significantly in V3R2N and remained stable in the other N- suppled plant groups (Fig. 4). During recovery, SOD activity levels in group R2 increased abruptly (about six folds) higher than in the nitrate-free control, whereas in group V3R2 the activity was only 1.9 folds higher (Fig. 3). The presence of nitrate during waterlogging caused a decrease in SOD activity in groups V3R2N and R2, this effect being greater in V3R2N, reaching values below nitrate controls and equivalent to the control without nitrate addition (Fig. 4).

CAT activity, although low, was stimulated in waterlogged groups, increasing from 2W in group V3R2, reaching values above R2. On the other hand, by supplying nitrate during the waterlogging, CAT activity was decreased in V3R2N to levels below R2N and controls with and without nitrate. At 5W the CAT activity remained high in group V3R2, being equivalent to R2 in front of the addition of nitrate and higher in the presence (Fig. 4). During recovery, group R2 showed an abrupt increase in CAT activity in relation to the control (7 folds) and significantly lower in group V3R2. In the presence of nitrate, the activities were equivalent in groups V3R2N and R2N, but lower than controls in the presence

and absence of nitrate. It is interesting to note that controls that received nitrate only in the R2 stage showed higher CAT and SOD activity than the other controls (Fig. 4).

APX was little responsive during the waterlogging of plants without nitrate supply, increasing only to 2W in relation to the control. The supply of ion caused a decrease in the V3R2N group compared to R2N and controls (without N and R2N) to 2W. The supply of nitrate during the waterlogging of group R2 caused a decrease in APX activity in relation to group V3R2N and other treatments. In addition, there was a great stimulus in enzyme activity in the R2N control at 5W (Fig. 5). During recovery, APX activity in group R2 increased about 10-fold relative to the control (nitrate-free) and V3R2, whereas relative to nitrate supply in waterlogging, activity in this group was equivalent to the control (nitrate-free) and superior to the other controls and group V3R2N (Fig. 5).

DAHR activity levels in group V3R2 at 2W both in the presence and absence of nitrate supply were equivalent to and significantly lower than group R2 and controls. At 5W the activity increased in group V3R2, being higher than the control and group R2 without nitrate supply and with the ion supply the activity was stimulated, but at equivalent levels to the other nitrate treatments (Fig. 5). At 5R the DAHR activity in group V3R2 increased slightly above the nitrate-free control, being equivalent to group V3R2N and nitrate-controlled groups (5RN). However, in group R2 the activity increased by about 6 and 8 folds with respect to the nitrate-free and nitrate-supplied control, respectively (Fig. 5).

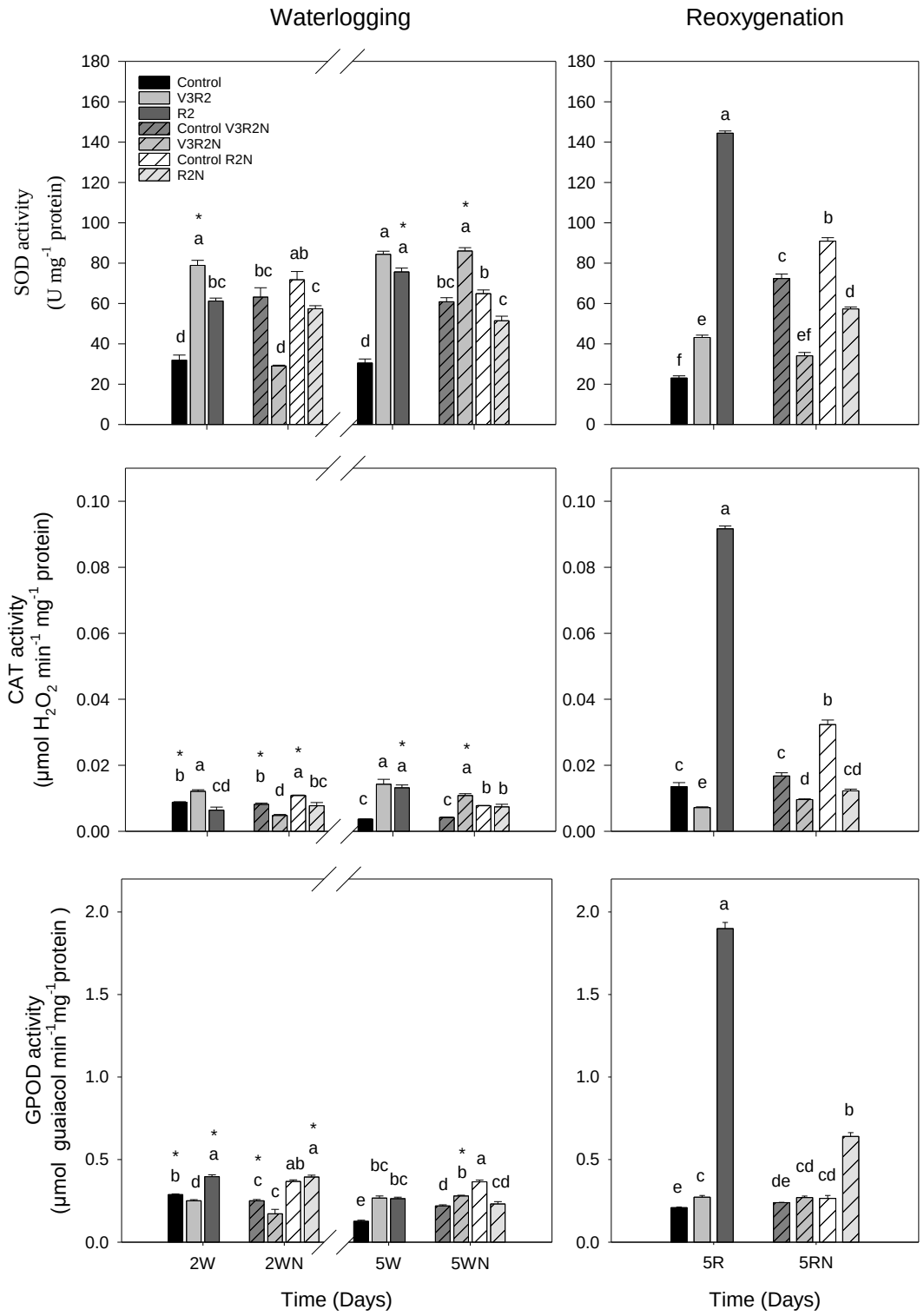


Figure 4. Superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOD) activities in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

GPOD activity has decreased in V3R2 plants in relation to the control, both in the absence and presence of nitrate. In group R2 activity has increased in relation to V3R2, and is also higher than the control without nitrate supply and the control V3R2N (Fig.5). At 5W the activity increased in relation to 2W, reaching values higher than the control without nitrate and activity levels equivalent to the others. In the R2 group, on the other hand, the activity increased strongly reaching values of approximately 9.5 and 2.4 folds in the absence and presence of nitrate, in relation to the nitrate-free control (Fig. 4).

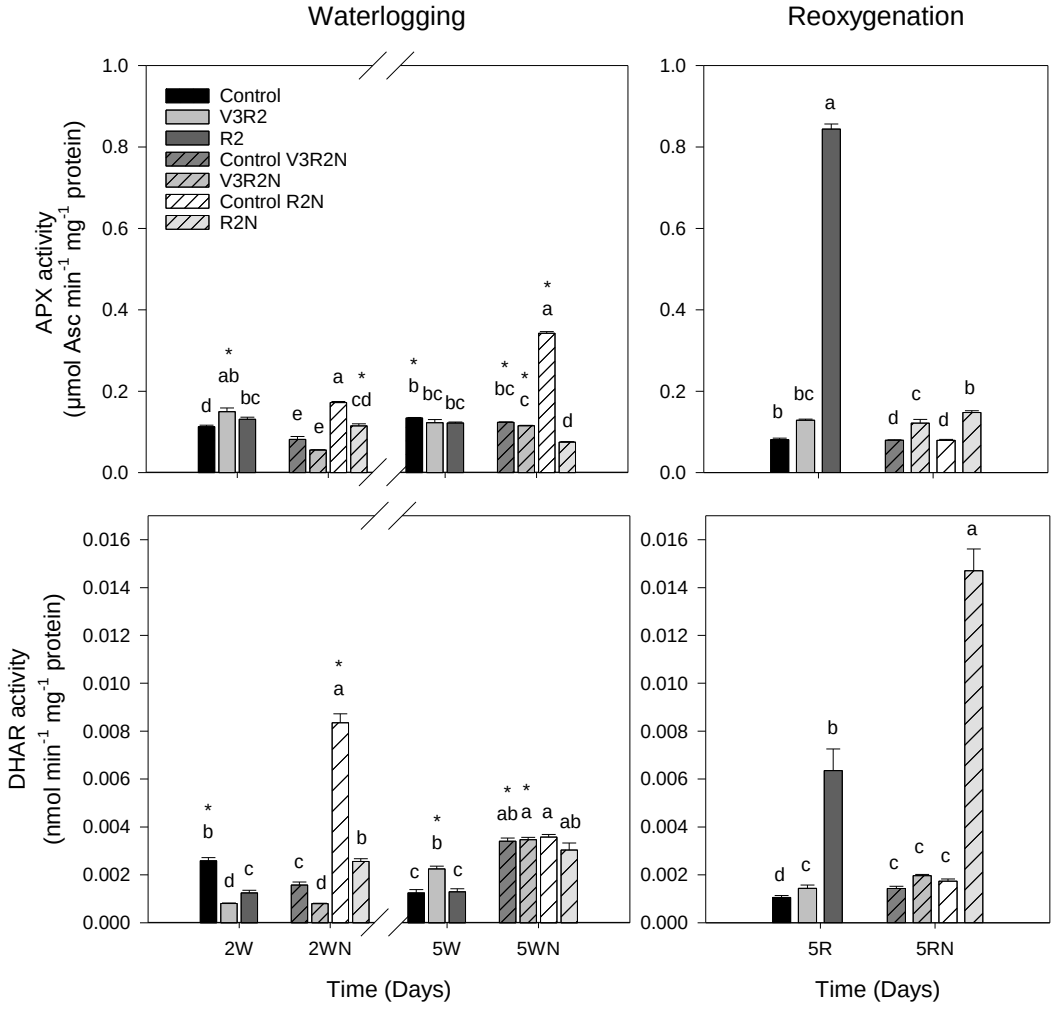


Figure 5. Ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR) activities in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

### *3.3.4.* Fermentative enzymes and Ala-AT activities

Fermentative enzyme activities increased in plants under waterlogging without nitrate supply, compared to waterlogged plants that received the ion effect that lasted for 5RN (Fig.6). LDH activity increased significantly (14.5 folds) at 2W and 5W (213 folds) in the R2 group compared to nitrate-free control and in reoxygenation, at 5R it decreased (twice). In contrast, in V3R2 plants LDH activity increased only at 5W (120 folds), decreasing in absolute values at 5R (1.5 folds), but at higher levels in relation to nitrate-free control and R2 group. When plants were nourished with nitrate during waterlogging, LDH activity was extremely low in both V3R2N and R2N groups, differing at 5W strongly from the respective groups (V3R2 and R2) when they did not receive nitrate (48.2 and 31.5 folds, respectively). At 5R, the activities of the waterlogged groups remained low and with lower activities than the groups of the waterlogged plants that did not receive nitrate. The R2 group maintained activity levels lower than the V3R2 group regardless of the addition of nitrate during the waterlogging (Fig. 6).

Similar to that observed for the LDH enzyme, ADH activity during waterlogging increased 103 folds at 2W in R2 group plants compared to the control (nitrate-free) and decreased (5.5 folds) at 5W, remaining superior to the control. At 5R it decreased 3.6 folds compared to the control. In plants of V3R2 group ADH activity increased slightly at 2W and abruptly (12.8 folds) at 5W compared to the nitrate-free control, reaching at 5R a value of about twice the control. In plants that received nitrate, ADH activity was little changed during waterlogging, reaching values slightly above the nitrate-free control at 5W in the R2N group. At 5RN, both V3R2N and R2N showed low ADH activity compared to controls (with and without nitrate). The activity in group V3R2N was slightly lower than R2 and about 11.5 folds lower than group V3R2 (without nitrate supply) (Fig. 6).

The variations in PDC activity during waterlogging followed those found for ADH, increasing by about 75 folds at 2W in group R2 maintaining an activity about 29 folds above nitrogen-free control at 5W. In group V3R2 a considerable increase of 100 folds over the 5W control was observed, differing from the nitrate-free control by 2.6 folds at 2W (Fig. 5). The variation in activity was very different from the treatments without nitrate supply during waterlogging. At 2W the activity

was minimal in both waterlogged groups (V3R2 and R2), practically equal to the controls. At 5RN, PDC activities in groups V3R2 and R2 were equal to controls Fig. 6).

The activity of Ala-AT increased around 135 folds at 5W in the V3R2 group, while in the R2 group there was a much less significant increase since 2W, reaching a value of 21.5 folds bad of the control without adding nitrate to 5W. The addition of nitrate during waterlogging hardly changed the activity of Ala-AT in both V3R2 and R2 (Fig. 6). At 5RN, Ala-AT activity levels decreased considerably in relation to that observed during waterlogging, showing values above controls in waterlogged groups, but lower in nitrate treated groups (V3R2N and R2N).

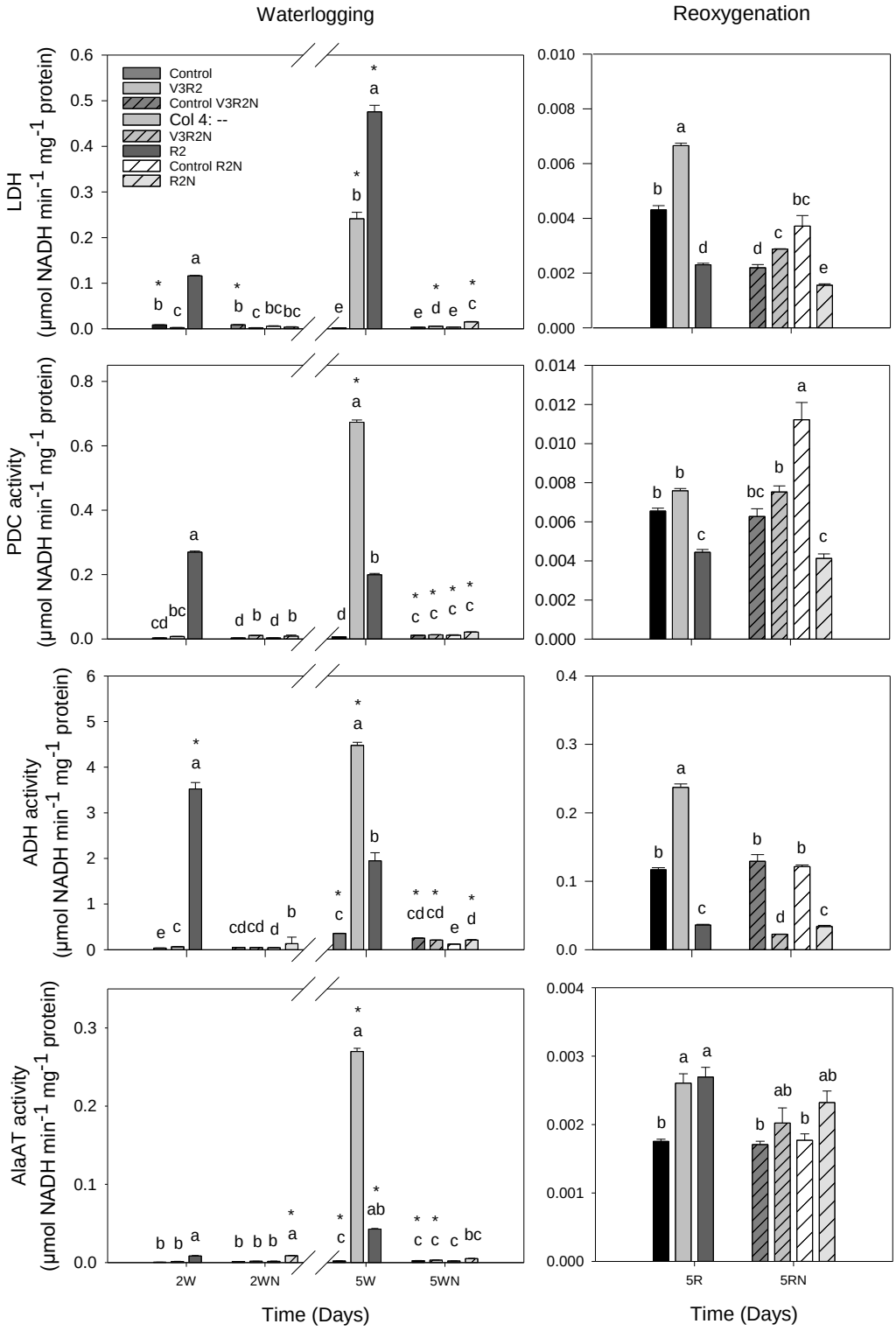


Figure 6. Lactate dehydrogenase (LDH), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH) and alanine aminotransferase (Ala-AT) activities in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

*3.3.5.* Levels of amino acids, total soluble sugars and sucrose

The concentration of total soluble amino acids (TAA) decreased in plants of group V3R2 to 2W in relation to the control (without nitrate supply), reaching values equivalent to 5W and in group R2, the levels remained stable. In the presence of nitrate, TAA concentrations increased abruptly in group R2N at 2W while remaining above the controls (with and without nitrate) at 5W. In group V3R2N the TAA contents were practically unchanged during waterlogging being higher than group V3R2 at 2WN and lower than the V3R2N control at 5WN (Fig. 6). At 5R, the TAA contents in group R2 were approximately 1.5 folds higher than the control without nitrate supply and in group V3R2N, slightly lower (Fig. 7). In front of the addition of nitrate during hypoxia, the TAA contents of R2N did not differ from group R2 (without nitrate supply), being superior to the other groups. In group V3R2N there was a less expressive increase in relation to group R2N, but significantly higher than group V3R2 and control without nitrate addition.

Total soluble sugar (TSS) levels for both V3R2 and R2 plants increased significantly (1.7 folds) at 2W compared to the nitrate-free control at 5W. While for plants V3R2N and R2N, with nitrate supply increased significantly at 2WN (about 1.7 and 2.5 folds higher than the controls), decreasing at 5WN for both plant groups. At this time of waterlogging, TSS concentrations were lower than the R2N control both for R2 and V3R2 and in the case of R2N they were also lower than the nitrate-free and V3R2N group treatments. At 5RN (reoxygenation), TSS concentrations in groups V3R2 and V3R2N did not differ, but were lower than the R2N control and equivalent to the other controls. In plants of group R2 the TSS contents did not differ from group V3R2, but decreased to values lower than the other treatments (Fig. 7).

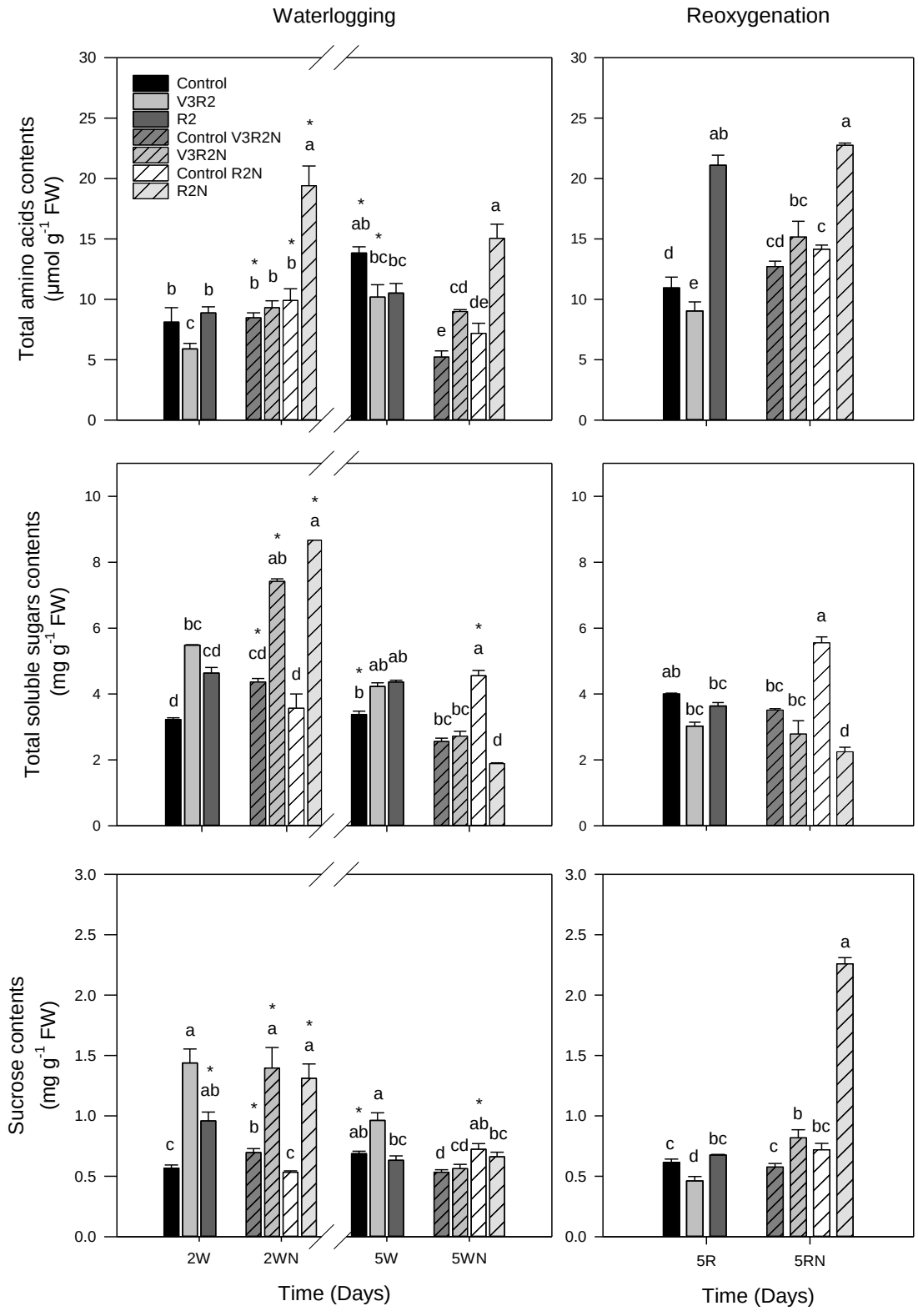


Figure7. Levels of total amino acids, total soluble sugars and sucrose in roots of soybean plants submitted to waterlogging associated to nitrate supply at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

Sucrose (SUC) in V3R2 plants increased about 2.5 folds at 2W, while in R2 group this increase was about 1.7 folds the control concentration (without nitrate supply). At 5W the SUC concentration in V3R2 plants decreased with the waterlogging time, but remained above the control (no nitrate supply) and in group R2 the SUC concentration remained at the control levels (Fig. 7). The addition of nitrate during waterlogging of plants in groups V3R2N and R2N maintained high concentrations of SUC in relation to the controls at 2W, but without differing from the groups waterlogged without nitrate (V3R2 and R2). At 5W, SUC levels in plants V3R2 and R2 decreased equally, both being lower than group V3R2 (2W) and presenting levels close to the other controls. In the recovery, V3R2 plants showed a decrease in SUC concentration compared to controls (without nitrate application) and R2. The supply of nitrate caused a slight increase in SUC contents in plants V3R2, and an abrupt increase in plants R2N (approximately four folds in relation to controls) (Fig. 7).

### *3.4.* Discussion

In relation to soybean cultivation, waterlogging stress is demonstrated in two ways in soybeans: the first is physical injury and the second is anaerobic stress (TEWARI; ARORA, 2016b). Under these conditions, the responses presented by the species to ensure survival involve the induction of several biochemical, physiological, morphoanatomical and even genetic mechanisms (REDDY et al., 2011; TAIZ; ZEIGER, 2002). The results by waterlogging stress generates a hypoxic situation, i.e., insufficient oxygen ($O_2$) concentration for maintaining normal or healthy respiration in roots, a change from aerobic to anaerobic path takes place, bringing changes in the respiratory metabolism of roots (MORALES-OLMEDO; ORTIZ; SELLÉS, 2015). These abrupt changes in respiratory metabolism in turn produce toxic metabolites like lactic acid ($C_3H_6O_3$) and ethanol ($C_2H_5OH$), which increase the activity of fermentative enzymes (BORELLA et al., 2014; HARTMAN; SASIDHARAN; VOESENEK, 2019; KOLB; JOLY, 2009).

Plants have developed several mechanisms to deal with the adverse effects of waterlogging, which can result from changes in many physiological and biochemical responses in plants. Plants require nitrogen (N) for growth, development and resistance against biotic and abiotic stresses (DA-SILVA; DO

AMARANTE, 2020a; GUPTA, 2020). This research was conducted under ambient light and temperature conditions, and indicates the stress responses of soybean plants under a single waterlogging stress event (at the reproductive stage R2) compared to the responses developed in plants under repeated stress (first in the vegetative stage V3 and then in the reproductive stage R2) associated or not with nitrate supply during stress events.

### *3.4.1.* Waterlogging priming and nitrate supply enhance the maintenance of cell homeostasis in $NO_3^-$ and NR activity under waterlogging stress

In the roots of V3R2 soybean plants, the $NO_3$- content was not altered during waterlogging and decreased at five days of recovery (Fig. 1), suggesting that the absorption of the ion by the roots was not affected during waterlogging and remained stable, which may be associated with the optimization of nitrate acquisition found under natural conditions, since this group of plants did not receive nitrate in their nutrition. Furthermore, compared to nitrate treatment, the lower ion content in group V3R2N compared to group R2N at 2WN may indicate a higher absorption and transport of nitrate compared to group R2N, supplemented only once with nitrate during waterlogging stress at R2 stage. Moreover, the variations in nitrate concentration in V3R2N group compared to R2 group at 5RN reinforce this hypothesis: under a lower availability of nitrate, the contents in the tissue were lower than in R2 and, when both groups were supplied with nitrate, levels were significantly higher in V3R2N (Fig. 1). The nitrate ion is first reduced to nitrite by the cytosolic enzyme nitrate reductase (NR) (CHAMIZO-AMPUDIA et al., 2017), and transported to plastid, where it is reduced to ammonium and assimilated into amino acids (SOUZA; MAZZAFERA; SODEK, 2016). NR activity in V3R2 group increased substantially at 2W and at levels equivalent to V3R2N group (supplied with nitrate), while in group R2, even in the presence of nitrate, this increase was less evident (Fig. 1), which may indicate that in plants V3R2 under waterlogging there was an acidification necessary to lead an activation and increase of NR activity (BOTREL; KAISER, 1997; STOIMENOVA et al., 2003). However, in R2 plants under anaerobic conditions, nitrate assimilation in the roots could be restricted, thus reducing the activity of the NR, which is responsible for nitrate reduction for further assimilation in amino acids (BRANDÃO; SODEK, 2009). In fact, at 5W, NR activity in group V3R2,

V3R2N and R2N decreased compared to 2W, but remained at activity levels above non-treated nitrate control (Fig. 1), indicating that nitrate assimilation was restricted with the advance of hypoxia period in the roots and nitrate reductase activity regulated by nitrate availability (WANY et al., 2019). However, at the reoxygenation period (5R), the activity levels of the waterlogged groups reached the levels of the non-treated nitrate control, being lower only than the R2N control, suggesting that the plants under reoxygenation, were able to activate the nitrite reductase, the enzyme necessary to catalyse the reduction $NO_3^-$ in $NO_2^-$ (BOTREL; MAGNE; KAISER, 1996), which facilitates nitrate assimilation (Fig. 1) under reoxygenation (REGGIANI; BERTINI; MATTANA, 1997). The increase of NR activity in plants V3R2 and R2 under hypoxia, probably made possible the activation of the futile cycle of NO production triggered the functioning of the non-symbiotic hemoglobin cycle1 (phytoglobin)-nitric oxide (Pb-NO), within which its active sequestration takes place by oxyhemoglobin which leads to the regeneration of $NAD^+$ for glycolysis and the production of ATP at substrate level (STOIMENOVA et al., 2007a) as well as alanine synthesis to compete with fermentation pathways and thus conserve carbon and nitrogen (IGAMBERDIEV et al., 2004; PUCCIARIELLO; PERATA, 2017b; WANY et al., 2019; ZABALZA et al., 2009a), and decrease in fermentative pathways ((OLIVEIRA; SODEK, 2013).

### *3.4.2.* Waterlogging priming and nitrate supply enhance ROS scavenging capacity under waterlogging stress

In the roots, the $H_2O_2$ content increased only at 2W for plants V3R2, and at 5W for R2, which is related to the higher SOD activity at these periods in the respective groups, occurring in response, a significant increase of $H_2O_2$ removing enzymes activity: CAT and APX for V3R2 and CAT and GPOD for R2 plants (Figs. 2, 3, 4) which corroborates other results obtained with soybean (DA-SILVA; DO AMARANTE, 2020b; GARCIA et al., 2020). This later response of the R2 group in relation to V3R2 may be due a 'priming' by waterlogging effect in which the V3R2 plants were submitted (initial priming at V3 stage by waterlogging for seven days before the stress by waterlogging at R2 stage). In relation to reoxygenation, V3R2 soybean plants showed a lower accumulation of $H_2O_2$ in relation to plants R2, that could be associated with variation of oxidative damage and activity of antioxidant enzymes

between groups V3R2 and R2 which possibly indicate the acquisition of tolerance by "priming effect" in plants V3R2.

In roots of V3R2N and R2N plants (supplied with nitrate), the decrease of $H_2O_2$ content during waterlogging could be associated with the accumulation of NO, since nitric oxide act as an antioxidant molecule in the hypoxic roots of soya (DA-SILVA; DO AMARANTE, 2020a) (Fig. 2). Possibly NO reacted directly with $O_2^{*-}$ to form peroxynitrite ($ONOO^-$) (GUPTA, 2020; HANCOCK; NEILL, 2019), thus, reducing the bioavailability of both, relieving the oxidative stress. In addition, NO behaves as a final electron acceptor in the mitochondrial chain, thus reducing electron leakage and ROS accumulation (GUPTA, 2020).

Malonaldehyde (MDA) has been used as a biomarker to assess oxidative damage. Lipid peroxidation was transient in both groups of waterlogged plants without nitrate supply during the hypoxia period, showing a slight increase at 2W and a reduction below control at 5W in plants V3R2, while in R2 group, this increase was considerable at 2W. Under reoxygenation (5R), the same response was maintained: MDA levels below the control in plants V3R2, and in R2 there was a slight increase (Fig. 2). This differentiated response between groups may be associated with waterlogging priming (LÄMKE; BÄURLE, 2017a), leading the V3R2 group to a greater adaptation to environmental variations.

Under the application of nitrate during waterlogging, MDA concentrations were initially (2WN) lower in plants V3R2N and R2N compared to the same groups that did not receive the ion and lower than the controls (Figure 2), which demonstrates the beneficial effect of nitrate application in reducing oxidative damage (BORELLA et al., 2019; DA-SILVA; DO AMARANTE, 2020a). Despite the increase in MDA at 5WN in V3R2N and R2N, the difference in lipid peroxidation levels increased between the two groups, with MDA levels at V3R2N remaining below the controls (with and without nitrate), in contrast to the R2 group, whose MDA levels were higher than the nitrate-free and R2N controls (Fig. 2). These results suggest that the "priming" effect persisted in group V3R2 even in the presence of nitrate which also helped reduce oxidative stress. Under reoxygenation, the oxidative damage followed differentiated in groups V3R2 and R2, whereas in the presence of nitrate, the MDA content increased for both

groups, reaching equivalent values, and a little above to their respective controls with nitrate and other nitrate-free treatments (Fig.2), which may be related in part to the lower activity of the antioxidant system in the groups waterlogged with nitrate at 5RN (Fig. 3, 4). This increase in lipid peroxidation in V3R2N group under nitrate application could be related to the increase in NO levels which is responsible for the NO respiration of NO in the increase internal oxygen levels tissues (WANY; FOYER; GUPTA, 2018).

Resistance to waterlogging stress may depend, at least in part, on improving the antioxidant defense system (BIN et al., 2010). Antioxidant enzyme activities have increased in plants under waterlogging compared to unstressed plants (Figs. 3, 4). In this sense, SOD activity increased in plants V3R2 and R2, being more significant in V3R2, suggesting that this group of plants developed a better cleaning capacity of the $O_2^{*-}$ radical, forming $H_2O_2$ and $O_2$. At 5R only the plants R2 still had levels of $H_2O_2$ above the control, which is related to the high SOD activity in this group of plants (Fig. 3). $H_2O_2$ is toxic and should be degraded by enzymes, such as CAT and APX considered the most important in the detoxification of this ROS (VOESENEK; BAILEY-SERRES, 2015).

CAT activity increased in V3R2 plants at 2W, and remained at levels above control at 5W, while in R2 group plants there was an increase at 5W, and in the recovery (at 5R) an abrupt increase, a period in which CAT activity was below control (Fig. 3). The difference in response time of the CAT activity compared to the stress time imposed on plants V3R2 and R2 reveals another possible aspect of the "priming effect" caused by waterlogging at stage V3 in group V3R2. Similarly, although the APX activity was higher than the control in both groups of plants at 2W, remaining at levels equivalent to 5W, there was a large increase in APX activity at 5R only in group R2, which reinforces this hypothesis of "priming" in group V3R2 (Fig 3).

GPOD, known as phenol peroxidase (guaiacol), is typically involved in the process of $H_2O_2$ purification associated with growth and development (CAVALCANTI et al., 2004) or the removal of $H_2O_2$ in the cell, and in the vacuoles in cooperation with ascorbate (BONIFACIO et al., 2011) to be converted to water through a peroxidase-dependent reaction using phenol as an electron donor (TAKAHAMA, 2004). GPOD activity increased in R2 plants at 2W while

remaining above control at 5W, a period in which activity was also above control in V3R2 group. During recovery (5R), GPOD activity increased significantly in plants in group R2, and in plants of V3R2 group it remained somewhat above the control (Figure 3), showing one more aspect that can differentiate acclimatised plants from those that were stressed only at R2 stage. V3R2 plants which had a waterlogging priming, had a better absorption capacity of $H_2O_2$.

DHAR activity, although lower than the 2W control, in groups V3R2 and R2, increased significantly at 5W, remaining slightly higher than the recovery control (5R). In contrast to group V3R2, there was a strong increase in DHAR activity in group R2 during this period (Fig 4). This difference in response possibly reveals distinct needs between the two groups in ascorbate regeneration, since this molecule besides being an important non-enzymatic antioxidant in plant cells (BLOKHINA, 2003), also acts as an electron donor for detoxification of $H_2O_2$ by the enzyme APX (RAHANTANIAINA et al., 2017).

Collectively, the variation in response of SOD, CAT, APX, GPOD and DHAR enzymes was sufficient for efficient control of ROS levels triggered by waterlogging stress and post-hypoxia reoxygenation, allowing that in group V3R2, MDA levels were reduced from the initial waterlogging and remained below control in reoxygenation, indicating a possible advantage of the "acclimatization" provided by the first waterlogging at stage V3 compared to group R2 (Figs. 2, 3, 4). On the other hand, the plants supplied with nitrate during the waterlogging did not increase the production of ROS, the presence of the ion was responsible for the maintenance of SOD activity unchanged or at low levels, compared to the waterlogged groups without receiving nitrate (V3R2 and R2) and also for the consequent maintenance or decrease of CAT, GPOD, APX and DHAR activity for nitrate supplied groups (V3R2N and R2N) (DA-SILVA; DO AMARANTE, 2020a). As NO and ROS are produced in the cell at the same time, it is important to consider their interaction and the ramifications of the chemistry that produces peroxynitrite (HANCOCK; NEILL, 2019), thus, reducing the bio-disponibility of NO and ROS. In addition, peroxynitrite can act by altering SOD activity (HOLZMEISTER et al., 2015) and CAT (BAUER, 2015) through nitration (CORPAS; RÍO; PALMA, 2019). Despite the low activity of antioxidant enzymes in plants under waterlogging and treated with nitrate, lipid peroxidation

has decreased, probably due to NO acting with an efficient antioxidant (HOLZMEISTER et al., 2015), preventing oxidative damage in plants under waterlogging.

*3.4.3.* Fermentation and Ala-AT activity in roots enhance plant tolerance to waterlogging stress and nitrate supply favouring the priming capacity.

When hypoxia sets in, the anoxic state replaces mitochondrial oxidative phosphorylation by anaerobic fermentation, avoiding pyruvate accumulation and contributing to $NAD^+$ cycling, and ATP production at substrate level (BORELLA et al., 2014; BUI et al., 2019). In contrast, fermentative enzyme activity increased in plants under waterlogging without nitrate supply compared to plants supplied with the ion under waterlogging condition (Fig. 5).

The LDH activity increased after five days of waterlogging (5W), and at recovery (5R) in V3R2 plants. However, in R2 plants, there was a significant increase from 2W and decreased at 5R. demonstrating that V3R2 is more efficient in responding to $O_2$ deficit, altering metabolism as a survival mechanism. This differentiated response in V3R2 plants indicates a rapid perception and shift from lactic to alcoholic fermentation represents an important indicator of the ability of soybean genotype to survive hypoxia without suffering extensive cellular damage (ZABALZA et al., 2009), this characteristic acquired in the V3R2 group of plants is probably a response linked to the "priming" effect of recurrent stress (HILKER et al., 2016; HILKER; SCHMÜLLING, 2019a). However, in R2 plants we observed early activation of fermentation in unprimed plants reversed by the addition of nitrate (Fig.5), possibly because of lactic acid accumulation could had lead to cell acidification (AROCA; PORCEL; RUIZ-LOZANO, 2012), resulting in inhibition of aquaporin's (TOURNAIRE-ROUX et al., 2003b) which has possibly increased significantly during waterlogging and decreased at recovery (GARCIA et al., 2020). Lactate produced in these plants in turn activated alcoholic fermentation (IRFAN et al., 2010; KOMATSU et al., 2010), inducing PDC activity that converts pyruvate to acetaldehyde, resulting in ethanol production by subsequent ADH enzyme activity. In contrast to R2 group, in V3R2 plants the increase in LDH activity was accompanied by significant increases in PDC and ADH activities at the end of waterlogging (5W) and at 5R.

The high activity of PDC and ADH enzymes in group V3R2 may be related to differentiated metabolic adaptation to R2 group, since alcoholic fermentation is one of the main metabolic strategies used for plant survival under $O_2$ deficit, and although it allows limited production of ATP (STOIMENOVA et al., 2007a), it represents carbon loss in the form of ethanol (TAMANG; FUKAO, 2015). Thus, this "late response" of R2 group could represent an interesting strategy for saving carbohydrate and obtaining energy for longer hypoxia periods. It is important to note that the high activities of NR in groups V3R2 and V3R2N possibly provided an adequate energy status for 2W, thus enabling in this group of "pre-acclimated" plants a further induction of fermentation activity, improving or at least sustaining glycolysis and contributing to a better tolerance to hypoxia (Figs. 1, 5). In V3R2N and R2N plants, supplied with nitrate before and between waterlogging events, the fermentative enzyme activities were severely restricted by nitrate treatment (Da Silva et al., 2020). This response may have been triggered by the direct participation of NR activity, induced under hypoxia through the nutrition of $NO_3^-$ in a sequence of reactions known as the NO/Pb cycle that converts $NO_3^-$ into $NO_2^-$ and finally into NO (GUPTA et al., 2020; IGAMBERDIEV; HILL, 2009), to generate $NAD(P)^+$ and consume protons, relieving alcoholic and lactic fermentation (OLIVEIRA; FRESCHI; SODEK, 2013; VAN DONGEN; LICAUSI, 2015a; WANY et al., 2019; WANY; FOYER; GUPTA, 2018) (Fig. 1, 5).

Ala-AT enzyme activity was stimulated during waterlogging, increasing slightly at 2W and 5W in group R2, while in group V3R2 the increase was significant only at 5W (Fig 5). Conversely, Ala-AT activity was restricted in nitrate- supplied waterlogged plants, probably due to the increased activity of LDH, PDC and ADH, which prevented the accumulation of the pyruvate that activates Ala-AT (VITOR; SODEK, 2019). The higher activity of Ala-AT in the V3R2 group of plants, at the end of the waterlogging period and at five days of recovery, is probably part of a tolerance mechanism, since the metabolism of alanine competes with the alcoholic and lactic acid fermentation pathways consuming pyruvate, contributing to a limitation of cytoplasmic acidification by lactic acid, and limiting the loss of carbon in the form of ethanol, facilitating the continuous operation of glycolysis during waterlogging. Furthermore, the activity

of Ala-AT during hypoxia caused by waterlogging, by linking glycolysis with the Krebs cycle, allows the continuous flow of α-keto acids into the Krebs cycle, allowing, via partial operation of the cycle, the increase of ATP synthesis at substrate level (ROCHA et al., 2010a; VITOR; SODEK, 2019). Additionally, the maintenance of Ala-AT activity under post-hypoxic conditions allows the use of part of the alanine accumulated during the waterlogging period, which functions as a source of carbon and nitrogen used during recovery from hypoxic stress (DA-SILVA; DO AMARANTE, 2020a; DE SOUSA; SODEK, 2003; GARCIA et al., 2020; SOUZA; MAZZAFERA; SODEK, 2016).

*3.4.4.* Carbohydrates and total amino acid levels in roots enhance plant tolerance to waterlogging stress and nitrate supply favouring the priming capacity.

The levels of total soluble sugars (TSS), sucrose (SUC), and total soluble amino acids (TAA) in waterlogged plants were varied between groups of waterlogged plants (Fig. 6). In the case of V3R2 plants, the concentrations of TAA in the roots submitted to waterlogging reduced at 2W and were equivalent to the control at 5W, followed by a decrease at reoxygenation (5R). However, in R2 plants the TAA concentration remained at control levels during waterlogging periods, and increased significantly at 5R (Fig.6). This temporary decrease in V3R2 plants may indicate that the synthesis of amino acids was inhibited because of the energy deficit imposed by waterlogging and possibly the amino acids produced in the shoots were used primarily for the development of reproductive structures rather than directed at the roots. It cannot be ruled out the hypothesis that at least a part of the amino acids was used as a source of carbon skeletons for numerous metabolic pathways, or even as respiratory substrates under carbon deficiency (photosynthesis drop in leaves and aerobic respiration in the roots), contributing to the production of energy (ATP synthesis) (Batista-Silva et al., 2019; Rocha et al., 2010b). Although the availability of soluble sugars and sucrose has increased in this period for both groups (V3R2 and R2), this hypothesis cannot be ruled out for longer waterlogging periods when the carbohydrate levels available for respiration have decreased (Fig. 6). In plants R2 at 5R, the TAA content increased considerably, which suggests a higher investment by the plant in root nitrogen metabolism, possibly involved in repairs,

compared to group V3R2, which could involve higher synthesis rates of amino acids or nitrogen compounds. Plants accumulate different sugars and amino acids to protect against stress (TEWARI; MISHRA, 2018). In addition, there may have been a higher transport of amino acids from the aerial part to the roots, reduced degradation of amino acids and restricted consumption due to a decrease in protein synthesis or production of secondary metabolites. In waterlogged plants, the supply of nitrate favoured a significant increase of TAA in the R2N group at two and five days of waterlogging, whereas in V3R2N there was a slight increase at 5WN, indicating that it is an effect of the external supply of nitrate that they are readily absorbed by the roots and can directly influence the internal cell concentrations of these molecules (MILLER et al., 2007; THOMAS; SODEK, 2006), and does not constitute the main strategy for tolerance to hypoxic stress in V3R2 plants. Under reoxygenation (5RN), the same difference in response has been maintained between the two groups, suggesting a higher flow of amino acids in the V3R2N group to the aerial part or even a higher rate of protein synthesis in the roots compared to the R2N group.

Oxygen deficiency resulted in increased production of total soluble sugars (TSS) in roots, with high levels at 2W. This increase in TSS could be related to low levels of pyruvate in the roots, and increased levels of sucrose (Fig. 6), probably attributable to respiratory metabolism and the need to supply the energy demand of cells (BORELLA et al., 2014), The decrease in TSS and sucrose at the reoxygenation period, seen in group V3R2, is probably related to the higher metabolic activity of the plant under normoxia, stimulating the aerobic respiratory process and the use of carbon skeletons produced to resume plant development.

The supply of nitrate during waterlogging resulted in a large increase in TSS and sucrose at 2WN in groups V3R2N and R2N, allowing the maintenance of higher concentrations of TSS in V3R2N plants (Fig. 6) at 5WN and recovery (5RN). These carbohydrates levels could maintain adequate levels of easily metabolisable (fermentable) sugars, resulting from decreasing fermentative metabolism as a consequence from nitrate reduction and activation of NO cycle in hypoxic roots (HOSSAIN; UDDIN, 2011; ROLLAND; BAENA-GONZALEZ; SHEEN, 2006) that is one of the adaptive mechanisms for waterlogging or

oxygen deficient environment (SAIRAM et al., 2009). In this period of reoxygenation, TSS levels in the R2N group fell below the controls (with and without nitrate), probably due to increased ATP consumption by cells, for maintenance of metabolism, which may be supported by a significant increase in sucrose in R2N compared to the discrete increase in the V3R2N group, sugar that possibly was available by the photosynthetic process to sustain growth resumption in this group of plants, which showed higher stress in relation to the V2R2N group (Figs. 2, 6). Furthermore, this result may be related to a higher efficiency of carbohydrate allocation from the photosynthetic process in acclimatized plants (V3R2N), due to the activation of glycolysis and the amount of root sugar reserve and the activity of sucrose hydrolytic enzymes that are determinant for the waterlogging tolerance of cultivated plants (KOMATSU; HIRAGA; YANAGAWA, 2012; KOMATSU; SAKATA; NANJO, 2015; TOUGOU et al., 2012b).

### *3.5.* Conclusions

We have demonstrated that the preparation of responses to water stress applied to soybean plants, improves the response to transient environmental stimuli (recurrent waterlogging) and it is as effective in triggering tolerance to waterlogging as to nitrate nutrition during waterlogging. Groups V3R2 and V3R2N showed some of the main metabolic aspects that have been modified as a result of the recurrent stress treatments on nitrate application, like as shown by the physiological parameters, compound levels, antioxidant and fermentative enzymes activities or carbohydrate levels, in this research. Nitrate application has been found to enhance the response of plants to water stress, and this response is even better in plants that have undergone recurrent stress priming.

Understanding the underlying mechanisms in "soybean" will ultimately enable us to improve stress tolerance in crop species. One possibility could be to explore the mechanisms of stress preparedness to induce a constitutive state, thereby increasing a crop's ability to tolerate stress and disease without incurring a penalty for accumulation of biomass and yield.

| Responses | Waterlogging | | Nitrate feeding | |
|---|---|---|---|---|
| | V3R2 | R2 | V3R2N | R2N |
| $NO_3$- |  |  |  |  |
| NR activity |  |  |  |  |
| $H_2O_2$ |  |  |  |  |
| Lipid peroxidation |  |  |  |  |
| SOD activity |  |  |  |  |
| CAT activity |  |  |  |  |
| GPOD activity |  |  |  |  |
| APX activity |  |  |  |  |
| DHAR activity |  |  |  |  |
| LDH activity |  |  |  |  |
| PCD activity |  |  |  |  |
| ADH activity |  |  |  |  |
| Ala-AT activity |  |  |  |  |
| TAA |  |  |  |  |
| TSS |  |  |  |  |
| Sucrose |  |  |  |  |

Fig. 8. The main metabolic effects of waterlogging associated with primed and unprimed plants, with and without nitrate supply (V3R2 vs V3R2N and R2 vs R2N). Arrows indicate increase (green arrows) or decrease (red arrows) and equal (blue equal) in the compound levels or in the activity of enzymes in comparison to non-supplied nitrate plants. The arrow thickness represents the intensity of the responses compared with non-supplied nitrate plants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

*3.6.* References

AGOSTINETTO, D. et al. Performance of transgenic soybean cultivars and weed control in function of application times and glyphosate formulations. **Planta Daninha**, v. 27, n. 4, p. 739–746, 2009.

AHMED, S. et al. Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. **Plant Science**, v. 163, n. 1, p. 117–123, 2002.

ALAM, I. et al. Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage. **Journal of Biosciences**, v. 35, n. 1, p. 49–62, mar. 2010.

AMARANTE, L. DO; LIMA, J. D.; SODEK, L. Growth and stress conditions cause similar changes in xylem amino acids for different legume species. **Environmental and Experimental Botany**, v. 58, n. 1–3, p. 123–129, 2006.

AMARANTE, L.; SODEK, L. Waterlogging effect on xylem sap glutamine of nodulated soybean. **BIOLOGIA PLANTARUM**, v. 50, n. 3, p. 405–410, 2006.

ANDRADE, C. A. et al. Hydrogen peroxide promotes the tolerance of soybeans to waterlogging. **Scientia Horticulturae**, v. 232, p. 40–45, 17 fev. 2018.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019a.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019b.

ANJUM, S. A. et al. Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. **Frontiers in Plant Science**, v. 08, 6 fev. 2017.

ARBONA, V. et al. Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. **Physiologia Plantarum**, v. 132, n. 4, p. 452–466, abr. 2008.

AROCA, R.; PORCEL, R.; RUIZ-LOZANO, J. M. Regulation of root water uptake under abiotic stress conditions. **Journal of Experimental Botany**, v. 63, n. 1, p. 43–57, 1 jan. 2012.

ASHRAF, M. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (Panicum antidotale Retz.) under salinity or waterlogging. **Plant Science**, v. 165, n. 1, p. 69–75, 1 jul. 2003.

AUGE, G. A. et al. Adjusting phenotypes via within- and across-generational plasticity. **New Phytologist**, v. 216, n. 2, p. 343–349, 2017.

AVOLA, G. et al. Gas exchange and photosynthetic water use efficiency in response to light, CO2 concentration and temperature in Vicia faba. **Journal of Plant Physiology**, v. 165, n. 8, p. 796–804, 26 maio 2008.

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

AZIZIAN, A.; SEPASKHAH, A. R. Maize response to water, salinity and nitrogen levels: Physiological growth parameters and gas exchange. **International Journal of Plant Production**, v. 8, n. 1, p. 131–162, 2013.

BAILEY-SERRES, J. et al. Making sense of low oxygen sensing. **Trends in Plant Science**, v. 17, n. 3, p. 129–138, mar. 2012.

BAILEY-SERRES, J.; LEE, S. C.; BRINTON, E. Waterproofing Crops: Effective Flooding Survival Strategies. **Plant Physiology**, v. 160, n. 4, p. 1698–1709, dez. 2012.

BAILEY-SERRES, J.; VOESENEK, L. A. C. J. Flooding Stress: Acclimations and Genetic Diversity. **Annual Review of Plant Biology**, v. 59, n. 1, p. 313–339, jun. 2008.

BALAKHNINA, T. I. Plant Responses to Soil Flooding. In: **Stress Responses in Plants**. Cham: Springer International Publishing, 2015. p. 115–142.

BANSAL, R. et al. Waterlogging tolerance in black gram [Vigna mungo (L.) Hepper] is associated with chlorophyll content and membrane integrity. **Indian Journal of Biochemistry & Biophysics**, v. 56, p. 81–85, 2019.

BANSAL, R.; SRIVASTAVA, J. P. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. **Russian Journal of Plant Physiology**, v. 62, n. 3, p. 322–327, 29 maio 2015.

BANTI, V. et al. Low Oxygen Response Mechanisms in Green Organisms. **International Journal of Molecular Sciences**, v. 14, n. 3, p. 4734–4761, 27 fev. 2013.

BARICKMAN, T. C.; SIMPSON, C. R.; SAMS, C. E. Waterlogging Causes Early Modification in the Physiological Performance, Carotenoids, Chlorophylls, Proline, and Soluble Sugars of Cucumber Plants. **Plants**, v. 8, n. 6, p. 160, 8 jun. 2019.

BATISTA, T. B. et al. Condicionamento fisiológico e stress sob alta umidade e temperatura na qualidade fisiológica de sementes de Brachiaria brizantha cv. MG-5. **Acta Scientiarum - Agronomy**, v. 38, n. 1, p. 123–127, 1 jan. 2016.

BAUER, G. Increasing the endogenous NO level causes catalase inactivation and reactivation of intercellular apoptosis signaling specifically in tumor cells. **Redox Biology**, v. 6, p. 353–371, 1 dez. 2015.

BAXTER, A.; MITTLER, R.; SUZUKI, N. ROS as key players in plant stress signalling. **Journal of Experimental Botany**, v. 65, n. 5, p. 1229–1240, 1 mar. 2014.

BERTRAND, A. et al. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. **Journal of Experimental Botany**, v. 54, n. 388, p. 1721–1730, 2003.

BEUTLER, A. N. et al. Soil hydric excess and soybean yield and development in Brazil. **Australian Journal of Crop Science**, v. 8, n. 10, p. 1461–1466, 2014.

BIELESKI, R. L.; TURNER, N. A. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography.

**Analytical Biochemistry**, v. 17, n. 2, p. 278–293, 1 nov. 1966.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. **Plant Physiology**, v. 116, n. 2, p. 651–658, 1998.

BIN, T. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, maio 2010.

BLOKHINA, O. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. **Annals of Botany**, v. 91, n. 2, p. 179–194, 1 jan. 2003.

BLOKHINA, O.; FAGERSTEDT, K. V. Oxidative metabolism, ROS and NO under oxygen deprivation. **Plant Physiology and Biochemistry**, v. 48, n. 5, p. 359–373, maio 2010.

BOARD, J. E. Waterlogging effects on plant nutrient concentrations in soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, maio 2008a.

BOARD, J. E. Waterlogging Effects on Plant Nutrient Concentrations in Soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, 13 maio 2008b.

BOARETTO, L. F. et al. Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. **Plant Physiology and Biochemistry**, v. 74, p. 165–175, jan. 2014.

BONIFACIO, A. et al. Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. **Plant, Cell and Environment**, v. 34, n. 10, p. 1705–1722, 2011.

BORELLA, J. et al. Waterlogging-induced changes in fermentative metabolism in roots and nodules of soybean genotypes. **Scientia Agricola**, v. 71, n. 6, p. 499–508, dez. 2014.

BORELLA, J. et al. Hypoxia-driven changes in glycolytic and tricarboxylic acid cycle metabolites of two nodulated soybean genotypes. **Environmental and Experimental Botany**, v. 133, p. 118–127, 1 jan. 2017.

BORELLA, J. et al. Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. **Scientia Agricola**, v. 76, n. 1, p. 51–62, 1 fev. 2019.

BOTREL, A.; KAISER, W. M. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. **Planta**, v. 201, n. 4, p. 496–501, 9 abr. 1997.

BOTREL, A.; MAGNE, C.; KAISER, W. M. Nitrate reduction, nitrite reduction and ammonium assimilation in barley roots in response to anoxia. **Plant Physiology and Biochemistry**, v. 34, n. 5, p. 645–652, 1996.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 7 maio 1976.

BRAMLEY, H. et al. The contrasting influence of short-term hypoxia on the

hydraulic properties of cells and roots of wheat and lupin. **Functional Plant Biology**, v. 37, n. 3, p. 183, 2010.

BRANDÃO, A. D.; SODEK, L. Nitrate uptake and metabolism by roots of soybean plants under oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 21, n. 1, p. 13–23, 2009.

BRITTO, D. T.; KRONZUCKER, H. J. NH4+ toxicity in higher plants: a critical review. **Journal of Plant Physiology**, v. 159, n. 6, p. 567–584, jan. 2002.

BRUCE, T. J. A. et al. Stressful “memories” of plants: Evidence and possible mechanisms. **Plant Science**, v. 173, n. 6, p. 603–608, 1 dez. 2007.

BUI, L. T. et al. Conservation of ethanol fermentation and its regulation in land plants. **Journal of Experimental Botany**, v. 70, n. 6, p. 1815–1827, 27 mar. 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, 1 nov. 1991.

CAPONE, R.; TIWARI, B. S.; LEVINE, A. Rapid transmission of oxidative and nitrosative stress signals from roots to shoots in Arabidopsis. **Plant Physiology and Biochemistry**, v. 42, n. 5, p. 425–428, 1 maio 2004.

CATALDO, D. A. et al. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis**, v. 6, n. 1, p. 71–80, 1 jan. 1975.

CAVALCANTI, F. R. et al. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. **New Phytologist**, v. 163, n. 3, p. 563–571, set. 2004.

CENTRITTO, M. et al. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. **Journal of Experimental Botany**, v. 60, n. 8, p. 2325–2339, 1 maio 2009.

CHAMIZO-AMPUDIA, A. et al. Nitrate Reductase Regulates Plant Nitric Oxide Homeostasis. **Trends in Plant Science**, v. 22, n. 2, p. 163–174, 1 fev. 2017.

CHO, J. W.; YAMAKAWA, T. Effects on growth and seed yield of small seed soybean cultivars of flooding conditions in paddy field. **Journal of the Faculty of Agriculture, Kyushu University**, v. 51, n. 2, p. 189–193, 2006.

COLLAKU, A.; HARRISON, S. A. Losses in Wheat Due to Waterlogging. **Crop Science**, v. 42, n. 2, p. 444–450, 1 mar. 2002.

COLMER, T. D.; VOESENEK, L. A. C. J. C. J. Flooding tolerance: suites of plant traits in variable environments. **Functional Plant Biology**, v. 36, n. 8, p. 665, 2009.

CONRATH, U. et al. Priming for Enhanced Defense. **Annual Review of Phytopathology**, v. 53, n. 1, p. 97–119, 4 ago. 2015.

CORPAS, F. J.; RÍO, L. A. DEL; PALMA, J. M. Impact of Nitric Oxide (NO) on the ROS Metabolism of Peroxisomes. **Plants**, v. 8, n. 2, p. 37, 10 fev. 2019.

COSKUN, D.; BRITTO, D. T.; KRONZUCKER, H. J. The nitrogen-potassium intersection: membranes, metabolism, and mechanism. **Plant, Cell & Environment**, v. 40, n. 10, p. 2029–2041, 1 out. 2017.

COUTINHO, I. D. et al. Flooded soybean metabolomic analysis reveals important primary and secondary metabolites involved in the hypoxia stress response and tolerance. **Environmental and Experimental Botany**, v. 153, p. 176–187, set. 2018.

CRAFTS-BRANDNER, S. J.; HARPER, J. E. Nitrate Reduction by Roots of Soybean (Glycine max [L.] Merr.) Seedlings. **Plant Physiology**, v. 69, n. 6, 1982.

CRAWFORD, N. M. Nitrate: nutrient and signal for plant growth. **The Plant Cell**, v. 7, n. 7, p. 859–868, jul. 1995.

CRAWFORD, N. M.; FORDE, B. G. Molecular and Developmental Biology of Inorganic Nitrogen Nutrition. **The Arabidopsis Book**, v. 1, p. e0011, jan. 2002.

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, 1 ago. 2020a.

DA-SILVA, C. J.; DO AMARANTE, L. Time-course biochemical analyses of soybean plants during waterlogging and reoxygenation. **Environmental and Experimental Botany**, v. 180, p. 104242, 1 dez. 2020b.

DA-SILVA, C. J.; MODOLO, L. V. Hydrogen sulfide: a new endogenous player in an old mechanism of plant tolerance to high salinity. **Acta Botanica Brasilica**, v. 32, n. 1, p. 150–160, 19 out. 2017.

DA ROCHA, T. S. M. et al. Performance of soybean in hydromorphic and nonhydromorphic soil under irrigated or rainfed conditions. **Pesquisa Agropecuaria Brasileira**, v. 52, n. 5, p. 293–302, 1 maio 2017.

DE AZEVEDO NETO, A. D. et al. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. **Environmental and Experimental Botany**, v. 56, n. 1, p. 87–94, maio 2006.

DE OLIVEIRA, W. J. et al. Leaf gas exchange in cowpea and CO2 efflux in soil irrigated with saline water. **Revista Brasileira de Engenharia Agricola e Ambiental**, v. 21, n. 1, p. 32–37, 2017.

DE SOUSA, C. A. F.; SODEK, L. Alanine metabolism and alanine aminotransferase activity in soybean (Glycine max) during hypoxia of the root system and subsequent return to normoxia. **Environmental and Experimental Botany**, v. 50, n. 1, p. 1–8, 1 ago. 2003.

DECHORGNAT, J. et al. From the soil to the seeds: the long journey of nitrate in plants. **Journal of Experimental Botany**, v. 62, n. 4, p. 1349–1359, 1 fev. 2011.

DEMONGEOT, J.; HASGUI, H.; THELLIER, M. Memory in plants: Boolean modeling of the learning and store/recall memory functions in response to environmental stimuli. **Journal of Theoretical Biology**, v. 467, p. 123–133, 21 abr. 2019.

DIAB, H.; LIMAMI, A. Reconfiguration of N Metabolism upon Hypoxia Stress and Recovery: Roles of Alanine Aminotransferase (AlaAT) and Glutamate Dehydrogenase (GDH). **Plants**, v. 5, n. 2, p. 25, 31 maio 2016.

DIETZ, K.-J.; MITTLER, R.; NOCTOR, G. Recent Progress in Understanding the Role of Reactive Oxygen Species in Plant Cell Signaling. **Plant Physiology**, v. 171, n. 3, 2016.

DO AMARAL, M. N. et al. Long-term transcriptional memory in rice plants submitted to salt shock. **Planta**, v. 251, n. 6, p. 111, 1 jun. 2020.

DONAT, M. G. et al. More extreme precipitation in the world's dry and wet regions. **Nature Climate Change**, v. 6, n. 5, p. 508–513, 7 maio 2016.

EIGENBROD, F. et al. Vulnerability of ecosystems to climate change moderated by habitat intactness. **Global Change Biology**, v. 21, n. 1, p. 275–286, 1 jan. 2015.

EMBRAPA SOJA. **Soja - Portal Embrapa**. Disponível em: <https://www.embrapa.br/soja/cultivos/soja1>. Acesso em: 21 jul. 2020.

EVANS, D. E.; GLADISH, D. K. Plant Responses to Waterlogging. In: **Encyclopedia of Applied Plant Sciences**. Second Edi ed. [s.l.] Elsevier, 2017. v. 1p. 36–39.

FARNESE, F. S. et al. When Bad Guys Become Good Ones: The Key Role of Reactive Oxygen Species and Nitric Oxide in the Plant Responses to Abiotic Stress. **Frontiers in Plant Science**, v. 7, n. APR2016, 12 abr. 2016.

FEHR, W. R.; AND C. E, C. "Stages of soybean development" (1977). Special Report. 80. p. 1–12, 1977.

FEHR, W. R. et al. Stage of Development Descriptions for Soybeans, Glycine Max (L.) Merrill 1. **Crop Science**, v. 11, n. 6, p. 929–931, nov. 1971.

FLETA-SORIANO, E.; MUNNÉ-BOSCH, S. Stress Memory and the Inevitable Effects of Drought: A Physiological Perspective. **Frontiers in Plant Science**, v. 7, n. February, p. 1–6, 15 fev. 2016.

FORDE, B. G.; CLARKSON, D. T. Nitrate and Ammonium Nutrition of Plants: Physiological and Molecular Perspectives. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 30p. 1–90.

FOYER, C. H. et al. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. **Physiologia Plantarum**, v. 100, n. 2, p. 241–254, jun. 1997.

FUKAO, T. et al. Submergence and Waterlogging Stress in Plants: A Review Highlighting Research Opportunities and Understudied Aspects. **Frontiers in Plant Science**, v. 10, p. 340, 22 mar. 2019.

FURTADO, G. DE F. et al. Alterações fisiológicas em feijão-caupi irrigado com água salina e adubação nitrogenada. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 8, n. 3, p. 175–181, 25 out. 2013.

GARCIA, N. et al. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. **Environmental and**

**Experimental Botany**, v. 172, p. 103975, 1 abr. 2020.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, v. 59, n. 2, p. 309–14, 1 fev. 1977.

GIBBS, J.; GREENWAY, H. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. **Functional Plant Biology**, v. 30, n. 1, p. 1, 2003.

GILL, P. K. et al. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. **BULG. J. PLANT PHYSIOL.**, v. 27, n. 2, p. 72–84, 2001.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909–930, dez. 2010.

GITHIRI, S. M. et al. QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. **Plant Breeding**, v. 125, n. 6, p. 613–618, 1 dez. 2006.

GIUNTOLI, B.; PERATA, P. Group VII Ethylene Response Factors in Arabidopsis: Regulation and Physiological Roles. **Plant Physiology**, v. 176, n. 2, 2018.

GONÇALVES, M. et al. Correlations and path analysis of common bean grain yield and its primary components. **Cropp Breeding and Applied Biotechnology**, v. 3, n. 3, p. 217–222, 30 set. 2003.

GOOD, A. G.; CROSBY, W. L. Anaerobic Induction of Alanine Aminotransferase in Barley Root Tissue. **Plant Physiology**, v. 90, n. 4, p. 1305–1309, 1 ago. 1989.

GOOD, A. G.; MUENCH, D. G. Purification and Characterization of an Anaerobically Induced Alanine Aminotransferase from Barley Roots. **Plant Physiology**, v. 99, n. 4, p. 1520–1525, 1 ago. 1992.

GRAHAM, D.; SMYDZUK, J. Use of anthrone in the quantitative determination of hexose phosphates. **Analytical Biochemistry**, v. 11, n. 2, p. 246–255, 1 maio 1965.

GUPTA, K. J. et al. The form of nitrogen nutrition affects resistance against Pseudomonas syringae pv. phaseolicola in tobacco. **Journal of Experimental Botany**, v. 64, n. 2, p. 553–568, jan. 2013.

GUPTA, K. J. et al. Nitric Oxide Is Required for Homeostasis of Oxygen and Reactive Oxygen Species in Barley Roots under Aerobic Conditions. **Molecular Plant**, v. 7, n. 4, p. 747–750, abr. 2014.

GUPTA, K. J. et al. The role of nitrite and nitric oxide under low oxygen conditions in plants. **New Phytologist**, v. 225, n. 3, p. 1143–1151, 11 fev. 2020.

GUPTA, K. J. **Nitrogen Metabolism in Plants**. Springer S ed. New York, NY: Springer New York, 2020. v. 2057

GUPTA, K. J.; IGAMBERDIEV, A. U. Reactive Nitrogen Species in Mitochondria and Their Implications in Plant Energy Status and Hypoxic Stress Tolerance. **Frontiers in Plant Science**, v. 7, n. MAR2016, p. 369, 24 mar. 2016.

GUPTA, K. J.; KAISER, W. M. Production and scavenging of nitric oxide by barley root mitochondria. **Plant and Cell Physiology**, v. 51, n. 4, p. 576–584, abr. 2010.

GUPTA, K. J.; ZABALZA, A.; VAN DONGEN, J. T. Regulation of respiration when the oxygen availability changes. **Physiologia Plantarum**, v. 137, n. 4, p. 383–391, 2009.

GUPTA, P. et al. Signaling cross talk between biotic and abiotic stress responses in soybean. In: **Abiotic and Biotic Stresses in Soybean Production**. [s.l.] Elsevier, 2016. v. 1p. 27–52.

HANCOCK, J. T.; NEILL, S. J. Nitric oxide: Its generation and interactions with other reactive signaling compounds. **Plants**, v. 8, n. 2, 1 fev. 2019.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984a.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984b.

HARTMAN, S.; SASIDHARAN, R.; VOESENEK, L. A. C. J. **The role of ethylene in metabolic acclimations to low oxygenNew Phytologist**, 2019.

HASANUZZAMAN, M. et al. Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor. In: **Crop Stress and its Management: Perspectives and Strategies**. Dordrecht: Springer Netherlands, 2012. v. 9789400722p. 261–315.

HEMANTARANJAN, A. Flooding: Abiotic Constraint Limiting Vegetable Productivity. **Advances in Plants & Agriculture Research**, v. 1, n. 3, 23 jul. 2014.

HILKER, M. et al. Priming and memory of stress responses in organisms lacking a nervous system. **Biological Reviews**, v. 91, n. 4, p. 1118–1133, nov. 2016.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, mar. 2019a.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, 1 mar. 2019b.

HINCHA, D. K.; ZUTHER, E. Introduction: Plant cold acclimation and freezing tolerance. **Methods in Molecular Biology**, v. 1166, p. 1–6, 2014.

HOLZMEISTER, C. et al. Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. **Journal of Experimental Botany**, v. 66, n. 3, p. 989–999, 1 fev. 2015.

HOSSAIN, A.; UDDIN, S. N. Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. **Australian Journal of Crop Science**, v. 5, n. 9 SPEC. ISSUE, p. 1094–1101, 2011.

HSU, F.-C. et al. Insights into Hypoxic Systemic Responses Based on Analyses of Transcriptional Regulation in Arabidopsis. **PLoS ONE**, v. 6, n. 12, p. e28888, 15 dez. 2011.

IGAMBERDIEV, A. U. et al. NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. **Planta**, v. 219, n. 1, p. 95–102, 22 maio 2004.

IGAMBERDIEV, A. U. Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. **Journal of Experimental Botany**, v. 55, n. 408, p. 2473–2482, 24 set. 2004.

IGAMBERDIEV, A. U. et al. The Haemoglobin/Nitric Oxide Cycle: Involvement in Flooding Stress and Effects on Hormone Signalling. **Annals of Botany**, v. 96, n. 4, p. 557–564, 1 set. 2005.

IGAMBERDIEV, A. U.; HILL, R. D. Plant mitochondrial function during anaerobiosis. **Annals of Botany**, v. 103, n. 2, p. 259–268, jan. 2009.

IQBAL, N.; NAZAR, R. **Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies**. New Delhi: Springer India, 2016.

IRFAN, M. et al. Physiological and biochemical changes in plants under waterlogging. **Protoplasma**, v. 241, n. 1–4, p. 3–17, 12 maio 2010.

ISMOND, K. P. et al. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. **Plant Physiology**, v. 132, n. 3, p. 1292–1302, 1 jul. 2003.

JENNINGS, A. C. The determination of dihydroxy phenolic compounds in extracts of plant tissues. **Analytical Biochemistry**, v. 118, n. 2, p. 396–398, dez. 1981.

JIMÉNEZ S., J. D. LA C.; MORENO F., L. P.; MAGNITSKIY, S. Plant responses to stress due to flooding. A review. **Revista Colombiana de Ciencias Hortícolas**, v. 6, n. 1, p. 96–109, 4 fev. 2013.

JITSUYAMA, Y. Hypoxia-Responsive Root Hydraulic Conductivity Influences Soybean Cultivar-Specific Waterlogging Tolerance. **American Journal of Plant Sciences**, v. 08, n. 04, p. 770–790, 3 mar. 2017.

JUSTINO, G. C.; SODEK, L. Recovery of nitrogen fixation after short-term flooding of the nodulated root system of soybean. **Journal of Plant Physiology**, v. 170, n. 3, p. 235–241, fev. 2013.

KANCHEVA, V. D. Phenolic antioxidants – radical-scavenging and chain-breaking activity: A comparative study*. **European Journal of Lipid Science and Technology**, v. 111, n. 11, p. 1072–1089, nov. 2009.

KATO-NOGUCHI, H. Pyruvate metabolism in rice coleoptiles under anaerobiosis. **Plant Growth Regulation**, v. 50, n. 1, p. 41–46, 23 nov. 2006.

KENNEDY, R. A.; RUMPHO, M. E.; FOX, T. C. Anaerobic metabolism in plants. **Plant Physiology**, v. 100, n. 1, p. 1–6, 1 set. 1992.

KEUNEN, E. et al. A mutant of the Arabidopsis thaliana LIPOXYGENASE1 gene shows altered signalling and oxidative stress related responses after cadmium exposure. **Plant Physiology and Biochemistry**, v. 63, p. 272–280, fev. 2013.

KHAN, M. I. R.; KHAN, N. A. **Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress**. Singapore:

Springer Singapore, 2017.

KINOSHITA, T.; SEKI, M. Epigenetic Memory for Stress Response and Adaptation in Plants. **Plant and Cell Physiology**, v. 55, n. 11, p. 1859–1863, nov. 2014.

KISHOREKUMAR, R. et al. An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants. In: **Methods in Molecular Biology**. [s.l.] Humana Press Inc., 2020. v. 2057p. 1–13.

KOCH, K. E. Carbohydrate-modulated gene expression in plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 47, n. 1, p. 509–540, 1996.

KOLB, R. M.; JOLY, C. A. Flooding tolerance of Tabebuia cassinoides: Metabolic, morphological and growth responses. **Flora - Morphology, Distribution, Functional Ecology of Plants**, v. 204, n. 7, p. 528–535, 1 jan. 2009.

KOMATSU, S. et al. A comprehensive analysis of the soybean genes and proteins expressed under flooding stress using transcriptome and proteome techniques. **Journal of Proteome Research**, v. 8, n. 10, p. 4766–4778, 2009.

KOMATSU, S. et al. Identification of flooding stress responsible cascades in root and hypocotyl of soybean using proteome analysis. **Amino Acids**, v. 38, n. 3, p. 729–738, 31 mar. 2010.

KOMATSU, S.; HIRAGA, S.; YANAGAWA, Y. Proteomics Techniques for the Development of Flood Tolerant Crops. **Journal of Proteome Research**, v. 11, n. 1, p. 68–78, 15 jan. 2012.

KOMATSU, S.; NANJO, Y.; NISHIMURA, M. Proteomic analysis of the flooding tolerance mechanism in mutant soybean. **Journal of Proteomics**, v. 79, p. 231–250, fev. 2013.

KOMATSU, S.; SAKATA, K.; NANJO, Y. 'Omics' techniques and their use to identify how soybean responds to flooding. **Journal of Analytical Science and Technology**, v. 6, n. 1, p. 9, 4 dez. 2015.

KONNERUP, D. et al. Waterlogging tolerance, tissue nitrogen and oxygen transport in the forage legume Melilotus siculus: a comparison of nodulated and nitrate-fed plants. **Annals of Botany**, v. 121, p. 699–709, 2018.

KOPSELL, D. E. et al. Ratio of calcium to magnesium influences biomass, elemental accumulations, and pigment concentrations in kale. **Journal of Plant Nutrition**, v. 36, n. 14, p. 2154–2165, 6 dez. 2013.

KREUZWIESER, J.; RENNENBERG, H. Molecular and physiological responses of trees to waterlogging stress. **Plant, Cell & Environment**, v. 37, n. 10, p. n/a-n/a, 1 maio 2014.

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, 30 abr. 2013a.

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, 30 abr. 2013b.

KUMUTHA, D. et al. Effect of waterlogging on carbohydrate metabolism in pigeon pea (Cajanus cajan L.): Upregulation of sucrose synthase and alcohol dehydrogenase. **Plant Science**, v. 175, n. 5, p. 706–716, 2008.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017a.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017b.

LASA, B. et al. Role of glutamate dehydrogenase and phosphoenolpyruvate carboxylase activity in ammonium nutrition tolerance in roots. **Plant Physiology and Biochemistry**, v. 40, n. 11, p. 969–976, 1 nov. 2002.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011a.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011b.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011c.

LI, X. et al. Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. **Plant and Soil**, v. 352, n. 1–2, p. 377–387, 14 mar. 2012.

LI, X.; LIU, F. Drought Stress Memory and Drought Stress Tolerance in Plants: Biochemical and Molecular Basis. In: **Drought Stress Tolerance in Plants, Vol 1**. Cham: Springer International Publishing, 2016. p. 17–44.

LICAUSI, F.; PERATA, P. Low Oxygen Signaling and Tolerance in Plants. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 50p. 139–198.

LIMAMI, A. M.; DIAB, H.; LOTHIER, J. Nitrogen metabolism in plants under low oxygen stress. **Planta**, v. 239, n. 3, p. 531–541, 27 mar. 2014.

LIN, K.-H. R. et al. Study of the root antioxidative system of tomatoes and eggplants under waterlogged conditions. **Plant Science**, v. 167, n. 2, p. 355–365, ago. 2004.

LINKEMER, G.; BOARD, J. E.; MUSGRAVE, M. E. Waterlogging effects on growth and yield components in late-planted soybean. **Crop Science**, v. 38, n. 6, p. 1576–1584, 1998.

LIU, B.; RENNENBERG, H.; KREUZWIESER, J. Hypoxia induces stem and leaf nitric oxide (NO) emission from poplar seedlings. **Planta**, v. 241, n. 3, p. 579–589, 2014.

LIU, Z. J.; GUO, Y. K.; BAI, J. G. Exogenous hydrogen peroxide changes

antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. **Journal of Plant Growth Regulation**, v. 29, n. 2, p. 171–183, 23 jun. 2010.

LOGAN, B. A.; ADAMS, W. W.; DEMMIG-ADAMS, B. Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. **Functional Plant Biology**, v. 34, n. 9, p. 853, 24 set. 2007.

LORETI, E.; PERATA, P. The Many Facets of Hypoxia in Plants. **Plants**, v. 9, n. 6, p. 745, 12 jun. 2020.

LORETI, E.; STRIKER, G. G. Plant Responses to Hypoxia: Signaling and Adaptation. **Plants**, v. 9, n. 12, p. 1704, 3 dez. 2020.

LORETI, E.; VAN VEEN, H.; PERATA, P. **Plant responses to flooding stressCurrent Opinion in Plant Biology**Elsevier Ltd, , 1 out. 2016.

MAIA, L. B.; MOURA, J. J. G. How Biology Handles Nitrite. **Chemical Reviews**, v. 114, n. 10, p. 5273–5357, 28 maio 2014.

MANRIQUE-GIL, I. et al. Nitric oxide function during oxygen deprivation in physiological and stress processes. **Journal of Experimental Botany**, 25 out. 2020.

MARTINEZ-MEDINA, A. et al. Recognizing Plant Defense Priming. **Trends in Plant Science**, v. 21, n. 10, p. 818–822, out. 2016.

MAUREL, C.; VERDOUCQ, L.; RODRIGUES, O. Aquaporins and plant transpiration. **Plant Cell and Environment**, v. 39, n. 11, p. 2580–2587, 2016.

MELZER, J. M.; KLEINHOFS, A.; WARNER, R. L. Nitrate reductase regulation: Effects of nitrate and light on nitrate reductase mRNA accumulation. **MGG Molecular & General Genetics**, v. 217, n. 2–3, p. 341–346, jun. 1989.

MEN, S. et al. Effects of supplemental nitrogen application on physiological characteristics, dry matter and nitrogen accumulation of winter rapeseed (Brassica napus L.) under waterlogging stress. **Scientific Reports**, v. 10, n. 1, p. 10201, 23 dez. 2020.

MILLER, A. J. et al. Amino acids and nitrate as signals for the regulation of nitrogen acquisition. **Journal of Experimental Botany**, v. 59, n. 1, p. 111–119, 18 dez. 2007.

MIRA, M.; HILL, R. D.; STASOLLA, C. Regulation of programmed cell death by phytoglobins. **Journal of Experimental Botany**, v. 67, n. 20, p. 5901–5908, 1 out. 2016.

MITTLER, R. ROS Are Good. **Trends in Plant Science**, v. 22, n. 1, p. 11–19, 1 jan. 2017.

MITTLER, R.; BLUMWALD, E. The roles of ROS and ABA in systemic acquired acclimation. **Plant Cell**, v. 27, n. 1, p. 64–70, 2015.

MIYASHITA, Y. et al. Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. **The Plant Journal**, v. 49, n. 6, p. 1108–1121, 22 fev. 2007.

MODOLO, L. V. et al. Nitrite as the major source of nitric oxide production by Arabidopsis thaliana in response to Pseudomonas syringae. **FEBS Letters**, v. 579, n. 17, p. 3814–3820, 4 jul. 2005.

MOHN, M.; THAQI, B.; FISCHER-SCHRADER, K. Isoform-Specific NO Synthesis by Arabidopsis thaliana Nitrate Reductase. **Plants**, v. 8, n. 3, p. 67, 16 mar. 2019.

MORALES-OLMEDO, M.; ORTIZ, M.; SELLÉS, G. Effects of transient soil waterlogging and its importance for rootstock selection. **Chilean journal of agricultural research**, v. 75, p. 45–56, 1 ago. 2015.

MORARD, P. et al. Nitrate uptake and nitrite release by tomato roots in response to anoxia. **Journal of Plant Physiology**, v. 161, n. 7, p. 855–865, jul. 2004.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, dez. 2008a.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, 15 dez. 2008b.

MUSTAFA, G.; KOMATSU, S. Quantitative proteomics reveals the effect of protein glycosylation in soybean root under flooding stress. **Frontiers in Plant Science**, v. 5, n. NOV, p. 627, 18 nov. 2014.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 2015a.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 1 jan. 2015b.

NAKANO, Y.; ASADA, K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. **Plant and Cell Physiology**, v. 22, n. May, p. 867–880, 1981.

NEILL, S. J.; DESIKAN, R.; HANCOCK, J. T. Nitric oxide signalling in plants. **New Phytologist**, v. 159, n. 1, p. 11–35, 1 jul. 2003.

NUNES MENOLLI LANZA, L.; FERREIRA LANZA, D. C.; SODEK, L. Utilization of 15NO3- by nodulated soybean plants under conditions of root hypoxia. **Physiology and Molecular Biology of Plants**, v. 20, n. 3, p. 287–293, 2014.

OLIVEIRA, H. C.; FRESCHI, L.; SODEK, L. Nitrogen metabolism and translocation in soybean plants subjected to root oxygen deficiency. **Plant Physiology and Biochemistry**, v. 66, p. 141–149, maio 2013.

OLIVEIRA, H. C.; SODEK, L. Effect of oxygen deficiency on nitrogen assimilation and amino acid metabolism of soybean root segments. **Amino Acids**, v. 44, n. 2, p. 743–755, 19 fev. 2013.

OOSTERHUIS, D. M. et al. Physiological responses of two soybean [Glycine max (L.) Merr] cultivars to short-term flooding. **Environmental and Experimental**

**Botany**, v. 30, n. 1, p. 85–92, 1 jan. 1990.

PARRA, M. et al. Increasing plant vigour and tomato fruit yield under salinity by inducing plant adaptation at the earliest seedling stage. **Environmental and Experimental Botany**, v. 60, n. 1, p. 77–85, 1 maio 2007.

PEDÓ, T. et al. Physiological attributes, growth and expression of vigor in soybean seeds under soil waterlogging. **African Journal of Agricultural Research**, v. 10, n. 39, p. 3791–3797, 2015.

PERAZZOLLI, M. et al. Arabidopsis Nonsymbiotic Hemoglobin AHb1 Modulates Nitric Oxide Bioactivity. **The Plant Cell**, v. 16, n. 10, p. 2785–2794, 1 out. 2004.

PETERMAN, E. J. et al. Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. **Biophysical Journal**, v. 69, n. 6, p. 2670–2678, 1995.

PHUKAN, U. J.; MISHRA, S.; SHUKLA, R. K. Waterlogging and submergence stress: affects and acclimation. **Critical Reviews in Biotechnology**, v. 36, n. 5, p. 956–966, 2 set. 2016.

PLANCHET, E. et al. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. **The Plant Journal**, v. 41, n. 5, p. 732–743, 2 fev. 2005.

PODSĘDEK, A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. **LWT - Food Science and Technology**, v. 40, n. 1, p. 1–11, 1 jan. 2007.

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. 212, 1 dez. 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. 117, 18 jul. 2020.

PUCCIARIELLO, C. et al. Plant responses to flooding. **Frontiers in Plant Science**, v. 5, n. MAY, p. 226, 23 maio 2014.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. **Plant, Cell & Environment**, v. 40, n. 4, p. 473–482, 13 abr. 2017a.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. . 13 abr. 2017 b, p. 473–482.

PUYANG, X. et al. Antioxidant responses to waterlogging stress and subsequent recovery in two Kentucky bluegrass (Poa pratensis L.) cultivars. **Acta Physiologiae Plantarum**, v. 37, n. 10, p. 197, 8 out. 2015.

R CORE TEAM. **A Language and Environment for Statistical ComputingR Foundation for Statistical Computing**Vienna, AustriaR Foundation for Statistical Computing, , 2020. Disponível em: <https://www.r-project.org/>. Acesso em: 27 dez. 2020

RAHANTANIAINA, M.-S. et al. Glutathione oxidation in response to intracellular H 2 O 2 : Key but overlapping roles for dehydroascorbate reductases. **Plant Signaling & Behavior**, v. 12, n. 8, p. e1356531, 3 ago. 2017.

REDDY, A. S. N. et al. Coping with Stresses: Roles of Calcium- and Calcium/Calmodulin-Regulated Gene Expression. **The Plant Cell**, v. 23, n. 6, p. 2010–2032, 1 jun. 2011.

REGGIANI, R. et al. Accumulation and Interconversion of Amino Acids in Rice Roots under Anoxia. **Plant and Cell Physiology**, v. 29, n. 6, p. 981–987, 1 set. 1988.

REGGIANI, R.; BERTINI, F.; MATTANA, M. Incorporation of nitrate nitrogen in rice seedlings transferred to anaerobic conditions. **Amino Acids**, v. 13, n. 2, p. 183–188, jun. 1997.

RHINE, M. D. et al. Yield and nutritional responses to waterlogging of soybean cultivars. **Irrigation Science**, v. 28, n. 2, p. 135–142, 8 jan. 2010.

RICOULT, C.; CLIQUET, J.-B.; LIMAMI, A. M. Stimulation of alanine amino transferase (AlaAT) gene expression and alanine accumulation in embryo axis of the model legume Medicago truncatula contribute to anoxia stress tolerance. **Physiologia Plantarum**, v. 123, n. 1, p. 30–39, 1 jan. 2005.

ROBERTS, J. K. et al. Mechanisms of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. **Proceedings of the National Academy of Sciences of the United States of America**, v. 81, n. 11, p. 3379–3383, 1 jun. 1984.

ROCHA, M. et al. Glycolysis and the Tricarboxylic Acid Cycle Are Linked by Alanine Aminotransferase during Hypoxia Induced by Waterlogging of Lotus japonicus. **Plant Physiology**, v. 152, n. 3, p. 1501–1513, mar. 2010a.

ROCHA, M. et al. Analysis of alanine aminotransferase in various organs of soybean (Glycine max) and in dependence of different nitrogen fertilisers during hypoxic stress. **Amino Acids**, v. 39, n. 4, p. 1043–1053, 23 out. 2010b.

ROLLAND, F.; BAENA-GONZALEZ, E.; SHEEN, J. SUGAR SENSING AND SIGNALING IN PLANTS: Conserved and Novel Mechanisms. **Annual Review of Plant Biology**, v. 57, n. 1, p. 675–709, jun. 2006.

SAHA, R. et al. Physiological and biochemical changes in waterlog tolerant sesame genotypes. **SAARC Journal of Agriculture**, v. 14, n. 2, p. 31–45, 23 jan. 2017.

SAIRAM, R. K. et al. Physiology and biochemistry of waterlogging tolerance in plants. **Biologia plantarum**, v. 52, n. 3, p. 401–412, 1 set. 2008.

SAIRAM, R. K. et al. Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (Vigna radiata). **Journal of Plant Physiology**, v. 166, n. 6, p. 602–616, 1 abr. 2009.

SARKER, U.; OBA, S. Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. **Scientific Reports**, v. 8, n. 1, 1 dez. 2018.

SASIDHARAN, R.; VOESENEK, L. A. C. J. Ethylene-Mediated Acclimations to Flooding Stress 1. v. 169, n. September, p. 3–12, 2015.

SCHÖFFEL, E. R. et al. EXCESSO HÍDRICO SOBRE OS COMPONENTES DO RENDIMENTO DA CULTURA DA SOJA. **Ciência Rural**, v. 31, n. 1, p. 7–12, 2001.

SHARMA, P. et al. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. **Journal of Botany**, v. 2012, p. 1–26, 24 abr. 2012.

SHARMA, R. et al. Recent Advances in Dissecting Stress-Regulatory Crosstalk in Rice. **Molecular Plant**, v. 6, n. 2, p. 250–260, 1 mar. 2013.

SILVA, T. A. DA et al. Condicionamento fisiológico de sementes de soja, componentes de produção e produtividade. **Ciência Rural**, v. 46, n. 2, p. 227–232, fev. 2016.

SINGH, N.; BHATLA, S. C. Hemoglobin as a probe for estimation of nitric oxide emission from plant tissues. **Plant Methods**, v. 15, n. 1, p. 39, 23 dez. 2019.

SINGH, S. K.; RAJA REDDY, K. Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (Vigna unguiculata [L.] Walp.) under drought. **Journal of Photochemistry and Photobiology B: Biology**, v. 105, n. 1, p. 40–50, 5 out. 2011.

SOARES, M. M. et al. Estresse hídrico e salino em sementes de soja classificadas em diferentes tamanhos. **Pesquisa Agropecuaria Tropical**, 2015.

SOJKA, R.; SCOTT, H. Aeration Measurement. In: **Encyclopedia of Soil Science, Second Edition**. [s.l.] CRC Press, 2005.

SOSBAI, S. S.-B. D. A. I. **ARROZ IRRIGADO: Recomendações Técnicas da Pesquisa para o Sul do Brasil**. XXXII REUNIÃO TÉCNICA DA CULTURA DO ARROZ IRRIGADO. **Anais**...Farroupilha- RS- Brasil: 2018Disponível em: <http://www.sosbai.com.br/>. Acesso em: 15 abr. 2020

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002a.

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002b.

SOUZA, R. P. et al. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. **Environmental and Experimental Botany**, v. 51, n. 1, p. 45–56, fev. 2004.

SOUZA, S. C. R.; MAZZAFERA, P.; SODEK, L. Flooding of the root system in soybean: biochemical and molecular aspects of N metabolism in the nodule during stress and recovery. **Amino Acids**, v. 48, n. 5, p. 1285–1295, 29 maio 2016.

SPRINGER, B. et al. The Shrunken gene on chromosome 9 of Zea mays L is

expressed in various plant tissues and encodes an anaerobic protein. **MGG Molecular & General Genetics**, v. 205, n. 3, p. 461–468, dez. 1986.

STOIMENOVA, M. et al. The role of nitrate reduction in the anoxic metabolism of roots II. Anoxic metabolism of tobacco roots with or without nitrate reductase activity. **Plant and Soil**, v. 253, n. 1, p. 155–167, jun. 2003.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, 6 jun. 2007a.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, jul. 2007b.

SULLIVAN, M. et al. Evaluating On-Farm Flooding Impacts on Soybean. **Crop Science**, v. 41, n. 1, p. 93–100, 1 jan. 2001.

SUZUKI, N. et al. Abiotic and biotic stress combinations. **New Phytologist**, 2014.

SWEETLOVE, L. J. et al. Lactate metabolism in potato tubers deficient in lactate dehydrogenase activity. **Plant, Cell & Environment**, v. 23, n. 8, p. 873–881, 25 ago. 2000.

TAIZ, L.; ZEIGER, E. **Plant Physiology. 3rd edn.** [s.l: s.n.]. v. 3

TAKAHAMA, U. Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: Physiological significance of the oxidation reactions. **Phytochemistry Reviews**, v. 3, n. 1–2, p. 207–219, jan. 2004.

TAMANG, B.; FUKAO, T. Plant Adaptation to Multiple Stresses during Submergence and Following Desubmergence. **International Journal of Molecular Sciences**, v. 16, n. 12, p. 30164–30180, 17 dez. 2015.

TAN, W. et al. Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. **Photosynthetica**, v. 46, n. 1, p. 21–27, mar. 2008.

TANG, B. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, 1 maio 2010.

TESNIERE, C. et al. Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. **Journal of Experimental Botany**, v. 57, n. 1, p. 91–99, 2006.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016a. v. 2p. 23–40.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016b. v. 2p. 23–40.

TEWARI, S.; MISHRA, A. Flooding Stress in Plants and Approaches to Overcome. In: **Plant Metabolites and Regulation Under Environmental Stress**. [s.l.] Elsevier, 2018. p. 355–366.

THOMAS, A. L.; GUERREIRO, S. M. C.; SODEK, L. Aerenchyma Formation and Recovery from Hypoxia of the Flooded Root System of Nodulated Soybean. **Annals of Botany**, v. 96, n. 7, p. 1191–1198, 1 dez. 2005.

THOMAS, A. L.; SODEK, L. Amino acid and ureide transport in the xylem of symbiotic soybean plants during short-term flooding of the root system in the presence of different sources of nitrogen. **Brazilian Journal of Plant Physiology**, v. 18, n. 2, p. 333–339, jun. 2006.

THOMASHOW, M. F. Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. **Plant Physiology**, v. 154, n. 2, p. 571–577, 2010.

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, 1 jun. 2019a.

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, 1 jun. 2019b.

TIMILSINA, A. et al. Potential Pathway of Nitrous Oxide Formation in Plants. **Frontiers in Plant Science**, v. 11, 31 jul. 2020.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012a.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012b.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003a.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003b.

TREWAVAS, A. Aspects of Plant Intelligence. **Annals of Botany**, v. 92, n. 1, p. 1–20, 9 maio 2003.

TREWAVAS, A. Green plants as intelligent organisms. **Trends in Plant Science**, v. 10, n. 9, p. 413–419, 1 set. 2005.

TRIPATHI, B. N.; MÜLLER, M. **Stress Responses in Plants**. Cham: Springer International Publishing, 2015.

UDVARDI, M.; POOLE, P. S. Transport and Metabolism in Legume-Rhizobia Symbioses. **Annual Review of Plant Biology**, v. 64, n. 1, p. 781–805, 29 abr. 2013.

UNGER, P. W.; KASPAR, T. C. Soil Compaction and Root Growth: A Review.

**Agronomy Journal**, v. 86, n. 5, p. 759–766, 1 set. 1994.

UNITED STATES DEPARTMENT OF AGRICULTURE. **World Agricultural Production**. [s.l: s.n.].

VAN AMERONGEN, H.; CHMELIOV, J. Instantaneous switching between different modes of non-photochemical quenching in plants. Consequences for increasing biomass production. **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, v. 1861, n. 4, p. 148119, 1 abr. 2020.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015a.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015b.

VAN HANDEL, E. Direct microdetermination of sucrose. **Analytical Biochemistry**, v. 22, n. 2, p. 280–283, 1 fev. 1968.

VAN NGUYEN, L. et al. Mapping quantitative trait loci for root development under hypoxia conditions in soybean (Glycine max L. Merr.). **Theoretical and Applied Genetics**, v. 130, n. 4, p. 743–755, 1 abr. 2017.

VANDOORNE, B. et al. Long term intermittent flooding stress affects plant growth and inulin synthesis of Cichorium intybus (var. sativum). **Plant and Soil**, v. 376, n. 1, p. 291–305, 29 nov. 2014.

VELIKOVA, V.; YORDANOV, I.; EDREVA, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. **Plant Science**, v. 151, n. 1, p. 59–66, 2002.

VITOR, S. C.; SODEK, L. Products of anaerobic metabolism in waterlogged roots of soybean are exported in the xylem. **Plant Science**, v. 284, p. 82–90, 1 jul. 2019.

VOESENEK, L. Flooding tolerance : O 2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flooding tolerance: O2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flood adaptive traits and processes: an overview. **New Phytologist**, v. 206, n. 1, p. 57–73, 7 abr. 2015.

VRIET, C.; HENNIG, L.; LALOI, C. Stress-induced chromatin changes in plants: of memories, metabolites and crop improvement. **Cellular and Molecular Life Sciences**, v. 72, n. 7, p. 1261–1273, 13 abr. 2015.

WALTER, J. et al. Do plants remember drought? Hints towards a drought-memory in grasses. **Environmental and Experimental Botany**, v. 71, n. 1, p. 34–40, 1 abr. 2011.

WALTER, J. et al. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. **Environmental and Experimental Botany**, v. 94, p. 3–8, 1 out. 2013.

WANG, X. et al. Physiological and proteomic mechanisms of waterlogging priming improves tolerance to waterlogging stress in wheat ( Triticum aestivum L.). **Environmental and Experimental Botany**, v. 132, p. 175–182, dez. 2016.

WANG, X.; LIU, F.; JIANG, D. Priming: A promising strategy for crop production in response to future climate. **Journal of Integrative Agriculture**, v. 16, n. 12, p. 2709–2716, dez. 2017.

WANY, A. et al. Nitrate nutrition influences multiple factors in order to increase energy efficiency under hypoxia in Arabidopsis. **Annals of Botany**, v. 123, n. 4, p. 691–705, 14 mar. 2019.

WANY, A.; FOYER, C. H.; GUPTA, K. J. Nitrate, NO and ROS Signaling in Stem Cell Homeostasis. **Trends in Plant Science**, v. 23, n. 12, p. 1041–1044, 1 dez. 2018.

WANY, A.; KUMARI, A.; GUPTA, K. J. Nitric oxide is essential for the development of aerenchyma in wheat roots under hypoxic stress. **Plant Cell and Environment**, v. 40, n. 12, p. 3002–3017, 1 dez. 2017.

WEI, W. et al. Morpho-anatomical and physiological responses to waterlogging of sesame (Sesamum indicum L.). **Plant Science**, v. 208, p. 102–111, 1 jul. 2013.

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, n. 3, p. 307–313, 1994.

YEMM, E. W.; COCKING, E. C.; RICKETTS, R. E. The determination of amino-acids with ninhydrin. **The Analyst**, v. 80, n. 948, p. 209–214, 1 jan. 1955.

YETISIR, H.; MEHMET, E. C. Some physiological and growth responses of watermelon [ C itrullus lanatus ( Thunb .) Matsum . and Nakai ] grafted onto Lagenaria siceraria to flooding. **Environmental and Experimental Botany**, v. 58, p. 1–8, 2006.

YIU, J.-C. et al. Changes in antioxidant properties and their relationship to paclobutrazol- induced flooding tolerance in Welsh onion. **Journal of the Science of Food and Agriculture**, v. 88, n. 7, p. 1222–1230, maio 2008.

YORDANOVA, R. Y.; CHRISTOV, K. N.; POPOVA, L. P. Antioxidative enzymes in barley plants subjected to soil flooding. **Environmental and Experimental Botany**, v. 51, n. 2, p. 93–101, 2004.

YU, F. et al. Comparative proteomic analysis revealing the complex network associated with waterlogging stress in maize ( *Zea mays* L.) seedling root cells. **PROTEOMICS**, v. 15, n. 1, p. 135–147, 1 jan. 2015.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, fev. 2009a.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, 1 fev. 2009b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, p. 290–298, 2019a.

ZHANG, J. et al. Sedum mexicanum ‘Gold Mound’ exhibits better adaptive characters in contrast to S. spurium ‘Coccineum’ when subjugated to sustained waterlogging stress. **Acta Horticulturae**, v. 1263, n. 1263, p. 141–148, nov. 2019b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, n. 2, p. 290–298, 14 dez. 2019c.

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, 2017.

ZHANG, X. et al. Physiological and transcriptional analyses of induced post-anthesis thermo-tolerance by heat-shock pretreatment on germinating seeds of winter wheat. **Environmental and Experimental Botany**, v. 131, p. 181–189, nov. 2016.

ZHOU, W. et al. Plant waterlogging/flooding stress responses: From seed germination to maturation. **Plant Physiology and Biochemistry**, v. 148, p. 228–236, 1 mar. 2020.

# 4. Chapter III - Priming and nitrate supply alter photosynthesis, antioxidant activity and metabolism of leaves of soybean plants submitted to hypoxia.

## 4.1. Introduction

Varying climatic conditions are posing a potential threat to biodiversity (EIGENBROD et al., 2015), inevitably increasing the likelihood of extreme changes affecting biotic parameters and inducing abiotic stressors (TEWARI; ARORA, 2016a; TEWARI; MISHRA, 2018). Flooding or waterlogging is one of the abiotic factors that climate change models predict will increase the frequency worldwide (PUCCIARIELLO et al., 2014), making waterlogging stress a major environmental threat to plants. The low rate of oxygen diffusion in water induces oxygen deficiency of the soil environment resulting in a limitation of oxygen availability to plants and soil microbes (ZHANG et al., 2019b), plants experience oxidative stress (DA-SILVA; DO AMARANTE, 2020a), lack of oxygen for the functioning of the mitochondrial respiratory chain involved in energy production (WANY et al., 2019), triggering a rapid depletion of carbohydrate reserves and accumulation of phytotoxins (EVANS; GLADISH, 2017; PUCCIARIELLO; PERATA, 2017a), limitation of water uptake and nutrient absorption, closure of stomata, reductions in pigment levels (GARCIA et al., 2020), followed by decreased photosynthesis, stunted growth (ANDRADE et al., 2018; POSSO et al., 2018), reduced leaf size, wilting, necrosis and productivity (TRIPATHI; MÜLLER, 2015). Under these circumstances, plants developed several strategies to combat hypoxia-induced stress. These processes include a series of physiological, biochemical and anatomical changes (BAILEY-SERRES et al., 2012).

Soybean culture is vulnerable to waterlogging stress which limits drastically growth and yield in many regions of the world (TOUGOU et al., 2012a; VAN NGUYEN et al., 2017), especially during vegetative and seed germination stages (GITHIRI et al., 2006). The cultivation of soybean (*Glycine max* L. Merr.) in soils subjected to waterlogging or waterlogging worldwide is affected with low production and yields (SOSBAI, 2018). The soybean is one of the most important legume crops, as it contains a high amount of protein content and isoflavones, used to animal, human nutrition, and various industrial products. It has been estimated that up to a 25% reduction in soy crop yield is due to waterlogging

injuries in Asia, North America, and other regions of the world (Mustafa and Komatsu, 2014). Approximately the loss is estimated at an average of 40-80% of soybean production, depending on the stage of development (PHUKAN; MISHRA; SHUKLA, 2016).

The soil waterlogging is mostly due to the occurrence of flat topography (COLLAKU; HARRISON, 2002), high water tables and poor drainage of claylike soils (JITSUYAMA, 2017). Causing the suppression of $O_2$ to the submerged organs, leading to the triggering of hypoxic stress in plants. Thus, the survival of plant species or development of tolerance to $O_2$ deficiency depends on a series of adaptive mechanisms that occur in three stages. Initially, the plant rapidly induces a series of signal transduction components. This is followed by metabolic adaptations, involving the primary carbon and nitrogen routes and, finally, depending on the tolerance of the species, there is the development of morphological changes such as aerenchyma and/or adventitious root formation (BAILEY-SERRES; VOESENEK, 2008; KREUZWIESER; RENNENBERG, 2014; THOMAS; GUERREIRO; SODEK, 2005).

Waterlogged soil can reduce the photosynthetic yield of crop plants (POSSO et al., 2018). In addition, it can inhibit plant growth by decreasing dry matter accumulation and nitrogen content by altering plant metabolism and nutrient availability (MEN et al., 2020), inducing oxidative stress, accelerating leaf senescence and causing crop yield loss (VOESENEK; BAILEY-SERRES, 2013). An increase in redox potential between waterlogged soil and plants, fostered by hypoxia, leads to the production of reactive oxygen species (ROS), which are necessary for intracellular signalling, but as their concentration increases, they disrupt normal plant metabolism through oxidation of membrane lipids (which cause lipid peroxidation), protein, DNA, RNA and nucleosides (ARBONA et al., 2008; GILL; TUTEJA, 2010; SAIRAM et al., 2008). In view of the effects of ROS, plants have developed a complex antioxidant system composed of enzymes such as SOD, GPOD, APX and CAT (FUKAO et al., 2019; HASANUZZAMAN et al., 2012), and non-enzymatic scavengers, such as carotenoids. Nitrogen fertilization has important regulatory effects on growth and yield of soybean (BORELLA et al., 2019; GUPTA, 2020), it also acts as a stimulating factor regulating levels of endogenous hormones, increases NO production and ATP synthesis while

decrease e lactate and ethanol levels, improving waterlogging tolerance in soybean (DA-SILVA; DO AMARANTE, 2020a). In common bean plants, reported that $NO_3^-$ uptake it induces significant increases in RN activity in leaf and root even under waterlogging conditions (POSSO et al., 2020), improves photosynthetic yield, improves energy efficiency under hypoxia, induction genes involved in oxygen detection combined with increased efficiency of the Pgb-NO cycle and fermentation pathways (WANY et al., 2019). Furthermore, the application of an adequate amount of nitrogen fertiliser has been shown to increase the dry matter content of plants, chlorophyll, photosynthesis rate and yield ((CRAWFORD, 1995; FORDE; CLARKSON, 1999).

However, under natural growth conditions, plants are often exposed to more than one stress, either simultaneously or sequentially (TEWARI; MISHRA, 2018). The ability to efficiently capture information is essential for plant survival, plants have developed the capacity of "stress memory", i.e. the memory of the first (preparatory) stress and the recovery of the remembered information when faced with a second stress with a prolonged period without stress between the two stress events. (BRUCE et al., 2007; HILKER et al., 2016; HILKER; SCHMÜLLING, 2019a; MARTINEZ-MEDINA et al., 2016). This ability to prepare for improved responses to inducible stress has been shown to prepare a plant for increased resistance to repeated environmental stresses such as resistance to heat, cold, drought or osmotic stress that occur later (BAXTER; MITTLER; SUZUKI, 2014; THOMASHOW, 2010; WALTER et al., 2013).

Althoug waterlogging tolerance studies have been carried out with different plant species such as in maize (YU et al., 2015), soybean (ANDRADE et al., 2018; DA-SILVA; DO AMARANTE, 2020b; GARCIA et al., 2020; KOMATSU; NANJO; NISHIMURA, 2013), wheat (WANG et al., 2016) and leguminous *Melilotus siculus* (KONNERUP et al., 2018), there is little information on acquired tolerance by recurrent hypoxic stress and the effects when associated with nitrate supply.

The constant rise of the soybean commercial value in the international market and the increase of the cultivated area in a series of climatic conditions and soils, including waterlogged soils in many areas of the world (BEUTLER et al., 2014), motivates us to recognize and understand some of the possible

metabolic mechanisms that comprise the responses of tolerance acquirement to waterlogging by recurrent stress associated with nitrate supply in soybean, in order to improve the production and the yield of this important crop under constant climatic variation. Our previous research suggested that priming mechanisms in plants under waterlogging (V3R2 group) compared to those that were not primed (R2 group), several metabolic factors were enhanced, and that priming occurs independently of nitrate. In the present work, we have been exploring whether or not nitrate enhances priming of a waterlogging. The results could contribute to a better understanding of the main physiological mechanisms and biochemical events (such as photosynthetic parameters, ROS content and antioxidant activity, nitrate reduction and accumulation of amino acids and carbohydrates) in the genotype BR11-6042, which may be relevant for the selection of plants tolerant to transient soil waterlogging and lead to explore practices to mitigate the hypoxic stress.

## 4.2. Material and methods

### 4.2.1. Plant growth conditions and experimental design

The experiment was performed with soybean plants genotype PELBR11-6042 RR, (sensitive to waterlogging according to Embrapa Soybean Breeding Program), allowing us to easily investigate biochemical changes from hypoxia. Seeds were inoculated with *Bradyrhizobium japonicum* strain SEMIA 5079 (BIOAGRO) and sown in boxes of 1x1.2x1m (36 plants per box) containing soil from lowland areas (Typical Hydromorphic Eutrophic Planosol). Soil fertility was corrected with 350 kg $ha^{-1}$ of commercial formulation NPK 00-25-25 and pH was corrected to 6.0.

After germination, plants were cultivated under natural condition. The waterlogging was imposed on soybean plants at V3 and R2. stage of growth (see Fehr et al. 1971). The cultivated plants were divided into N-free waterlogging and N-waterlogged plants, with their respective control. In N waterlogged plants, nitrogen was applied (25.78 g $KNO_3$ diluted in 250 mL of water per box) three days before waterlogging and three days after waterlogging has started. The waterlogged plants were separated in four groups, two of them without nitrate supply waterlogged: V3R2 group (soybean plants initially waterlogged at V3

stage for five days and posteriorly for five days at R2 stage) and R2 group (waterlogging only at R2 stage for five days). The other two groups were supplied with nitrate prior and during waterlogging was performed: V3R2N group (waterlogged as V3R2 group associated to N supply) and R2N (waterlogged only at R2 stage associated with N supply). Waterlogging was performed by keeping the water at 2-3 cm above the soil surface, through a water valve to control the water flow during the stress period. Posteriorly, the boxes were drained and the plants were allowed to recover for five days.

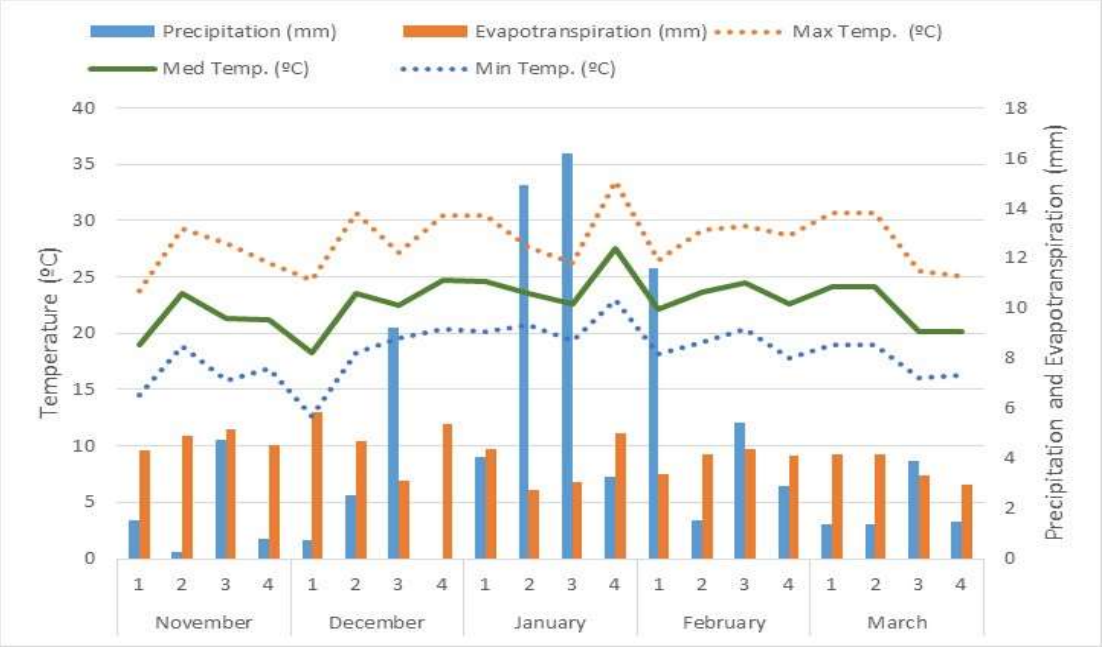


Figure 1. Minimum daily temperature (Min Temp), medium daily temperature (Med Temp) and maximum daily temperature (Max Temp) (lines) expressed in ºC, precipitation (blue bars) and evapotranspiration (orange bars) in mm, for the corresponding experimental period (2018/2019 agricultural year). Source: Agroclimatological Station Pelotas (Capão do Leão), Embrapa Temperate Climate Agricultural Research Center, Pelotas, RS, Brazil. Available at: http://agromet.cpact.embrapa.br/.

Three groups of plants were carried out as controls: a control group without N-$NO_3^-$ supply (Control) e two with N-$NO_3^-$ supply, one during periods of waterlogging at V3 and R2 stages (Control V3R2N) and the other, supplied with N only at R2 stage (Control R2N). In these groups, soil moisture was kept at field capacity during the whole growth period.

The role of intermittent stress in association with nitrate supply in the tolerance of plants to waterlogging in root system was investigated by sampling roots segments from treatments at two and five days of waterlogging stress and five days of recovery for analysis of levels of nitrate, nitrate reductase activity,

antioxidant enzyme activities, hydrogen peroxide and lipid peroxidation, fermentative enzymes activity and amino acids, total soluble sugars and sucrose. Samples of leaves (upper most expanded) from three plants were collected, carefully cleaned weighed and immediately stored at -86°C for subsequent biochemical analysis.

4.2.2. Gas exchange

Gas exchange was measured in the second uppermost mature leaf, fully expanded, with a Model LI-6400XT infrared portable $CO_2$ analyser (LI-COR, Inc.; Lincoln, NE, EUA), with measurements taken between 10:00 and 11:00 AM. The conditions of the leaf chamber were controlled so as to obtain a concentration of 380 µmol $CO_2$ $mol^{-1}$ and flux density of photosynthetically active photons of 1,500 µmol photons $m^{-2}$ $s^{-1}$. The following variables were measured: net assimilation rate of $CO_2$ (A); stomatal conductance (gs); transpiration rate (E); and internal $CO_2$ concentration (Ci). The IRGA data were used to calculate the intrinsic water use efficiency ($WUE_i$) (Eq. 1) and the instantaneous carboxylation efficiency ($CE_i$) (Eq. 2).

$WUE_i = A/gs$ (1)

$CE_i = A/Ci$ (2)

4.2.3. Photosynthetic pigments

Chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids were extracted from leaves using dimethyl sulfoxide as described by Wellburn (1994).

4.2.4. Nitrate concentration

Nitrate was extracted according to (BIELESKI; TURNER, 1966). Briefly, frozen leaves (0.250 g) were ground into a powder with liquid nitrogen, and the metabolites extracted for 24 h with 10 mL of a methanol:chloroform:water (12/5/3; v/v/v) solution. After centrifugation at 600 × *g* for 10 min, one volume of chloroform and 1.5 vol. of water were added to four volumes of the supernatant. The aqueous phase was collected after 24 h and used for the analysis. Nitrate was determined at 410 nm in plant tissue was performed according to the salicylic acid method (CATALDO et al., 1975).

### 4.2.5. Nitrate reductase activity

The activity nitrate reductase (NR; EC 1.6.6.1) was evaluated according to Modolo et al., (2005), with modifications. Leaves (0.5 g) were ground in the presence of 1 mL of 50 mM MOPS buffer (pH 7.5) containing 1 mM phenylmethanesulfonyl fluoride. Homogenate was centrifuged at 13,000 × g at 4 °C for 10 min and the supernatant collected for analysis. The supernatant was incubated for 30 min with an equal volume of 50 mM MOPS (pH 7.5) containing 1 mM EDTA, 10 mM $KNO_3$, and 1 mM NADH. The reaction was stopped by adding 1 M zinc acetate followed by centrifugation at 10,000 × g for 5 min. The supernatant was then homogenized with Griess reagent containing 0.5% (w/v) sulphanilamide prepared in 1.25% (v/v) $H_3PO_4$ and 0.5% (w/v) naphthyl ethylene-diamine dihydrochloride. The final supernatant was analyzed at 540 nm for determining the levels of nitrite. A standard curve was prepared using $NaNO_2$.

### 4.2.6. Antioxidant enzyme activity

Antioxidant enzymes were extracted according to Giannopolitis and Ries, (1977). Frozen leaves (0.25 g) were ground in liquid nitrogen and homogenized with 10% (w:w) polyvinylpolypyrrolidone (PVPP) in 1.5 mL of extraction buffer (100 mM phosphate buffer pH 7.8 containing 0,1 mM EDTA, 10 mM ascorbic acid). The homogenate was centrifuged at 12.000 × g for 20 min at 4 °C and the supernatant was collected for further analysis of SOD (Superoxide dismutase), CAT (Catalase), APX (Ascorbate peroxidase) and GPOD (guaiacol peroxidase). DHAR (Dehydroascorbate reductase) was extracted with 1 mL of 50 mM MES/KOH buffer (pH 6.0), containing 40 mM KCl, 2 mM $CaCl_2$, and 1 mM L-ascorbic acid (AsA) (Murshed et al., 2008). All enzyme extraction procedures were performed at 4º C.

The SOD (EC 1.15.1.1) activity was measured by the ability of enzyme to inhibit the photoreduction of nitroblue tetrazolium by 50% at 560 nm (GIANNOPOLITIS; RIES, 1977). The CAT (EC 1.11.1.6) activity was assessed by the decrease in absorbance at 240 nm, monitored by the consumption of $H_2O_2$. Specific activity was calculated using the molar extinction coefficient ε =36 $mM^{-1}$ $cm^{-1}$) (DE AZEVEDO NETO et al., 2006) The APX (EC 1.11.1.11) activity was assayed by monitoring of the rate of oxidation of ascorbate at 290 nm (NAKANO;

ASADA, 1981). Specific activity was calculated using the molar extinction coefficient ε = 2.8 $mM^{-1}$ $cm^{-1}$. The GPOD (EC 1.11.1.7) activity was assayed by monitoring the tetraguaiacol production at 470 nm. The DHAR (EC 1.8.5.1) activity was measured by the addition of DHA (freshly prepared) and the increase in the absorbance at 265 nm was monitored. Specific activity was calculated using the molar extinction coefficient ε =14 $mM^{-1}$ $cm^{-1}$ (MURSHED; LOPEZ-LAURI; SALLANON, 2008b). The protein levels were estimated by Bradford's assay using a BSA (bovine serum albumin) standard curve (BRADFORD, 1976).

4.2.7. Hydrogen peroxide and lipid peroxidation levels

The levels of $H_2O_2$ were determined according to Velikova et al. (2002). Leaves (0.250 g) were ground into powder in liquid nitrogen and homogenized in 0.1% (w/v) trichloroacetic acid and centrifuged at 13,000 × g at 4 °C for 20 min. Aliquots of 300 µL of supernatant were added to the reaction medium containing 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide. Samples were incubated at 30 °C for 30 min, and the absorbance was determined at 390 nm. The $H_2O_2$ concentrations were estimated based on a calibration curve. Lipid peroxidation was determined by measuring the levels of malonyldialdehyde (MDA) according to the method of CAKMAK; HORST (1991). Aliquots of the supernatant were added to the reaction medium containing 0.5% (w/v) thiobarbituric acid (TBA) and 10% (w/v) trichloroacetic acid (TCA), and then incubated at 90 $^oC$. After 20 min, the reaction was stopped in an CEi bath for 10 min. The absorbance was measured at 535 nm and 600 nm and the concentration of MDA-reactive TBA was calculated using the molar extinction coefficient (Ɛ) of 155 $mM^{-1}$ $cm^{-1}$.

4.2.8. Activity of fermentative pathway enzymes and alanine aminotransferase

Frozen leaves (0.250 *g*) were ground into a powder and extracted with 50 mM Tris-HCl (pH 7.5), containing 1 mM dithiothreitol and 3 % (w/w) PVPP. Plant extract homogenate was centrifuged at 12,000 × g at 4 °C for 20 min, and an aliquot of the supernatant was desalted using a PD-10 column (BORELLA et al., 2014).

The desalted leaves samples were collected for further analysis of LDH (Lactate dehydrogenase; EC 1.1.1.17), PDC (Pyruvate decarboxylase; EC 4.1.1.17), ADH (Alcohol dehydrogenase EC 1.1.1.1), and Ala-AT (Alanine aminotransferase; EC 2.6.1.2). All the enzymatic assays were performed by monitoring the oxidation of NADH at 340 nm. The LDH assay was monitored in pyruvate → lactate direction in a reaction mix containing 50 mM buffer Tris-HCl (pH 7.5), 0.6 mM NADH, 3.0 µM KCN, 0.2 mM 4-methylpyrazole and 10 mM Na-pyruvate to initiate the reaction (HANSON; JACOBSEN; ZWAR, 1984b). PDC and ADH activity were evaluate as described by Hanson et al. (1984). The reaction medium to determine PDC activity was composed by 50 mM MES buffer (pH 6.0), 0.2 mM NADH, 0.5 mM thiamine pyrophosphate, 1 mM magnesium chloride, 20 mM oxamic acid, 5 units of ADH, and 10 mM Na-pyruvate. ADH activity was determinate using 50 mM phosphate buffer (pH 7.0), 0.6 mM NADH, and 5.0 mM acetaldehyde. The Ala-AT activity was evaluated according to Sousa and Sodek (2003) using 10 mM L-alanine, 5 mM 2-oxoglutarate, 0.6 mM NADH, 50 mM Tris-HCl (pH 7.5), and 5 units of LDH (GOOD; MUENCH, 1992).

### 4.2.9. Amino acids, total soluble sugars and sucrose contents

The quantification of amino acids, total soluble sugars and sucrose was performed in the same extract obtained for nitrate (2.2 item).

The determination of total soluble amino acids (YEMM; COCKING; RICKETTS, 1955), total soluble sugars (GRAHAM; SMYDZUK, 1965) and the contents of sucrose was measured following the method of (VAN HANDEL, 1968).

### 4.2.10. Experimental design and statistical analysis

The experimental design was completely randomized consisting of four repetitions for each treatment and the experimental unit was considered three plants per box. Data were analysed by Shapiro-Wilk for normality test and by Levene's test to verify homoscedasticity, followed by ANOVA analysis. The post hoc comparisons were tested using a Tukey or *t*-test at the significance level of $p < 0.05$ lyses with RStudio software (R CORE TEAM, 2020).

## 4.3. Results

### 4.3.1. Leaf gas exchange parameters

The effect of waterlogging and waterlogging with nitrate supply on the rate of carbon assimilation *(A)* in soybean plants with priming stress, V3R2, and R2 is shown in Figure 2A. The V3R2 plants behave as if they were under normoxia at 2W of waterlogging, at five days decreased 1.3 fold compared with the control However the plants R2 reduced at 2W and 5W days of hypoxia reduced 3.0 and 2.5 folds, respectively compared to the control. Although V3R2 and R2 decreased under the influence of waterlogging, the differences with respect to time between 2W and 5W, for each of them were not significant, and the V3R2plants maintained *A* about two fold higher than the R2. At the re-oxygenation period, *A* of the V3R2 plants reached the control level and in R2 plants was 1.25 folds lower. Under waterlogged conditions with nitrate application, V3R2N plants decreased 1.5 and 1.17 folds the *A* compared to the controlCV3R2N at 2WN and 5WN days, respectively. In R2N plants, the rate of CO2 assimilation was strongly reduced by 2.74 and 1,23 folds at same period, 2WN and 5WN, compared to the CR2N. Although. *A* has decreased in V3R2N and R2N plants under the influence of $NO_3^-$, the differences with respect to time between 2WN and 5WN, for V3R2N were not significant, unlike in R2N increased markedly at 5WN. At reoxygenation period, the CO2 assimilation rate of the V3R2N and R2N reached the controls rates (with and without nitrate supply) (Fig. 2A).

Waterlogging effects on stomatal conductance *($g_s$)* values in V3R2 and R2 plants were contrasting. In V3R2 soybean plants, the gs values were similar to control levels at 2W and 5W, while in R2 decreased nine fold at 2W and kept the same value at 5W. Under re-oxygenation conditions (5R), $g_s$ in V3R2 was the same as the control and in R2 plants it was slightly lower than the control and V3R2. Under the influence of nitrate supply $g_s$ was similar to the controls, in V3R2N plants during the waterlogging periods and reoxygenation while in R2N group plants decreased near to 1,5 fold below V3R2N at 2WN and increased to a half this group at 5WNre not reaching the control without nitrate supply. At reoxygenation (5RN), plants from both groups show no difference in stomatal conductance, reaching the level of all controls (Fig. 2B).

Transpiration rate (E) variations during waterlogging were very similar to $g_s$ for the two groups of plants, V3R2 and R2, with and without nitrate supply. In V3R2 plants, E rates did not differ from control during waterlogging, and in R2 decreased to 13 fold at 2W and 10-fold at 5W. When plants were fed with nitrate, E in V3R2 plants were the same as controls with nitrate (V3R2N and R2N) during waterlogging but in comparison to control without nitrate, decreased at 2W and recovered the same rate at 5WN. Under re-oxygenation (5R), E in both groups, V3R2 and R2 were similar and had no difference from controls with or without nitrate supply (Fig. 2C).

The intercellular concentration of $CO_2$ (Ci) in V3R2 soybean plants, measured at 2W and 5W of hypoxia shown the same contents of control. However, in R2 plants, it decreased significantly,1.27 and 1.3 times at 2W and 5W, respectively less than the control and V3R2 plants, while at 5R, the Ci of the R2 and V3R2 groups were similar to the control. In plants with $NO_3^-$ supply- the Ci of V3R2 plants was equivalent to controls at 2WN and slightly higher than controls with nitrate at 5WN, while in plants of the R2N group the Ci was the same as controls and slightly lower than V3R2 and V3R2N at 5W. At re-oxygenation conditions the Ci in V3R2N group was similar or R2N group and higher than V3R2 and controls (Fig.2D).

The intrinsic water use efficiency (WUEi) showed differences in V3R2 and R2 soybean plants under waterlogging (Figure 3A). In V3R2 plants WUEi values kept as the control and were similar at 2W and 5W, as well as at 5R (reoxygenation). However, in R2 plants the WUEi (A/gs) increased by 4,62-fold at 2W and 3,9-fold at 5W in comparison to the control, and at 5 days of reoxygenation decreased to the control and V3R2 levels. Under supply with $NO_3^-$ WUEi was not altered during waterlogging in V3R2N kepping values closed to all controls at fifth day of stress. At 5RN the WUEi decreased in comparison to the controls (with and without nitrate feeding). While in R2N it increased significantly 1,5 folds on the second and fifth day of stress respectively but at levels below R2 (without nitrate supply). However, at 5RN it decreased to levels below R2N control and kept similar to other treatments.

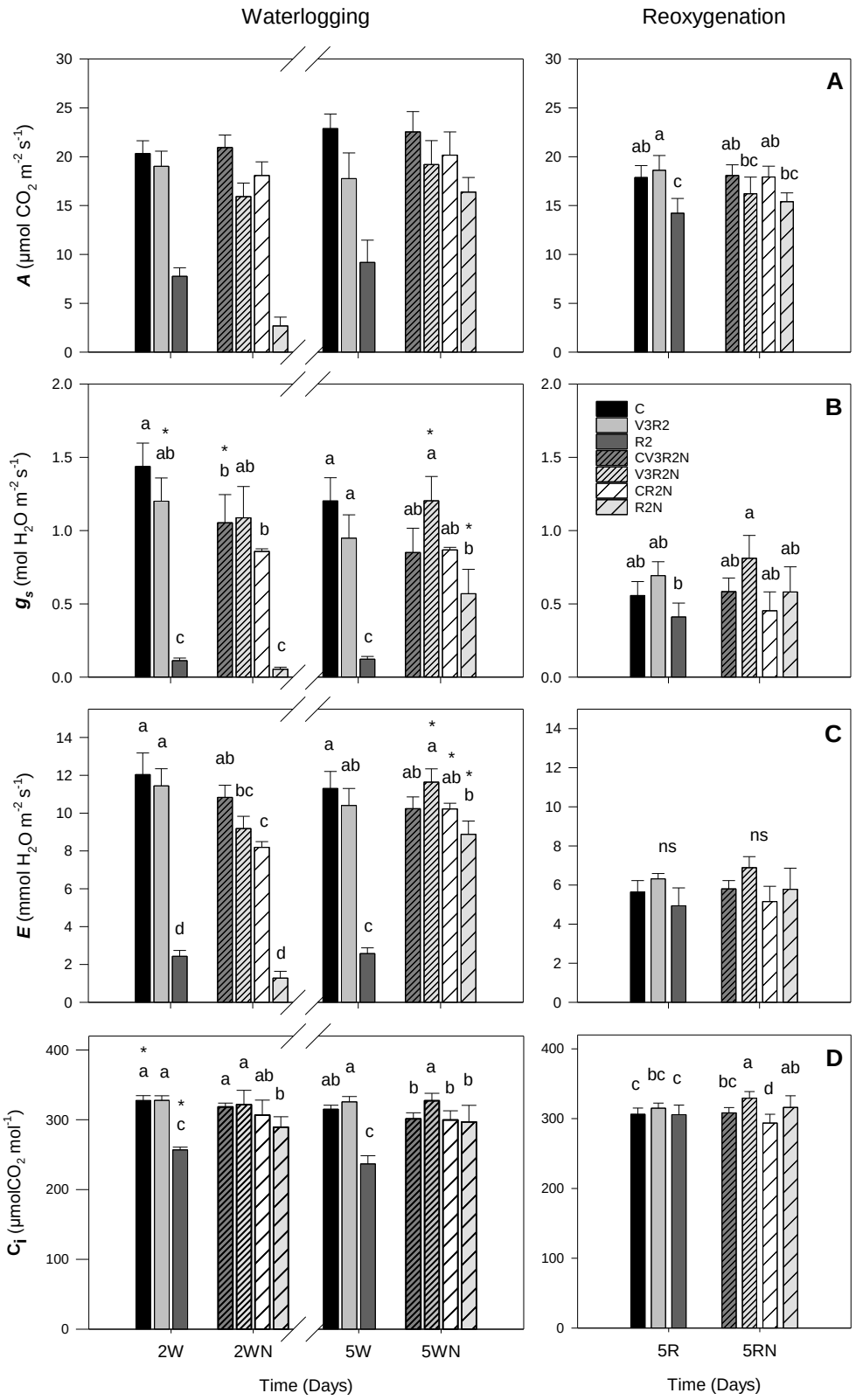


Figure. 2. Gas exchange in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

The efficiency of instantaneous carboxylation (CEi) reduced after five days of waterlogging in V3R2 plants, while at reoxygenation (5R) it recovered to the control levels. In contrast, in R2 plants the CEi decreased approximately two fold in comparison to the control at 2W and 5W, keeping levels below V3R2 plants. At reoxygenation, levels of CEi were not recovered, and maintained inferior of V3R2 levels (Fig. 3B). In plants supplied with nitrate, the CEi decreased in V3R2N plants (compared to controls without nitrate supply and CV3R2N) during waterlogging but reached similar levels at recovery, while for the R2N plants it was a drastic reduction (6,3 fold) when compared to the controls (supplied with nitrate or not) at 2WN and kept below (1,2 fold) at 5WN. At 5R, the CEi levels of R2N plants were inferior in comparison to CR2N control and equivalent to other treatments (Fig. 3B).

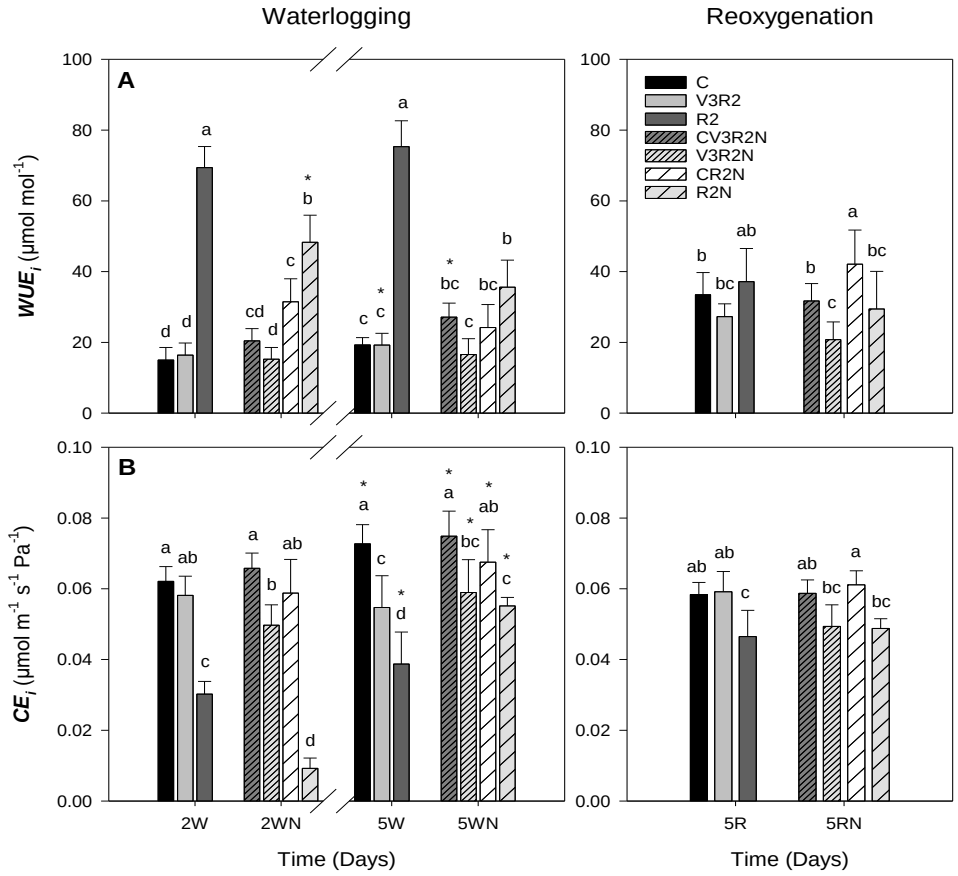


Figure 3. Intrinsic water use efficiency (WUEi) (A), and instantaneous carboxylation efficiency (CEi) (B) in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

4.2.1. Photosynthetic pigments

The influence of waterlogging in association with nitrate supply on the content of chlorophyll a *(Chl a)*, chlorophyll b *(Chl b)*, total carotenoids (*total Car*) and total chlorophylls (*total Chl*) of V3R2 and R2 plants are shown in Table 1. The level of *Chl a* in V3R2 plants decreased at 2W (1.5 fold) and 5W (1.7 fold), and in plant R2 decreased at 5W (1.2 fold) in comparison to control (without nitrate supply). After five days of reoxygenation the plants of the two groups reached control levels. However, the waterlogging with $NO_3^-$ supply influences the level of *"Chl a"* in V3R2N plants at 2W and 5W, registering levels equal to the control with nitrate, while in R2N plants the levels of *Chl a* initially was similar to the control at 2W, later it increased (1.5 fold) at 5W compared to the control CR2N. At re-oxygenation (5RN) the *Chl a* content in V3R2N plants did not recover controls supplied with nitrate (1,5 fold lower than CV3R2N) and the R2N plants managed to restore the controls levels (Table 1).

Chlorophyll *b* contents in the groups V3R2 and R2, subjected to waterlogging presented a variable response. In V3R2 plants they decreased at 5W to levels below controls and at 5R these plants achieve the same contents as the controls. However, in plants of the R2 group, the *Chl b*, was significantly higher than in the controls at 2W and had no difference at 5W. At 5R it increased to levels higher than in CV3R2N and CR2N. While in waterlogged plants with $NO_3^-$ supply, V3R2N plants showed no difference in *Chl b* content when compared to their respective controls, during hypoxia c and in reoxygenation. In R2N plants *Chl b* levels did not change compared to controls at 2W and were higher than control (without nitrate) and CR2N at 5W. Under reoxygenation levels were lower than control without nitrate and higher than V3R2CN (Table 1).

The influence of waterlogging on the total chlorophyll (*total Chl*) in V3R2 and R2 plants is shown in table 1. In V3R2 plants the levels of total *Chl* kept similar to controls at 2W and decreased significantly at 5W days. While in R2 plants total *Chl* levels maintained similar to controls during waterlogging (2W and 5W). However, in both groups of V3R2 and R2 plants under five days of reoxygenation, levels of total *Chl* were similar to the controls. Meanwhile, in waterlogged plants supplied with $NO_3^-$, the total Chl levels in V3R2N plants did not alter at 2W and 5W in comparison to the controls. In R2N levels kept

equivalent to controls at 2W and increased to contents higher than controls at 5W. Under reoxygenation total Chl decreased to control levels (Table 1).

Table 1. Chlorophyll a *(Chl a*), chlorophyll b (*Chl b*), total carotenoids (*total Car*) and total chlorophylls (*total Chl)* levels in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N) or V3 and R2 stage (V3R2N) or without nitrate feeding (R2; V3R2) and re-oxygenation. and re-oxygenation. Non-waterlogged plants with or without nitrate feeding were used as controls. Data are mean values ± SD (n = 4). The same lowercase letters within the same row and uppercase in the column indicate no significant difference according to Tukey test (P=0.05). W; WN= Waterlogging, R; RN= Reoxygenation.

| **Parameters** | **Time (d)** | **C** | **CV3R2N** | **CR2N** | **V3R2** | **V3R2N** | **R2** | **R2N** |
|---|---|---|---|---|---|---|---|---|
| **Chl a (mg·g$^{-1}$ FW)** | 2W | 1.09 aB | 1.16 a | 1.07 a | 0.74 bB | 1.10 aA | 1.25 aB | 1.20 aB |
| | 5W | 1.32 aA | 1.16 bc | 1.04 cd | 0.78 dB | 1.14 bcA | 1.12 bcB | 1.50 aA |
| | 5R | 0.99 bcB | 1.13 b | 1.07 b | 1.16 bA | 0.75 cB | 1.61 aA | 1.13 bB |
| **CV** | 11,11% | | | | | | | |
| **Chl b (mg·g$^{-1}$ FW)** | 2W | 0.80 b | 0.78 b | 0.88 b | 0.77 bA | 0.79 b | 0.84 a | 1.12 bA |
| | 5W | 0.83 b | 0.82 b | 0.92 ab | 0.58 cAB | 0.74 bc | 0.79 b | 1.11 aA |
| | 5R | 0.88 ab | 0.66 c | 0.74 b | 0.69 bcB | 0.69 bc | 0.92 a | 0.75 bB |
| **CV** | 12.12 % | | | | | | | |
| **Total Chl (mg·g$^{-1}$ FW)** | 2W | 1.89 ab | 1.94 ab | 1.95 ab | 1.51 bAB | 1.89 abA | 2.10 aB | 2.33 aA |
| | 5W | 2.15 b | 1.98 b | 1.96 b | 1.36 cB | 1.88 bA | 1.92 bB | 2.61 aA |
| | 5R | 1.87 b | 1.79 b | 1.81 b | 1.85 bA | 1.44 bB | 2.54 aA | 1.88 bB |
| **CV** | 10.64 % | | | | | | | |
| **Total Car (mg·g$^{-1}$ FW)** | 2W | 0.15 abA | 0.19 aA | 0.10 c | 0.05 dB | 0.05 dB | 0.08 cdB | 0.15 bA |
| | 5W | 0.13 aA | 0.11 aB | 0.12 a | 0.05 bB | 0.11 aA | 0.11 aA | 0.15 aA |
| | 5R | 0.07 cB | 0.08 bcC | 0.11 ab | 0.13 aA | 0.14 aA | 0.12 aA | 0.08 bcB |
| **CV** | 15.93 % | | | | | | | |

In relation to the total carotenoids (total Car) contents, the plants of the V3R2 group present a decrease (3 and 2.6 times) at 2W and 5W respectively, increasing at 5R (1.86 fold) in comparison to the control (without nitrate supply). While in plants of the R2 group subjected to waterlogging, it decreased (1.8 times) at 2W and increased to control levels at 5W and kept similar at 5R. While in plants with $NO_3^-$ supply, V3R2N group plants decreased (3.8 times) total Car at 2W, while at 5W showed content similar to the control, increasing by 1.8 times the control at reoxygenation (5R). In R2N plants at 2W total Car increased 1.5 times the CR2N content, later at 5W total Car content reduced to a level similar

to the control, while in reoxygenation R2N plants managed to reach similar levels of control and decreased compared to V3R2N plants by 1.8 folds.

4.2.2. Nitrate concentration and NR activity

The effect of the waterlogging on the $NO_3^-$ concentration in the leaves of V3R2 and R2 plants is shown in Figure 4A. In V3R2 plants the $NO_3^-$ levels at two days of stress decreased by 1.7 fold, later at five days of stress they increased significantly (2.1 fold) in relation to the control, meanwhile at recovery they were re-established to nitrate-free control levels. However, in R2 plants during waterlogging, the nitrate content has not presented significant difference when compared to nitrate-free control as well as at the reoxygenation period (5R). Under $NO_3^-$ application, in V3R2N plants the contents decrease significantly about 1,65 and 1,2 fold at two and five days of waterlogging, and kept 1,2 fold lower at reoxygenation period when compared to the CV3R2N. However, in the leaves of R2N plants nitrate supply did not change nitrate concentration during waterlogging in comparison to CR2N but at 5R (reoxygenation) levels increased by 1,7 fold.

Nitrate reductase (NR) activity revealed contrasting responses in leaves of non-supplied nitrate groups subjected to waterlogging. In V3R2 plants, NR activity was similar to non-treated nitrate control under hypoxia, increasing significantly at the reoxygenation 2.5 fold compared to the non-treated nitrate control. R2 plants increased 2,7 and 2 folds respectively at two and five days of hypoxia, compared to the control (without nitrate) and V3R2 plants and kept higher at 5R (1,8 fold higher than control). While in plants treated with $NO_3^-$, the NR activity in V3R2N plants increased 1,7 fold in comparison to V3R2N at two days of hypoxia and increased to similar activity at 5W. At reoxygenation NR activity increased 1,4 fold in comparison to CV3R2N. In R2N plants the NR activity increased significantly by 1,4 at 2W and increased to control levels at 5W. Under reoxygenation NR increased by 1,8 fold the CR2N level and reached an activity higher than in V3R2N plants and the controls during waterlogging and reoxygenation periods (Fig. 4B).

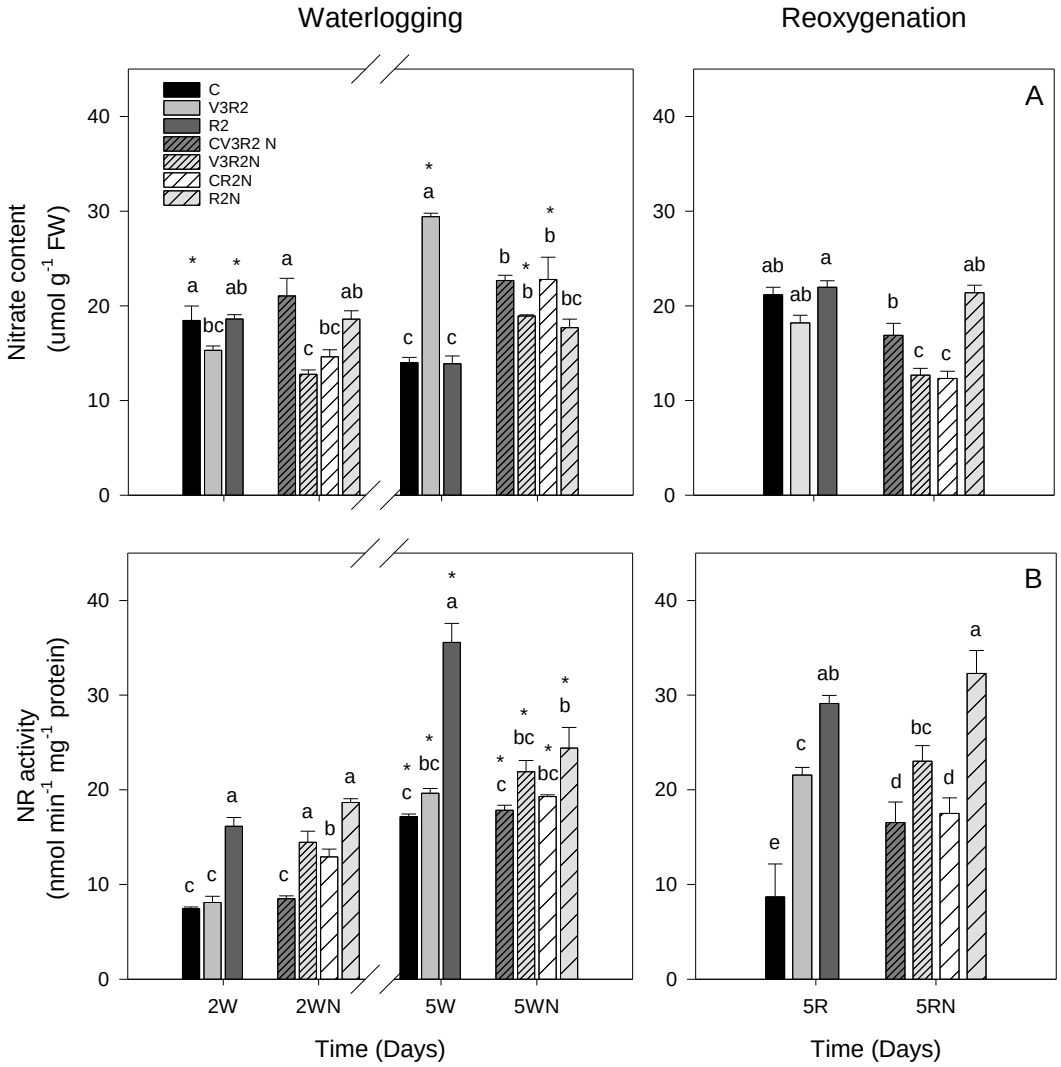


Figure 4. Levels of nitrate (A) and nitrate reductase (NR) activity (B) in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

## 4.2.3. Peroxide of hydrogen and lipid peroxidation.

The effect of hypoxia caused an increase in the levels of $H_2O_2$ and MDA in the leaves of soybean plants of the V3R2 and R2 groups, regardless of nitrate supply. In V3R2 plants the waterlogging resulted in significant increases by 1.6 and 1.16 fold, at two and five days of stress respectively, increasing also at 5R (reoxygenation period) by once fold compared to the nitrate non-treated control. In the waterlogged R2 plants the levels of $H_2O_2$ were high, increasing significantly by 1.5 and 1.42 fold at 2W and 5W, keeping higher than control at the

reoxygenation time (2-fold). R2 group presented higher levels of hydrogen peroxide than V3R2 at the end of waterlogging and reoxygenation. However, in the waterlogged plants supplied with nitrate, the V3R2N plants presented a significant increase during waterlogging in comparison to CV3R2N, reaching higher contents than V3R2 (nitrate non-treated plants) at 2W and decreasing to the same levels at 5W and 5R (reoxygenation period) when the levels were equivalent to the CV3R2N. While in the R2N plants, H2O2 levels remained similar to the CR2N control during waterlogging and at reoxygenation period, but kept significantly lower than R2 plants (nitrate non-treated) and showing the same level of V3R2 plants at 5W and 5R (Fig. 5A). Compared to soybean leaves under hypoxia, soybean leaves supplied with $NO_3^-$ produced lower levels of $H_2O_2$.

Lipid peroxidation increased significantly during hypoxia, and a significant difference was observed between treatments, indicating possible damages in membranes, mainly in nitrate non-treated plants (Fig. 5B). In V3R2 plants the MDA content was affected by the waterlogging, increasing by 3.6 times after two days of stress, and by 1.3 times after five days compared to the free-nitrate control, decreasing to control levels at re-oxygenation. While in R2 plants the amount of MDA increased significantly (4.7 and 1.4 folds) at two and five days of stress respectively, at five days of reoxygenation the MDA levels remained largely the same as nitrate non-treated control. However, the MDA content in V3R2N plants supplied as nitrate, increased 1.7 fold and 1.2 fold at two and five days of stress, respectively, in comparison to the CV3R2N control, when decreased to lower levels than V3R2 plants (nitrate non-treated). At five days of reoxygenation decreased in 1.4 fold the CV3R2N levels. MDA levels in R2N plants were similar to the CR2N control levels during waterlogging, but kept lower than R2 and equivalent to V3R2 levels, and at reoxygenation time the MDA content increased (1.5 fold) by above CR2N, reaching higher levels than V3R2N and treatments without nitrate (Fig. 5B).

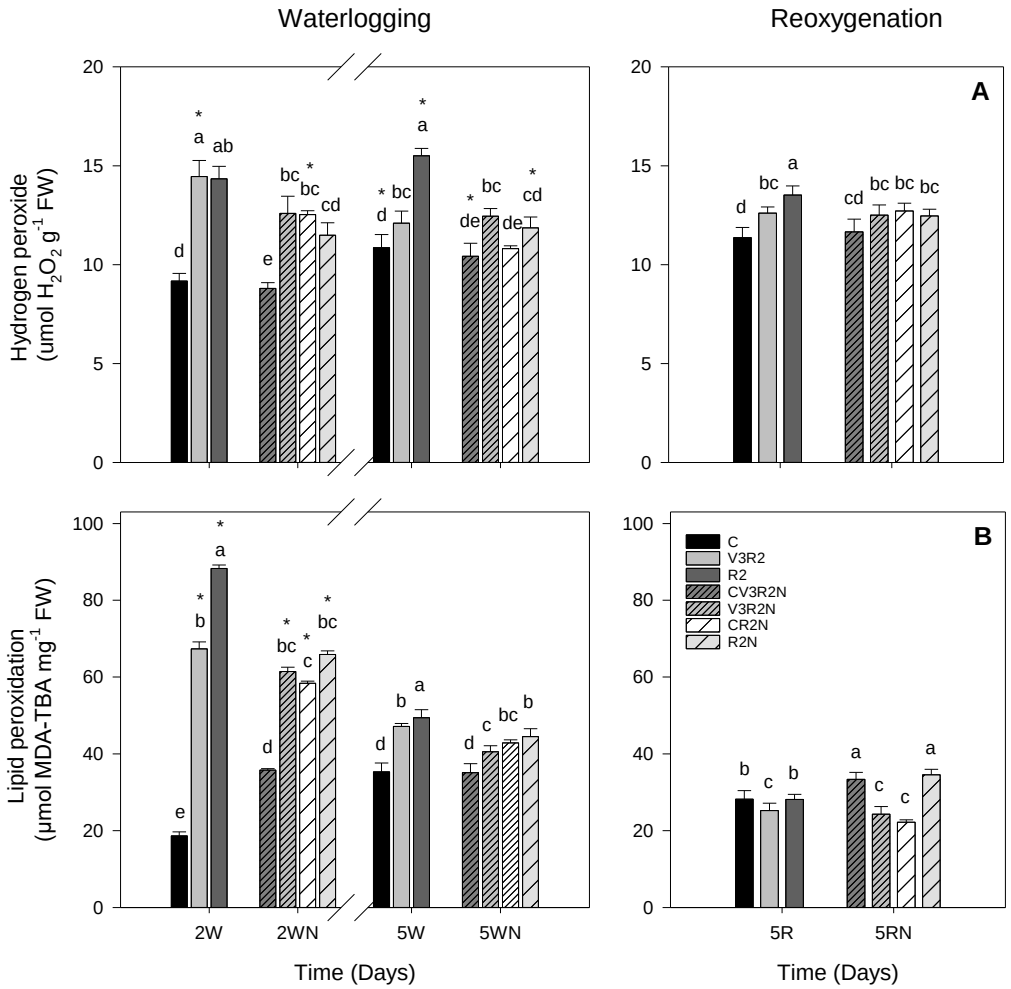


Figure 5. Levels of $H_2O_2$ (A) and lipid peroxidation (B) in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

### 4.2.4. Enzymatic antioxidant activity

The effect of root waterlogging on the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOD), ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR) in leaves of soybean plants of V3R2 and R2 groups with and without $NO_3^-$ supply, are shown in Figure 5. SOD activity in V3R2 leaves under waterlogging stress increased significantly by 1.3 and 1.4 fold, the control on days 2W, and 5W respectively, and at the reoxygenation decreased to levels similar to the control (non treated nitrate plants). While in R2 leaves it increased by 1.2-fold at 2W, decreasing 3-fold at 5W and 1.5 fold at reoxygenation when compared to the

nitrate non-treated control. However, the effect of $NO_3^-$ on SOD activity shows that in the case of V3R2N plants at 2W, SOD activity remained stable at levels similar to those of the control CV3R2N, and at 5W it decreased by 1.3-fold, reaching a level 3.5 fold below V3R2 group, and increased to CV3R2 levels at the reoxygenation (5RN). While in the R2N leaves SOD activity increased by 2-fold; decreasing 5-fold at 2WN and 5WN respectively in relation to the CR2N. At the reoxygenation time SOD activity in these plants reached the control (CR2N) levels. At the end of waterlogging (5W), SOD activity in R2N plants was lower than R2 and V3R2 and V3R2N groups. During waterlogging, SOD activity in R2 plants continued below V3R2 independently of nitrate supply (Fig. 6A).

CAT activity in V3R2 plants increased only at 5W of waterlogging and at five days of reoxygenation (1.6 fold) compared with the control (without nitrate supply), whereas in the R2 it did not show significant differences during waterlogging and reoxygenation. The effect of nitrate on CAT activity in V3R2N and R2N plants was an increase in both groups at the beginning of stress (2WN), followed by a decrease at 5W of stress and an increase at the reoxygenation (5RN) compared to their respective controls (CV3R2N and CR2N), reaching in the two groups, CAT activity levels higher in comparison to stressed plants not fed with nitrate (V3R2 and R2 groups) (Fig. 6B).

GPOD activity increased significantly at 5W and reoxygenation (5R) in V3R2 and R2 plants, reaching higher levels in R2 group. On the other hand, with the application of $NO_3^-$, the GPOD activity was variable between stressed groups, in V3R2N plants the GPOD activity decreased at 2WN (1,46 fold) and increased significantly (1.2 fold) at 5WN in comparison do CV3R2N, increasing at 5RN. In R2N plants, GPOD activity increased (2fold) at 2WN, decreasing to control (CR2N) levels at 5WN and below the control at 5RN (Reoxygenation). ls than CR2N (Fig. 6C).

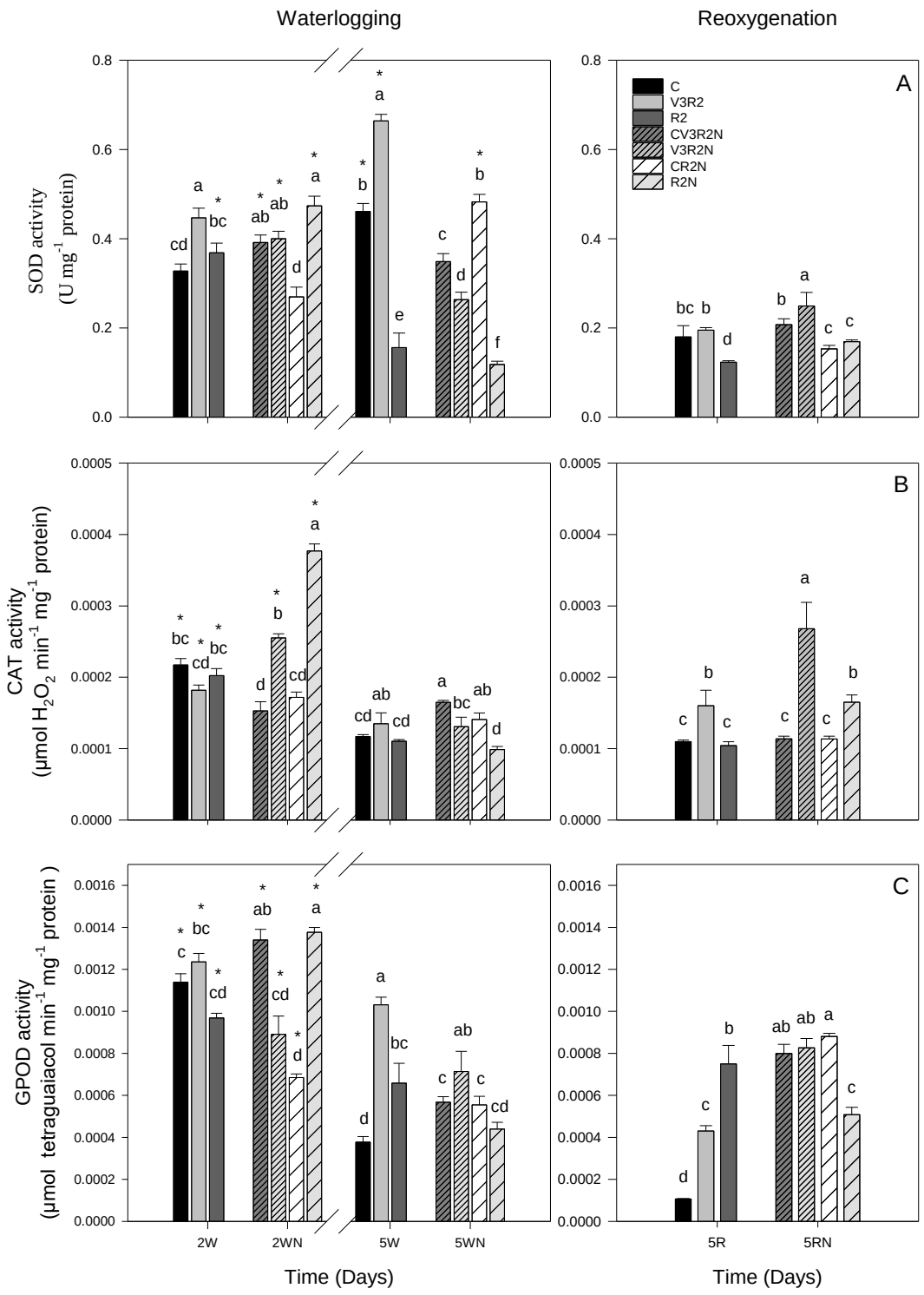


Figure 6**.** Superoxide dismutase (SOD) (A), catalase (CAT) (B) and guaiacol peroxidase (GPOD) (C) activities in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

APX activity increased 5.2 and 1.7 fold at 2W and 5W (days of waterlogging), decreasing 1.8 fold at the reoxygenation period compared with the control without nitrate. While in R2 plants the activity increased 1.9 fold at the beginning of the hypoxia (2W) decreasing to levels equivalent to control at 5W, and remained at control levels at the end of the reoxygenation period. The $NO_3^-$ supplied plants during waterlogging showed a variable response of the APX activity, so in V3R2N plants it remained at similar levels to the control at 2WN, decreased significantly at 5WN and reached control (CV3R2N) levels at 5RN (reoxygenation period). In R2N plants the APX activity remained at similar control (CR2N) levels during waterlogging and recovery (Fig. 7A).

DHAR activity in the leaves of waterlogged plants decreased in V3R2 and R2 plants. In V3R2 group DHAR activity decreased at 5W while in R2 plants, waterlogging inhibited the activity since 2W and remained constant at 5W. Under reoxygenation, activity levels of both groups of plants remained below control (non-treated nitrate). Under nitrate supply, DHAR activity decreased markedly in V3R2N during waterlogging by 2,6 fold at 2WN and 3 fold at 5WN in comparison to CV3R2N and remained below control at 5RN. In R2N group DHAR activity was similar to CR2N control during waterlogging and recovery periods. (Fig. 7B).

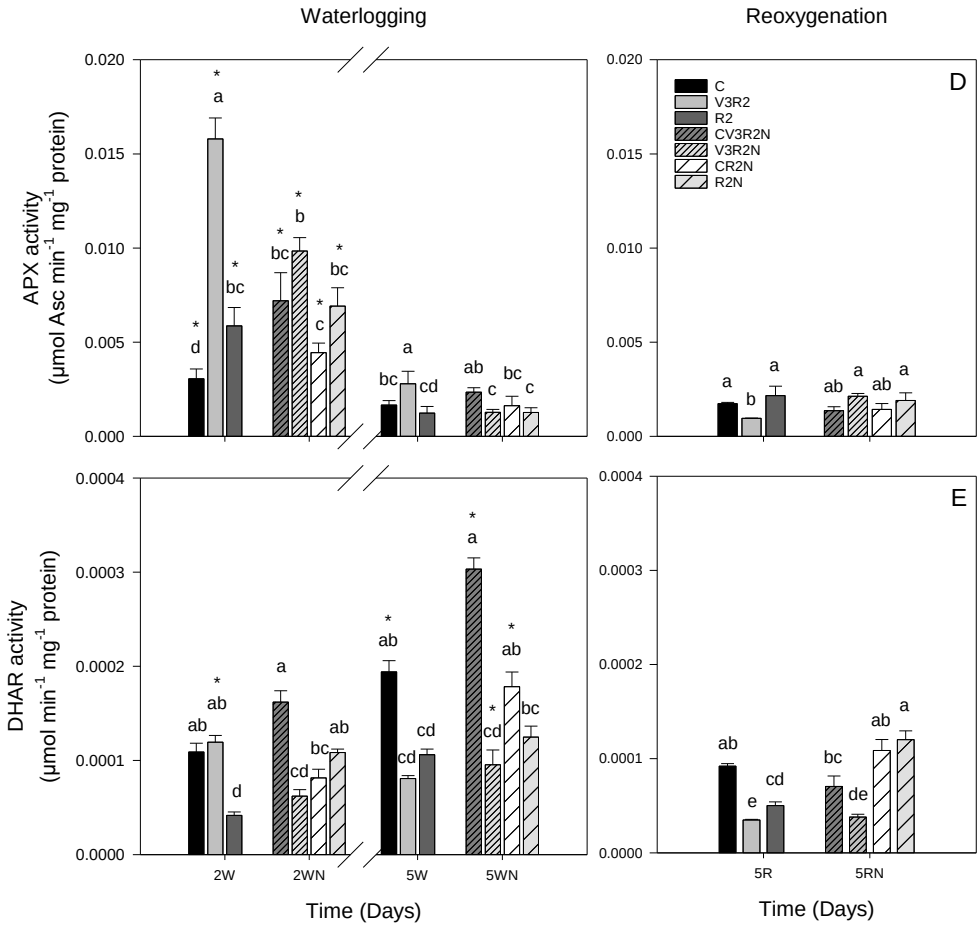


Figure 7**.** Ascorbate peroxidase (APX) (A) and dehydroascorbate reductase (DHAR) (B) activities in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

### 4.2.5. Amino acid and carbohydrates contents

Levels of total amino acids (TAA) total soluble sugars (TSS) and sucrose (SUC) in the leaves of soybean plants of the V3R2 and R2 groups subjected to waterlogging in association or not with $NO_3^-$ supply is shown in Figure 8.

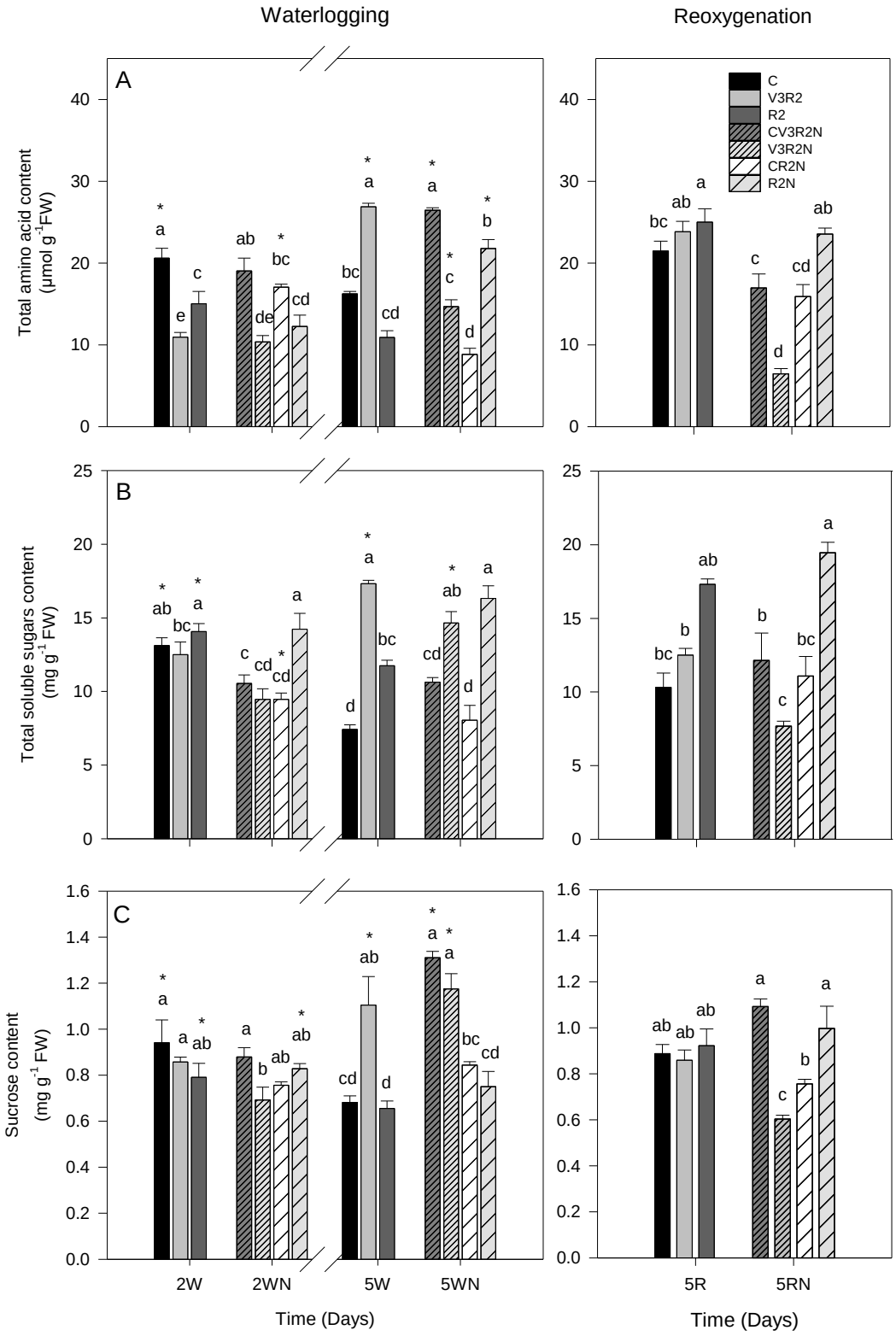


Figure 8. Levels of total amino acids (A), total soluble sugars (B) and sucrose (C) in leaves of soybean plants in response to waterlogging associated with nitrate feeding at R2 stage (R2N and V3R2N) or without nitrate supply (R2; V3R2) during two and five days (2W and 2WN; 5W and 5WN) of stress and five days of recovery (5R and 5RN). Controls with or without nitrate supply at the same periods of waterlogging were carried out. Values are mean ± SE, n = 4. Asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; $P < 0.05$) and distinct letters indicate significant differences between treatments for each waterlogging and reoxygenation time.

The concentration of TAA in the leaves of the waterlogged plants of the V3R2 group decreased at the beginning of the stress (1.88 fold), subsequently increased 1.65 fold at 5W, and reached the control levels at 5R (days of reoxygenation) in comparison to the non-treated nitrate control, while in the R2 leaves the TAA content decreased significantly (1.37 fold) during the whole waterlogging stress period, increasing at the reoxygenation stage (1.2 fold) when compared to the control. The effect of $NO_3^-$ supply in waterlogged V3R2N plants resulted in a significant decrease by 1.9 and 2.6 fold less than the control CV3R2N, on days 2WN, 5WN and 5RN, respectively. In R2N plants TAA levels increased significantly (2.46 fold) at 5WN of waterlogging stress, and remained higher (1.48 fold) than control (CR2N) during reoxygenation (5RN) (Fig.8A).

Total soluble sugar (TSS) levels for both V3R2 and R2 plants significantly increased during hypoxia. In V3R2 plants increased 2.3 fold at the 5W and reached the control level at 5R (reoxygenation period) in comparison to the control (without nitrate). While in the R2 plants, TSS levels increased 1,6 fold at 5W and at 5R increased 1.7 fold. However, in plants treated with $NO_3^-$, V3R2N group significantly increased TSS levels (1.4 fold) at 5 days of stress, decreasing at 5RN (reoxygenation) when compared to the control CV3R2N. Unlike, TSS levels in the R2N plants significantly increased by 1.46 and 2 fold at 2WN and 5WN days of waterlogging, respectively and 1.7 fold at 5RN compared to the control CR2N (Fig.8B).

The effects of waterlogging on sucrose (SUC) content in leaves of waterlogged plants, V3R2 and R2, were distinct. SUC levels in the V3R2 leaves increased by 1.6 fold at 5W, and at 5R reached levels similar to the control. In R2 plants under hypoxia SUC levels were not altered, remaining similar to those of the control at 5R. The waterlogged plants with $NO_3^-$ supply, V3R2N and R2N showed some differences during waterlogging and recovery. In V3R2N SUC decreased at 2W and increased at 5W reaching the same levels of CV3R2N and at 5RN, levels were markedly lower than CV3R2N (1.6 fold). In R2N group, SUC levels were equivalent to the CR2N control during waterlogging and increased at 5RN (1.3 fold) (Fig. 8C).

## 4.4. Discussion

Clearly, soil waterlogging or deeper immersion occurs when water enters the soil faster than it can drain under gravity, as presented in the soils of this experiment. The adverse effect of soil waterlogging on soybean growth has been pointed out by numerous studies attributing it to the lack of oxygen to root metabolism and the decrease in biological nitrogen fixation (AMARANTE; SODEK, 2006; BORELLA et al., 2017; GARCIA et al., 2020). Thus, two vital processes of the plant, respiration and photosynthesis, are affected (SASIDHARAN; VOESENEK, 2015) Nitrogen is one of the most important and key nutrient controlling many aspects of plant metabolism and development (DA-SILVA; DO AMARANTE, 2020a; GUPTA, 2020). It is very important to understand how metabolic pathways are modulated both under normal growth conditions and intermittent stress by waterlogging (one primed stress in vegetative phase V3 and another in reproductive phase R2), and the effects resulted from the application of $NO_3^-$ during the events of stress, under light and ambient temperature conditions.

### 4.4.1. Physiological parameters in plants of soybean V3R2 and R2 are sensitive to waterlogging and $NO_3^-$ supply

Waterlogging creates an environment where an excess of water limits aerobic respiration and photosynthesis, causing a decrease in the energy supply. Here, we show the involvement of stress memory associated to nitrate supply in the waterlogged roots of soybean plants and the possible extension of effects on the shoot to maintain the photosynthetic process by kepping the dynamic dissipation of photosynthetic energy (Figure.1). V3R2 soybean plants that received intermittent stress showed a slight decrease in the maintenance of the $CO_2$ assimilation rate, while $g_s$, *E* and *Ci* remained at the control levels. Similarly, in the waterlogged plants supplied with $NO_3^-$ (V3R2N) the variation in these parameters not changed. While in R2 plants they were severely inhibited during hypoxia and in plants supplied with nitrate (R2N) there was an improvement in *A*, $g_s$, *E* and *Ci* during waterlogging without reach the control levels. The maintenance of the nitrate-mediated photosynthetic process, may be related to the increase of NR activity in leaves (UDVARDI; POOLE, 2013) induced by nitrate

transported from soil (NUNES MENOLLI LANZA; FERREIRA LANZA; SODEK, 2014) (Fig. 3) avoiding the accumulation of reducers when stomata are closed (CAPONE; TIWARI; LEVINE, 2004; NEILL; DESIKAN; HANCOCK, 2003) (Fig. 1B) and decreasing oxidative damages (Fig. 4) in photosynthetic machinery (POSSO et al., 2020). Another important effect in plant roots, could be associated with the NO cycle operating in the NAD+ recycling and consequent plant tolerance against hypoxia stress (VAN DONGEN; LICAUSI, 2015b; WANY et al., 2019) , alleviating root acidification by decreasing fermentation (ZABALZA et al., 2009b) and allowing a better water uptake by roots and transport under hypoxia conditions (DA-SILVA; DO AMARANTE, 2020a) wich probably resulted in a better hydric status in the leaves of nitrate-treated plants. In the case of plants subjected to intermittent stress, V3R2, we believe that they developed compensation mechanisms triggered in the first stress such as improved water absorption by the roots and maintenance of water potential during the hypoxia, which may have contributed to keep $g_s$ and *E* similar to those of control. Carotenoids probably acted as an antioxidant defence protecting the photosynthetic apparatus from ROS (LI et al., 2012) and may have been an important antioxidant defence for V3R2N. The lower content of photosynthetic pigments, as well as decreases in A, may be caused by the accumulation of ROS (Fig. 1 A, 4A), mainly in R2 plants. The reduced stomatal conductance (Figure 1B) was probably caused by an increase in $H_2O_2$ content and lactate in roots, causing cell acidification that may inhibit the activity of aquaporins or water channel proteins, reducing hydraulic conductivity and affecting directly the stomatal conductance (MAUREL; VERDOUCQ; RODRIGUES, 2016). Besides that, the decrease in stomatal conductance, limited the diffusion of CO2 from the air into the carboxylation sites of Rubisco enzyme ((BANSAL; SRIVASTAVA, 2015; POSSO et al., 2018), and reduced $CO_2$ fixation (Fig. 1B) in R2 plants, which resulted in a decrease of intercellular $CO_2$ concentration (Ci; Fig. 1D) which could be associated to the additional effect of a reduction in the respiration rate though $CO_2$.

Additionally, the content of $H_2O_2$ increased in the leaves of all groups under waterlogging, being higher in the R2 plants, which probably had less water absorption, and less maintenance of the photosynthetic apparatus that are the

main factors that limit plant growth (BARICKMAN; SIMPSON; SAMS, 2019). For waterlogged R2 plants without and with nitrate application, photosynthetic activity decreased (MUTAVA et al., 2015b). However, during waterlogging, nitrate supplied plants (R2N) restored partially A at same level of V3R2 and V3R2N, suggesting that nitrate supply could compensate at least in part, the waterlogging tolerance mechanisms developed by V3R2 group.

CHO; YAMAKAWA (2006) reported that soybean leaves and branches are the first parts to respond to flooding stress. The decrease in photosynthetic activity with longer exposure to waterlogging may be caused by the reduction in chlorophyll and transpiration (TEWARI; ARORA, 2016a). In fact, in these plants a visible yellowing of the leaves was not during the waterlogging, which indicates a degradation of the chlorophyll. Reductions in chlorophyll content may be related to nitrogen deficiency. Since N2 fixation is sensitive to the low $O_2$ condition that occurs during hypoxia, soybean nodules have shown impaired N2 fixation activity when submitted to waterlogging (JUSTINO; SODEK, 2013; SOUZA; MAZZAFERA; SODEK, 2016). Under these conditions, a change in N metabolism has been observed (SOUZA; MAZZAFERA; SODEK, 2016) and in the export of N2 fixation products in xylem (AMARANTE; LIMA; SODEK, 2006). In addition, carotenoid content also decreased. R2N plants showed normal levels of *chl a*, *chl b* and carotenoids under hypoxia. The carotenoids probably acted as an antioxidant defence protecting chlorophylls and the photosynthetic apparatus from ROS (BANSAL; SRIVASTAVA, 2015; LI et al., 2012)and may have been an important antioxidant defence role for V3R2N, R2, R2N groups.

### 4.4.2. WUEi and CEi are sensitive to waterlogging and $NO_3^-$ supply

Despite the reductions in stomatal conductance and internal $CO_2$ concentration, which are denominators in the determination of WUEi and CEi, respectively, these variables behaved differently. WUEi uses, as denominators, variables related to the passage of water through the leaf. The CEi has, as denominator, a variable related to the $CO_2$ flow. This occurs because the concentration gradient between the plant and the atmosphere is much stronger for water than for $CO_2$, and $CO_2$ diffuses more

slowly because it finds greater resistance in the plant, causing the variables related to water suffer greater reductions compared to those related to $CO_2$.

The V3R2 plants submitted to waterlogging during vegetative and reproductive stages did not present alterations in their intrinsic efficiency of water use (WUEi); the $g_s$ also remained without alteration (Fig. 1B; 2A), which suggests that these plants developed a mechanism of water absorption that could be favoured by high activity of the aquaporins, presented an osmotic adjustment that enable a greater loss of water, i.e., the water potential behaved the same as a plant under normoxia (Figure 2A). On the other hand, the nitrate supply in V3R2N plants had no influence on the intrinsic WUE (WUEi) during the whole experiment, when compared to V3R2 plants.

The instantaneous carboxylation efficiency (*CEi*) in soybean plants under the increase of the hypoxia days, of group not supplied with nitrate (V3R2) or treated with nitrate (V3R2N) had no reduction but decreased similarly in relation to the control (not treated with nitrate). The same response occurred in both groups during reoxygenation (Fig. 2B). The *CEi* is closely related to stomatal opening and closure. Stomatal conductance was observed to be similar to that control plant, as well as photosynthesis; therefore, there was no stomatal closure, which allowed a greater diffusion of $CO_2$ to the substomatal chamber and, as a consequence, a higher internal concentration of $CO_2$ (Fig. 1D). This response of V3R2 plants may be related to the “preparatory stress” at V3 stage ( Fig. S1), their behaviour differed strongly from R2 plants, which suggests that they developed a “stress memory” (LÄMKE; BÄURLE, 2017b).

In the case of waterlogged R2 plants, the reduction of the stomatal conductance (Fig.1B), resulted in a lower loss of water by the plant due to the stomata closure, which hindered water absorption by the roots due to acidosis and the decrease in the activity of the aquaporins and the decrease of the leaf water potential. As a consequence of the decrease in stomata conductance and a lower $CO_2$ fixation, a significant increase in the WUEI was observed (Fig. 1A, B; 2B). AZIZIAN; SEPASKHAH (2014) observed an increase in intrinsic water use efficiency values by growing corn in saline water. In *Panicum antidotale* under flooding and salinity conditions, higher values of intrinsic water use efficiency were observed for salinity conditions (ASHRAF, 2003)However, in R2N

plants, as observed, $NO_3^-$ helped increase the net assimilation rate of photosynthesis (Fig. 1A), although it continued to be lower, it also increased the stomatal conductance (Fi. 1B), favouring the increase of WUEi (Fig. 2A), which was lower than R2 plants under waterlogging. As it has been observed, the increase in the time (days) of waterlogging caused a decrease in transpiration and photosynthesis rates due to the closure of the stomata; however, the effect of the hypoxia caused greater reductions in the stomatal conductance than in the net photosynthesis, which resulted in higher WUEi values, favouring the increase of WUEI values with the increase of waterlogging in R2 plants. From a physiological perspective, a high WUEi value is considered to be a mechanism of improvement that improves the productivity and survival of waterlogged plants (CENTRITTO et al., 2009).

The instantaneous carboxylation efficiency (*CEi*) in R2 plants, decreased markedly during waterlogging (Fig. 2B). The *CEi* variations, which is closely related to the opening and closing of the stomata, resulted from the reduction of the stomatal conductance that caused decreases of photosynthesis (Fig. 1A, B); the closing of the stomata also caused less diffusion of $CO_2$ into the sub stomatal chamber and, as a consequence, a lower internal concentration of $CO_2$ (Fig. 1D). Although there was a decrease in both variables, the reduction of the internal $CO_2$ concentration was lower than that of net photosynthesis, which explains the decrease of *CEi* (Fig. 1A, D; 2B). Similar behaviour was shown by R2N plants (supplied with nitrate), although they were lower than R2 at the beginning of waterlogging. These results (of R2N plants) are related to studies carried out by SOARES et al (2015), and FURTADO et al (2013) and DE OLIVEIRA et al (2017) which observed a reduction in the efficiency of instantaneous carboxylation in cowpea plants under salt stress. Rodrigues et al (2014) observed a reduction in the efficiency of instantaneous carboxylation with increasing salt concentration in castor bean plants. It is understood that the supply of $NO_3^-$ has similar effects to the different levels of salinity of the water used in irrigation (FURTADO et al., 2013).

### 4.4.3. Nitrate contents and NR activity are sensitive to waterlogging and $NO_3^-$ supply

The leaves of waterlogged plants, V3R2, which received a first stress at V3 stage, (Fig. S2) increased $NO_3^-$ contents during waterlogging, while in leaves of R2 plants they were maintained at control levels, while nitrate reductase (NR) activity was not altered in V3R2 plants and increased in R2 during waterlogging (Fig. 3A, B). We believe that V3R2 plants developed a waterlogging tolerance mechanism, managing to assimilate and have a higher concentration of nitrate and therefore keeping a NR activity enough to nitrate reduction that resulted in an increase in TAA, sucrose, TSS levels during waterlogging (Fig. 7A, B, C), in higher levels than R2. While in plants supplied with nitrate the presence of ion led to the uptake and induction of NR metabolization of this, in both groups, V3R2 and R2 (Figure 3A, B), resulting in a lower level of TAA in V3R2 plants (Fig. 7A), possibly due a higher mobilization of N-assimilates from the shoots or even in a higher assimilation of nitrate in the roots of this group of plants, in comparison to R2N group, since the concentration of sugars and sucrose was high in both groups (7 A, B) and probably not limiting to N assimilation, or it have evolved several adaptive responses to compensate for the energy loss caused by oxygen deprivation (GUPTA; ZABALZA; VAN DONGEN, 2009)

Nitrate reductase (NR) is an important enzyme involved in N metabolism, essential to the assimilation of nitrate through the conversion of nitrate into nitrite (CHAMIZO-AMPUDIA et al., 2017; KISHOREKUMAR et al., 2020), which activity is mostly regulated by the availability of nitrate (MELZER; KLEINHOFS; WARNER, 1989). In the two groups of plants cultivated without and with $NO_3^-$ supply, we observed an increase in NR activity under hypoxia, being much more in R2 plants than V3R2. Therefore, the higher the NR activity, the higher the accumulation of NO, using nitrate as substrate (DA-SILVA; DO AMARANTE, 2020a). $NO_2^-$ produced from $NO_3^-$ can act as a substrate for NO production by RN activity and by the mitochondrial electron transport chain (MOHN; THAQI; FISCHER-SCHRADER, 2019; WANY et al., 2019), activated by acidification and dephosphorylation triggered at hypoxia in roots (WANY et al., 2019). NR is a component of the Pb-NO cycle and a potential source of NO from nitrite under hypoxia. In addition, cytosolic phytoglobins (Pb) are capable of collecting NO

and oxidizing it to $NO_3^-$ (IGAMBERDIEV et al., 2005). The functioning of this pathway can therefore play a role in the exchange of NO between mitochondria and cytosol. This Pb-NO cycle contributes both to the oxidation of NADH and NADPH and to the synthesis of ATP under hypoxia (STOIMENOVA et al., 2007b), increasing the energy efficiency in situations of hypoxic stress such as V3R2 plants with intermittent stress, R2 and other groups of plants with nitrate supply (Fig. 3B). Although the alleviation of hypoxic root metabolism by nitrate supply through Pb-NO cycle could impact positively nitrate supplied plants, reflecting in an improvement of gas exchange parameters in non-primed plants (R2N group) (Fig. 1A,B,C,D), part of nitrate transported to the shoot could reduce the impact of hypoxia on leaf metabolism by NO production, however the accumulation of foliar NO can also result from either non-enzymatic or oxidative pathways ((DA-SILVA; MODOLO, 2017).

4.4.4. ROS are sensitive to waterlogging and $NO_3^-$ supply

Reactive oxygen species (ROS) play numerous important roles in plant development and environmental responses. Under waterlogging stress, the primarily damages occur in the mitochondrial electron transport chain (ETC) in hypoxic roots, resulting in additional ROS generation (TEWARI; ARORA, 2016a). In leaves, the redox imbalance generated by an over reduction in the electron transport chain of chloroplasts due to a decrease in ATP and NADPH consumption via Calvin-Benson cycle also leads to electron escape that react with oxygen to produce ROS (BLOKHINA; FAGERSTEDT, 2010). Levels of $H_2O_2$ in leaves increased in all groups of plants, V3R2, R2, V3R2N and R2N, during waterlogging and reoxygenation (Fig. 4A). The overproduction of ROS in leaves of waterlogged plants (Fig. 4A) may be a result of impaired photosynthesis (Fig. 1A), resulting from reduced stomatal opening (Fig. 1B) and pigment damage (Table 1) (GARCIA et al., 2020). In addition, plants under waterlogging supplied with nitrate were less likely to produce ROS and lipid peroxidation compared to their respective waterlogged plants (Figure 4A, B). Compared to soybean leaves under hypoxia, soybean leaves supplied with $NO_3^-$ produced lower levels of $H_2O_2$.

Malondialdehyde (MDA) is an index of lipid peroxidation in response to stress. Our results revealed that R2 plants accumulated higher MDA content in response to water saturation stress compared to V3R2 even at reoxygenation

period (Fig. 4B). This response of the V3R2 plants could be attributed to the priming effect (HILKER; SCHMÜLLING, 2019b; KINOSHITA; SEKI, 2014; LÄMKE; BÄURLE, 2017b), which caused a metabolic reprogramming (these plants did not increase MDA production in the roots during hypoxia or reoxygenation; data not shown). In plants with nitrate application V3R2N and R2N, MDA remained at lower levels than those found in waterlogged plants without nitrate application, indicating that nitrate helped to decrease lipid peroxidation under those conditions (DA-SILVA; DO AMARANTE, 2020a; WANY; FOYER; GUPTA, 2018). In hypoxic roots, nitrate can alleviate oxidative stress through NO synthesis and Pb-NO cycle, involving production of $NO_2$- by NR and subsequent reduction to NO by CTE (DA-SILVA; DO AMARANTE, 2020a). Possibly as it is known that nitric oxide (NO) also increases internal oxygen levels and controls reactive oxygen species production (ROS) (GUPTA et al., 2014). However, accumulation of foliar NO in plants exposed to waterlogging can be explained by increases in the NR activity during waterlogging is likely to be due to the production of NO by NR (DA-SILVA; DO AMARANTE, 2020a). NR can also indirectly contribute to NO accumulation by catalyzing the formation of the substrate (i.e. nitrite) for other NO biosynthesis pathways, such as the mitochondrial electron transport chain, the plasma membrane-bound nitrite: NO reductase, or the xanthine oxidoreductase (DA-SILVA; MODOLO, 2017).

### 4.4.5. Antioxidant system is sensitive to waterlogging and $NO_3^-$ supply

Although the concentration of $H_2O_2$ was increased upon waterlogging stress in leaves of plants without and with nitrate supply (Figure 4), the activities of antioxidant enzymes were increased as well, which may contribute to alleviate oxidative stress (Fig.5, Fig. 6). Waterlogging may cause plant senescence, because of the decrease in $CO_2$ concentration and increase in the concentration of ROS (PUYANG et al., 2015), and the stress resistance of waterlogging may depend, at least in part, on the improvement of the antioxidant defence system (BIN et al., 2010).

The superoxide dismutase (SOD) activity increased in V3R2 plants significantly during waterlogging and reoxygenation, unlike in R2 plants that decreased during the whole experiment, suggesting that the V3R2 plants

developed a better capacity of cleaning the $O_2^-$ radical, forming $H_2O_2$ and $O_2$, which is considered the first line of defence (BLOKHINA; FAGERSTEDT, 2010; DIETZ; MITTLER; NOCTOR, 2016; MITTLER, 2017). The transient increase of ROS levels in the leaves, highly reactive molecules that must be controlled to avoid cell damage (BAXTER; MITTLER; SUZUKI, 2014; FARNESE et al., 2016; GILL; TUTEJA, 2010). To nullify the damaging effects of ROS, plants are equipped with a set of antioxidant and enzymes that act together to alleviate cell damage under oxidative stress conditions (ANEE et al., 2019b). In this way, plants that had a first waterlogging stress in stage V3 and a second stress in stage R2 (V3R2) could effectively alleviate oxidative damage of leaf cells by maintaining relatively higher activities of ROS scavenging enzymes than plants submitted to a single waterlogging treatment (R2) (Figure 5A, B, C). The enzymatic antioxidant defence in V3R2 plants include CAT, GPOD, APX and DHAR. Catalase (CAT) is the enzyme that actively catalyzes the $H_2O_2$ scavenging reaction. The increasing trend in $H_2O_2$ accumulation with increased stress duration is partly the result of decreased CAT activity as the $H_2O_2$scavenging enzyme (HASANUZZAMAN et al., 2012). Guaiacol peroxidases (GPOD) are heme-containing proteins that preferably oxidize aromatic electron donors such as guaiacol and pyrogallol at the expense of $H_2O_2$ (SHARMA et al., 2012). Guaiacol peroxidase is associated with many important biosynthetic processes and defense against abiotic and biotic stresses. In this experiment, GPOD activity increased in a time-dependent manner with increased duration of waterlogging, which is supported by other studies on sesame (SAHA et al., 2017). The enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR, and GR) readily and efficiently catalyze ROS detoxification with the help of vital components AsA (ascorbic acid) and GSH (glutathione); after scavenging ROS, AsA and GSH are recycled [61–63]. The ascorbate peroxidase (APX) catalyzes the reduction of $H_2O_2$ to $H_2O$ by using AsA [64,65]. Dehydroascorbate reductase (DHAR) regenerates AsA from its oxidized state (DHA) and regulates the cellular AsA redox state (MURSHED; LOPEZ-LAURI; SALLANON, 2008a). In our study, it was observed that APX activity increased with stress duration, while DHAR activity reduced in a time-dependent manner (Figure 6D, E).

The supply of nitrate for plants (Fig. 5; 6), efficiently increased the induction of SOD CAT, GPOD, APX, and DHAR enzymes in V3R2 and R2 groups to counteract the possible effects of ROS production in leaves, mainly in R2N plants. In fact, the increases of activity in this group of enzymes were more pronounced in R2 group, possibly part of nitrate consumption in this group of plants was used to NO production that stimulated enzymatic antioxidant activity (BORELLA et al., 2019; DA-SILVA; DO AMARANTE, 2020a) and compensate the priming effect in V3R2 group that presented lower oxidative stress (Figure 4B). The application of nitrate helped to alleviate oxidative damage in the leaves by maintaining the relatively higher activities of the ROS scavenging enzymes at the onset of stress and reoxygenation, allowing a decrease of ROS to control levels (Fig. 4A). In the case of V3R2N plants, nitrate inhibited the activity of SOD and DHAR, with the consequent lower activity of CAT, GPOD and APX (Fig. 5A, B, C; 6A, B). We believe that the action of NO and ROS produced in the plant at the same time, managed to interact chemically, producing peroxynitrite (HANCOCK; NEILL, 2019), This peroxynitrite can act inhibiting the activity of SOD (HOLZMEISTER et al., 2015), through nitration (CORPAS; RÍO; PALMA, 2019). Despite the low activity of antioxidant enzymes in plants under waterlogging and treated with nitrate, the lipid peroxidation decreased, probably due to the action of NO acting with an efficient antioxidant, preventing oxidative damage in plants under cultivation (DA-SILVA; DO AMARANTE, 2020a).

### 4.4.6. Amino acids and carbohydrate levels are sensitive to waterlogging and $NO_3^-$ supply

Under waterlogged conditions, plants experience energy and carbohydrate deprivation due to reduced photosynthesis and aerobic respiration. The content of total amino acids (TAA) (Fig. 6A), total soluble sugars (TSS) (Fig. 6B) and sucrose (Fig. 6C) in the leaves increased under hypoxia, and expressive increases were detected on the fifth day of waterlogging, in V3R2 and R2N plants, not change substantially under reoxygenation. However, a decrease in TSS and sucrose content caused by glycolysis stimulation was expected, as an attempt to supply the plant's ATP demand, due to the decrease in $O_2$ availability in the roots. The accumulation of pyruvate (final product in the glycolytic pathway) can trigger the fermentation pathway, which is key for the regeneration of NAD+ used as a

coenzyme in the glycolytic pathway. Thus, it is possible that the increase of non-structural carbohydrates in V3R2 that would previously meet the demands of vegetative growth, occurs due to the restriction of translocation of assimilates to the roots due to lack of available $O_2$, which is corroborated by the fact that there is no increase in carbohydrates in the roots (Date not shown). Interestingly, variations in TSS in leaves match assimilation $CO_2$ rates, corroborate with the hypothesis that flow of carbohydrates was impaired during waterlogging (Fig. 1A, 7B). Sugars not only function as osmoprotectors during stress, but also as substrates for growth and regulators of gene expression (IQBAL; NAZAR, 2016; KOCH, 1996). The variations in the sugar content of the leaves can still be attributed to different degrees of starch degradation, to the allocation of carbon in the plant biomass and to the use of those carbohydrates in the respiratory metabolism, depending on the age of the plant (FUKAO et al., 2019). Increased sucrose and fructans are essentially involved in plant tolerance to stress, including waterlogging (KEUNEN et al., 2013), while low sugar levels are observed in other stress conditions such as high light irradiation, heavy metals and nutrient deficiency (GILL et al., 2001). Although there was a reduction of amino acids in V3 in the leaves (Sup. Fig. S3), it suggests that these molecules may have been removed to the roots, thus revealing an interconnection between amino acids in the roots as is the case of Alanine, the main amino acid transported and accumulated under oxygen deficiency in soybean roots (DE SOUSA; SODEK, 2003), the same behaviour of AAT was observed in R2 plants. Submerged V3R2 plants could harbour a large amount of soluble sugars and amino acids as a result of improved fatty acid and starch catabolism (BAILEY-SERRES; LEE; BRINTON, 2012; HSU et al., 2011), we could say that these plants acquired a stress memory effect, managing better the synthesis and allocation of carbohydrates and amino acids. The alterations of TAA, TSS and sucrose from the end of hypoxia and reoxygenation could reinforce this hypothesis.

However, in plants with a supply of nitrate, in R2N plants, nitrate stimulated an increase in levels of AAT and, TSS in V3R2 and R2 groups while sucrose was not changed during waterlogging. It is possible that in V3R2 group, amino acids could be directed to the protein synthesis or N-organic molecules, or even N0,

once nitrate contents and NR activity were similar in both groups of plants by the end of waterlogging (Figure 3A, B) and TSS as well (Figure 7B), probably enough to supply the energy demand for nitrogen assimilation. and an increase in enzymatic activity and the theory of carbohydrates, due to glycolysis activation and low regulation of sucrose degrading enzymes (KOMATSU; HIRAGA; YANAGAWA, 2012; KOMATSU; SAKATA; NANJO, 2015)On the other hand, the decrease in $NO_3^-$ in V3R2 (Figure 3A) at reoxygenation, followed by a decrease in TAA contents as well as TSS and sucrose levels (Fig. 7A, B, C) contrasting to R2 group, suggest that sites of nitrate assimilation and allocation of organic molecules differed substantially between the two groups when they are supplied with nitrate.

## 4.5. Conclusions

This conclusion supported by the data in chap. II (Root), demonstrating that priming of water stress responses applied to 'soybean' plants, specifically in the leaves, enhances acclimation to transient environmental stimuli (recurrent waterlogging), being as efficient in triggering tolerance to waterlogging as to waterlogging associated with nitrate nutrition. The V3R2 and V3R2N groups showed positive waterlogging tolerance considering some physiological indexes such as gas exchange parameters, organic compound levels, and in a lower extension, oxidative stress and enzyme antioxidant activity. The application of nitrate seems to improve the tolerance of the R2 group, without altering the priming effect of the V3R2 group, but rather favouring the priming effect.

Understanding the underlying mechanisms in soybean will ultimately allow us to improve stress tolerance in crop species. One possibility could be to explore the mechanisms of stress priming to induce a constitutive priming state, thus increasing the ability of a crop to tolerate stress and disease without incurring a penalty for biomass accumulation and yield.

## 4.6. References


AGOSTINETTO, D. et al. Performance of transgenic soybean cultivars and weed control in function of application times and glyphosate formulations. **Planta Daninha**, v. 27, n. 4, p. 739–746, 2009.

AHMED, S. et al. Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. **Plant Science**, v. 163, n. 1, p. 117–123, 2002.

ALAM, I. et al. Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage. **Journal of Biosciences**, v. 35, n. 1, p. 49–62, mar. 2010.

AMARANTE, L. DO; LIMA, J. D.; SODEK, L. Growth and stress conditions cause similar changes in xylem amino acids for different legume species. **Environmental and Experimental Botany**, v. 58, n. 1–3, p. 123–129, 2006.

AMARANTE, L.; SODEK, L. Waterlogging effect on xylem sap glutamine of nodulated soybean. **BIOLOGIA PLANTARUM**, v. 50, n. 3, p. 405–410, 2006.

ANDRADE, C. A. et al. Hydrogen peroxide promotes the tolerance of soybeans to waterlogging. **Scientia Horticulturae**, v. 232, p. 40–45, 17 fev. 2018.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019a.

ANEE et al. Oxidative Damage and Antioxidant Defense in Sesamum indicum after Different Waterlogging Durations. **Plants**, v. 8, n. 7, p. 196, 29 jun. 2019b.

ANJUM, S. A. et al. Drought Induced Changes in Growth, Osmolyte Accumulation and Antioxidant Metabolism of Three Maize Hybrids. **Frontiers in Plant Science**, v. 08, 6 fev. 2017.

ARBONA, V. et al. Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. **Physiologia Plantarum**, v. 132, n. 4, p. 452–466, abr. 2008.

AROCA, R.; PORCEL, R.; RUIZ-LOZANO, J. M. Regulation of root water uptake under abiotic stress conditions. **Journal of Experimental Botany**, v. 63, n. 1, p. 43–57, 1 jan. 2012.

ASHRAF, M. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (Panicum antidotale Retz.) under salinity or waterlogging. **Plant Science**, v. 165, n. 1, p. 69–75, 1 jul. 2003.

AUGE, G. A. et al. Adjusting phenotypes via within- and across-generational plasticity. **New Phytologist**, v. 216, n. 2, p. 343–349, 2017.

AVOLA, G. et al. Gas exchange and photosynthetic water use efficiency in response to light, CO2 concentration and temperature in Vicia faba. **Journal of Plant Physiology**, v. 165, n. 8, p. 796–804, 26 maio 2008.

AZEVEDO, R. A. et al. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. **Physiologia Plantarum**, v.

104, n. 2, p. 280–292, out. 1998.

AZIZIAN, A.; SEPASKHAH, A. R. Maize response to water, salinity and nitrogen levels: Physiological growth parameters and gas exchange. **International Journal of Plant Production**, v. 8, n. 1, p. 131–162, 2013.

BAILEY-SERRES, J. et al. Making sense of low oxygen sensing. **Trends in Plant Science**, v. 17, n. 3, p. 129–138, mar. 2012.

BAILEY-SERRES, J.; LEE, S. C.; BRINTON, E. Waterproofing Crops: Effective Flooding Survival Strategies. **Plant Physiology**, v. 160, n. 4, p. 1698–1709, dez. 2012.

BAILEY-SERRES, J.; VOESENEK, L. A. C. J. Flooding Stress: Acclimations and Genetic Diversity. **Annual Review of Plant Biology**, v. 59, n. 1, p. 313–339, jun. 2008.

BALAKHNINA, T. I. Plant Responses to Soil Flooding. In: **Stress Responses in Plants**. Cham: Springer International Publishing, 2015. p. 115–142.

BANSAL, R. et al. Waterlogging tolerance in black gram [Vigna mungo (L.) Hepper] is associated with chlorophyll content and membrane integrity. **Indian Journal of Biochemistry & Biophysics**, v. 56, p. 81–85, 2019.

BANSAL, R.; SRIVASTAVA, J. P. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. **Russian Journal of Plant Physiology**, v. 62, n. 3, p. 322–327, 29 maio 2015.

BANTI, V. et al. Low Oxygen Response Mechanisms in Green Organisms. **International Journal of Molecular Sciences**, v. 14, n. 3, p. 4734–4761, 27 fev. 2013.

BARICKMAN, T. C.; SIMPSON, C. R.; SAMS, C. E. Waterlogging Causes Early Modification in the Physiological Performance, Carotenoids, Chlorophylls, Proline, and Soluble Sugars of Cucumber Plants. **Plants**, v. 8, n. 6, p. 160, 8 jun. 2019.

BATISTA, T. B. et al. Condicionamento fisiológico e stress sob alta umidade e temperatura na qualidade fisiológica de sementes de Brachiaria brizantha cv. MG-5. **Acta Scientiarum - Agronomy**, v. 38, n. 1, p. 123–127, 1 jan. 2016.

BAUER, G. Increasing the endogenous NO level causes catalase inactivation and reactivation of intercellular apoptosis signaling specifically in tumor cells. **Redox Biology**, v. 6, p. 353–371, 1 dez. 2015.

BAXTER, A.; MITTLER, R.; SUZUKI, N. ROS as key players in plant stress signalling. **Journal of Experimental Botany**, v. 65, n. 5, p. 1229–1240, 1 mar. 2014.

BERTRAND, A. et al. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. **Journal of Experimental Botany**, v. 54, n. 388, p. 1721–1730, 2003.

BEUTLER, A. N. et al. Soil hydric excess and soybean yield and development in Brazil. **Australian Journal of Crop Science**, v. 8, n. 10, p. 1461–1466, 2014.

BIELESKI, R. L.; TURNER, N. A. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. **Analytical Biochemistry**, v. 17, n. 2, p. 278–293, 1 nov. 1966.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. **Plant Physiology**, v. 116, n. 2, p. 651–658, 1998.

BIN, T. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, maio 2010.

BLOKHINA, O. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. **Annals of Botany**, v. 91, n. 2, p. 179–194, 1 jan. 2003.

BLOKHINA, O.; FAGERSTEDT, K. V. Oxidative metabolism, ROS and NO under oxygen deprivation. **Plant Physiology and Biochemistry**, v. 48, n. 5, p. 359–373, maio 2010.

BOARD, J. E. Waterlogging effects on plant nutrient concentrations in soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, maio 2008a.

BOARD, J. E. Waterlogging Effects on Plant Nutrient Concentrations in Soybean. **Journal of Plant Nutrition**, v. 31, n. 5, p. 828–838, 13 maio 2008b.

BOARETTO, L. F. et al. Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. **Plant Physiology and Biochemistry**, v. 74, p. 165–175, jan. 2014.

BONIFACIO, A. et al. Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. **Plant, Cell and Environment**, v. 34, n. 10, p. 1705–1722, 2011.

BORELLA, J. et al. Waterlogging-induced changes in fermentative metabolism in roots and nodules of soybean genotypes. **Scientia Agricola**, v. 71, n. 6, p. 499–508, dez. 2014.

BORELLA, J. et al. Hypoxia-driven changes in glycolytic and tricarboxylic acid cycle metabolites of two nodulated soybean genotypes. **Environmental and Experimental Botany**, v. 133, p. 118–127, 1 jan. 2017.

BORELLA, J. et al. Nitrogen source influences the antioxidative system of soybean plants under hypoxia and re-oxygenation. **Scientia Agricola**, v. 76, n. 1, p. 51–62, 1 fev. 2019.

BOTREL, A.; KAISER, W. M. Nitrate reductase activation state in barley roots in relation to the energy and carbohydrate status. **Planta**, v. 201, n. 4, p. 496–501, 9 abr. 1997.

BOTREL, A.; MAGNE, C.; KAISER, W. M. Nitrate reduction, nitrite reduction and ammonium assimilation in barley roots in response to anoxia. **Plant Physiology and Biochemistry**, v. 34, n. 5, p. 645–652, 1996.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.

**Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 7 maio 1976.

BRAMLEY, H. et al. The contrasting influence of short-term hypoxia on the hydraulic properties of cells and roots of wheat and lupin. **Functional Plant Biology**, v. 37, n. 3, p. 183, 2010.

BRANDÃO, A. D.; SODEK, L. Nitrate uptake and metabolism by roots of soybean plants under oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 21, n. 1, p. 13–23, 2009.

BRITTO, D. T.; KRONZUCKER, H. J. NH4+ toxicity in higher plants: a critical review. **Journal of Plant Physiology**, v. 159, n. 6, p. 567–584, jan. 2002.

BRUCE, T. J. A. et al. Stressful “memories” of plants: Evidence and possible mechanisms. **Plant Science**, v. 173, n. 6, p. 603–608, 1 dez. 2007.

BUI, L. T. et al. Conservation of ethanol fermentation and its regulation in land plants. **Journal of Experimental Botany**, v. 70, n. 6, p. 1815–1827, 27 mar. 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, 1 nov. 1991.

CAPONE, R.; TIWARI, B. S.; LEVINE, A. Rapid transmission of oxidative and nitrosative stress signals from roots to shoots in Arabidopsis. **Plant Physiology and Biochemistry**, v. 42, n. 5, p. 425–428, 1 maio 2004.

CATALDO, D. A. et al. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis**, v. 6, n. 1, p. 71–80, 1 jan. 1975.

CAVALCANTI, F. R. et al. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. **New Phytologist**, v. 163, n. 3, p. 563–571, set. 2004.

CENTRITTO, M. et al. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. **Journal of Experimental Botany**, v. 60, n. 8, p. 2325–2339, 1 maio 2009.

CHAMIZO-AMPUDIA, A. et al. Nitrate Reductase Regulates Plant Nitric Oxide Homeostasis. **Trends in Plant Science**, v. 22, n. 2, p. 163–174, 1 fev. 2017.

CHO, J. W.; YAMAKAWA, T. Effects on growth and seed yield of small seed soybean cultivars of flooding conditions in paddy field. **Journal of the Faculty of Agriculture, Kyushu University**, v. 51, n. 2, p. 189–193, 2006.

COLLAKU, A.; HARRISON, S. A. Losses in Wheat Due to Waterlogging. **Crop Science**, v. 42, n. 2, p. 444–450, 1 mar. 2002.

COLMER, T. D.; VOESENEK, L. A. C. J. C. J. Flooding tolerance: suites of plant traits in variable environments. **Functional Plant Biology**, v. 36, n. 8, p. 665, 2009.

CONRATH, U. et al. Priming for Enhanced Defense. **Annual Review of**

**Phytopathology**, v. 53, n. 1, p. 97–119, 4 ago. 2015.

CORPAS, F. J.; RÍO, L. A. DEL; PALMA, J. M. Impact of Nitric Oxide (NO) on the ROS Metabolism of Peroxisomes. **Plants**, v. 8, n. 2, p. 37, 10 fev. 2019.

COSKUN, D.; BRITTO, D. T.; KRONZUCKER, H. J. The nitrogen-potassium intersection: membranes, metabolism, and mechanism. **Plant, Cell & Environment**, v. 40, n. 10, p. 2029–2041, 1 out. 2017.

COUTINHO, I. D. et al. Flooded soybean metabolomic analysis reveals important primary and secondary metabolites involved in the hypoxia stress response and tolerance. **Environmental and Experimental Botany**, v. 153, p. 176–187, set. 2018.

CRAFTS-BRANDNER, S. J.; HARPER, J. E. Nitrate Reduction by Roots of Soybean (Glycine max [L.] Merr.) Seedlings. **Plant Physiology**, v. 69, n. 6, 1982.

CRAWFORD, N. M. Nitrate: nutrient and signal for plant growth. **The Plant Cell**, v. 7, n. 7, p. 859–868, jul. 1995.

CRAWFORD, N. M.; FORDE, B. G. Molecular and Developmental Biology of Inorganic Nitrogen Nutrition. **The Arabidopsis Book**, v. 1, p. e0011, jan. 2002.

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, 1 ago. 2020a.

DA-SILVA, C. J.; DO AMARANTE, L. Time-course biochemical analyses of soybean plants during waterlogging and reoxygenation. **Environmental and Experimental Botany**, v. 180, p. 104242, 1 dez. 2020b.

DA-SILVA, C. J.; MODOLO, L. V. Hydrogen sulfide: a new endogenous player in an old mechanism of plant tolerance to high salinity. **Acta Botanica Brasilica**, v. 32, n. 1, p. 150–160, 19 out. 2017.

DA ROCHA, T. S. M. et al. Performance of soybean in hydromorphic and nonhydromorphic soil under irrigated or rainfed conditions. **Pesquisa Agropecuaria Brasileira**, v. 52, n. 5, p. 293–302, 1 maio 2017.

DE AZEVEDO NETO, A. D. et al. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. **Environmental and Experimental Botany**, v. 56, n. 1, p. 87–94, maio 2006.

DE OLIVEIRA, W. J. et al. Leaf gas exchange in cowpea and CO2 efflux in soil irrigated with saline water. **Revista Brasileira de Engenharia Agricola e Ambiental**, v. 21, n. 1, p. 32–37, 2017.

DE SOUSA, C. A. F.; SODEK, L. Alanine metabolism and alanine aminotransferase activity in soybean (Glycine max) during hypoxia of the root system and subsequent return to normoxia. **Environmental and Experimental Botany**, v. 50, n. 1, p. 1–8, 1 ago. 2003.

DECHORGNAT, J. et al. From the soil to the seeds: the long journey of nitrate in plants. **Journal of Experimental Botany**, v. 62, n. 4, p. 1349–1359, 1 fev.

2011.

DEMONGEOT, J.; HASGUI, H.; THELLIER, M. Memory in plants: Boolean modeling of the learning and store/recall memory functions in response to environmental stimuli. **Journal of Theoretical Biology**, v. 467, p. 123–133, 21 abr. 2019.

DIAB, H.; LIMAMI, A. Reconfiguration of N Metabolism upon Hypoxia Stress and Recovery: Roles of Alanine Aminotransferase (AlaAT) and Glutamate Dehydrogenase (GDH). **Plants**, v. 5, n. 2, p. 25, 31 maio 2016.

DIETZ, K.-J.; MITTLER, R.; NOCTOR, G. Recent Progress in Understanding the Role of Reactive Oxygen Species in Plant Cell Signaling. **Plant Physiology**, v. 171, n. 3, 2016.

DO AMARAL, M. N. et al. Long-term transcriptional memory in rice plants submitted to salt shock. **Planta**, v. 251, n. 6, p. 111, 1 jun. 2020.

DONAT, M. G. et al. More extreme precipitation in the world's dry and wet regions. **Nature Climate Change**, v. 6, n. 5, p. 508–513, 7 maio 2016.

EIGENBROD, F. et al. Vulnerability of ecosystems to climate change moderated by habitat intactness. **Global Change Biology**, v. 21, n. 1, p. 275–286, 1 jan. 2015.

EMBRAPA SOJA. **Soja - Portal Embrapa**. Disponível em: <https://www.embrapa.br/soja/cultivos/soja1>. Acesso em: 21 jul. 2020.

EVANS, D. E.; GLADISH, D. K. Plant Responses to Waterlogging. In: **Encyclopedia of Applied Plant Sciences**. Second Edi ed. [s.l.] Elsevier, 2017. v. 1p. 36–39.

FARNESE, F. S. et al. When Bad Guys Become Good Ones: The Key Role of Reactive Oxygen Species and Nitric Oxide in the Plant Responses to Abiotic Stress. **Frontiers in Plant Science**, v. 7, n. APR2016, 12 abr. 2016.

FEHR, W. R.; AND C. E, C. "Stages of soybean development" (1977). Special Report. 80. p. 1–12, 1977.

FEHR, W. R. et al. Stage of Development Descriptions for Soybeans, Glycine Max (L.) Merrill 1. **Crop Science**, v. 11, n. 6, p. 929–931, nov. 1971.

FLETA-SORIANO, E.; MUNNÉ-BOSCH, S. Stress Memory and the Inevitable Effects of Drought: A Physiological Perspective. **Frontiers in Plant Science**, v. 7, n. February, p. 1–6, 15 fev. 2016.

FORDE, B. G.; CLARKSON, D. T. Nitrate and Ammonium Nutrition of Plants: Physiological and Molecular Perspectives. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 30p. 1–90.

FOYER, C. H. et al. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. **Physiologia Plantarum**, v. 100, n. 2, p. 241–254, jun. 1997.

FUKAO, T. et al. Submergence and Waterlogging Stress in Plants: A Review Highlighting Research Opportunities and Understudied Aspects. **Frontiers in Plant Science**, v. 10, p. 340, 22 mar. 2019.

FURTADO, G. DE F. et al. Alterações fisiológicas em feijão-caupi irrigado com água salina e adubação nitrogenada. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 8, n. 3, p. 175–181, 25 out. 2013.

GARCIA, N. et al. Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. **Environmental and Experimental Botany**, v. 172, p. 103975, 1 abr. 2020.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: I. Occurrence in higher plants. **Plant physiology**, v. 59, n. 2, p. 309–14, 1 fev. 1977.

GIBBS, J.; GREENWAY, H. Review: Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. **Functional Plant Biology**, v. 30, n. 1, p. 1, 2003.

GILL, P. K. et al. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. **BULG. J. PLANT PHYSIOL.**, v. 27, n. 2, p. 72–84, 2001.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909–930, dez. 2010.

GITHIRI, S. M. et al. QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. **Plant Breeding**, v. 125, n. 6, p. 613–618, 1 dez. 2006.

GIUNTOLI, B.; PERATA, P. Group VII Ethylene Response Factors in Arabidopsis: Regulation and Physiological Roles. **Plant Physiology**, v. 176, n. 2, 2018.

GONÇALVES, M. et al. Correlations and path analysis of common bean grain yield and its primary components. **Cropp Breeding and Applied Biotechnology**, v. 3, n. 3, p. 217–222, 30 set. 2003.

GOOD, A. G.; CROSBY, W. L. Anaerobic Induction of Alanine Aminotransferase in Barley Root Tissue. **Plant Physiology**, v. 90, n. 4, p. 1305–1309, 1 ago. 1989.

GOOD, A. G.; MUENCH, D. G. Purification and Characterization of an Anaerobically Induced Alanine Aminotransferase from Barley Roots. **Plant Physiology**, v. 99, n. 4, p. 1520–1525, 1 ago. 1992.

GRAHAM, D.; SMYDZUK, J. Use of anthrone in the quantitative determination of hexose phosphates. **Analytical Biochemistry**, v. 11, n. 2, p. 246–255, 1 maio 1965.

GUPTA, K. J. et al. The form of nitrogen nutrition affects resistance against Pseudomonas syringae pv. phaseolicola in tobacco. **Journal of Experimental Botany**, v. 64, n. 2, p. 553–568, jan. 2013.

GUPTA, K. J. et al. Nitric Oxide Is Required for Homeostasis of Oxygen and Reactive Oxygen Species in Barley Roots under Aerobic Conditions. **Molecular Plant**, v. 7, n. 4, p. 747–750, abr. 2014.

GUPTA, K. J. et al. The role of nitrite and nitric oxide under low oxygen conditions in plants. **New Phytologist**, v. 225, n. 3, p. 1143–1151, 11 fev. 2020.

GUPTA, K. J. **Nitrogen Metabolism in Plants**. Springer S ed. New York, NY: Springer New York, 2020. v. 2057

GUPTA, K. J.; IGAMBERDIEV, A. U. Reactive Nitrogen Species in Mitochondria and Their Implications in Plant Energy Status and Hypoxic Stress Tolerance. **Frontiers in Plant Science**, v. 7, n. MAR2016, p. 369, 24 mar. 2016.

GUPTA, K. J.; KAISER, W. M. Production and scavenging of nitric oxide by barley root mitochondria. **Plant and Cell Physiology**, v. 51, n. 4, p. 576–584, abr. 2010.

GUPTA, K. J.; ZABALZA, A.; VAN DONGEN, J. T. Regulation of respiration when the oxygen availability changes. **Physiologia Plantarum**, v. 137, n. 4, p. 383–391, 2009.

GUPTA, P. et al. Signaling cross talk between biotic and abiotic stress responses in soybean. In: **Abiotic and Biotic Stresses in Soybean Production**. [s.l.] Elsevier, 2016. v. 1p. 27–52.

HANCOCK, J. T.; NEILL, S. J. Nitric oxide: Its generation and interactions with other reactive signaling compounds. **Plants**, v. 8, n. 2, 1 fev. 2019.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984a.

HANSON, A. D.; JACOBSEN, J. V; ZWAR, J. A. Regulated Expression of Three Alcohol Dehydrogenase Genes in Barley Aleurone Layers. **Plant Physiology**, v. 75, n. 3, p. 573–581, 1 jul. 1984b.

HARTMAN, S.; SASIDHARAN, R.; VOESENEK, L. A. C. J. **The role of ethylene in metabolic acclimations to low oxygenNew Phytologist**, 2019.

HASANUZZAMAN, M. et al. Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor. In: **Crop Stress and its Management: Perspectives and Strategies**. Dordrecht: Springer Netherlands, 2012. v. 9789400722p. 261–315.

HEMANTARANJAN, A. Flooding: Abiotic Constraint Limiting Vegetable Productivity. **Advances in Plants & Agriculture Research**, v. 1, n. 3, 23 jul. 2014.

HILKER, M. et al. Priming and memory of stress responses in organisms lacking a nervous system. **Biological Reviews**, v. 91, n. 4, p. 1118–1133, nov. 2016.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, mar. 2019a.

HILKER, M.; SCHMÜLLING, T. Stress priming, memory, and signalling in plants. **Plant, Cell & Environment**, v. 42, n. 3, p. 753–761, 1 mar. 2019b.

HINCHA, D. K.; ZUTHER, E. Introduction: Plant cold acclimation and freezing tolerance. **Methods in Molecular Biology**, v. 1166, p. 1–6, 2014.

HOLZMEISTER, C. et al. Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. **Journal of**

**Experimental Botany**, v. 66, n. 3, p. 989–999, 1 fev. 2015.

HOSSAIN, A.; UDDIN, S. N. Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. **Australian Journal of Crop Science**, v. 5, n. 9 SPEC. ISSUE, p. 1094–1101, 2011.

HSU, F.-C. et al. Insights into Hypoxic Systemic Responses Based on Analyses of Transcriptional Regulation in Arabidopsis. **PLoS ONE**, v. 6, n. 12, p. e28888, 15 dez. 2011.

IGAMBERDIEV, A. U. et al. NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. **Planta**, v. 219, n. 1, p. 95–102, 22 maio 2004.

IGAMBERDIEV, A. U. Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. **Journal of Experimental Botany**, v. 55, n. 408, p. 2473–2482, 24 set. 2004.

IGAMBERDIEV, A. U. et al. The Haemoglobin/Nitric Oxide Cycle: Involvement in Flooding Stress and Effects on Hormone Signalling. **Annals of Botany**, v. 96, n. 4, p. 557–564, 1 set. 2005.

IGAMBERDIEV, A. U.; HILL, R. D. Plant mitochondrial function during anaerobiosis. **Annals of Botany**, v. 103, n. 2, p. 259–268, jan. 2009.

IQBAL, N.; NAZAR, R. **Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies**. New Delhi: Springer India, 2016.

IRFAN, M. et al. Physiological and biochemical changes in plants under waterlogging. **Protoplasma**, v. 241, n. 1–4, p. 3–17, 12 maio 2010.

ISMOND, K. P. et al. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. **Plant Physiology**, v. 132, n. 3, p. 1292–1302, 1 jul. 2003.

JENNINGS, A. C. The determination of dihydroxy phenolic compounds in extracts of plant tissues. **Analytical Biochemistry**, v. 118, n. 2, p. 396–398, dez. 1981.

JIMÉNEZ S., J. D. LA C.; MORENO F., L. P.; MAGNITSKIY, S. Plant responses to stress due to flooding. A review. **Revista Colombiana de Ciencias Hortícolas**, v. 6, n. 1, p. 96–109, 4 fev. 2013.

JITSUYAMA, Y. Hypoxia-Responsive Root Hydraulic Conductivity Influences Soybean Cultivar-Specific Waterlogging Tolerance. **American Journal of Plant Sciences**, v. 08, n. 04, p. 770–790, 3 mar. 2017.

JUSTINO, G. C.; SODEK, L. Recovery of nitrogen fixation after short-term flooding of the nodulated root system of soybean. **Journal of Plant Physiology**, v. 170, n. 3, p. 235–241, fev. 2013.

KANCHEVA, V. D. Phenolic antioxidants – radical-scavenging and chain-breaking activity: A comparative study*. **European Journal of Lipid Science and Technology**, v. 111, n. 11, p. 1072–1089, nov. 2009.

KATO-NOGUCHI, H. Pyruvate metabolism in rice coleoptiles under

anaerobiosis. **Plant Growth Regulation**, v. 50, n. 1, p. 41–46, 23 nov. 2006.

KENNEDY, R. A.; RUMPHO, M. E.; FOX, T. C. Anaerobic metabolism in plants. **Plant Physiology**, v. 100, n. 1, p. 1–6, 1 set. 1992.

KEUNEN, E. et al. A mutant of the Arabidopsis thaliana LIPOXYGENASE1 gene shows altered signalling and oxidative stress related responses after cadmium exposure. **Plant Physiology and Biochemistry**, v. 63, p. 272–280, fev. 2013.

KHAN, M. I. R.; KHAN, N. A. **Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress**. Singapore: Springer Singapore, 2017.

KINOSHITA, T.; SEKI, M. Epigenetic Memory for Stress Response and Adaptation in Plants. **Plant and Cell Physiology**, v. 55, n. 11, p. 1859–1863, nov. 2014.

KISHOREKUMAR, R. et al. An Overview of Important Enzymes Involved in Nitrogen Assimilation of Plants. In: **Methods in Molecular Biology**. [s.l.] Humana Press Inc., 2020. v. 2057p. 1–13.

KOCH, K. E. Carbohydrate-modulated gene expression in plants. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 47, n. 1, p. 509–540, 1996.

KOLB, R. M.; JOLY, C. A. Flooding tolerance of Tabebuia cassinoides: Metabolic, morphological and growth responses. **Flora - Morphology, Distribution, Functional Ecology of Plants**, v. 204, n. 7, p. 528–535, 1 jan. 2009.

KOMATSU, S. et al. A comprehensive analysis of the soybean genes and proteins expressed under flooding stress using transcriptome and proteome techniques. **Journal of Proteome Research**, v. 8, n. 10, p. 4766–4778, 2009.

KOMATSU, S. et al. Identification of flooding stress responsible cascades in root and hypocotyl of soybean using proteome analysis. **Amino Acids**, v. 38, n. 3, p. 729–738, 31 mar. 2010.

KOMATSU, S.; HIRAGA, S.; YANAGAWA, Y. Proteomics Techniques for the Development of Flood Tolerant Crops. **Journal of Proteome Research**, v. 11, n. 1, p. 68–78, 15 jan. 2012.

KOMATSU, S.; NANJO, Y.; NISHIMURA, M. Proteomic analysis of the flooding tolerance mechanism in mutant soybean. **Journal of Proteomics**, v. 79, p. 231–250, fev. 2013.

KOMATSU, S.; SAKATA, K.; NANJO, Y. ‘Omics’ techniques and their use to identify how soybean responds to flooding. **Journal of Analytical Science and Technology**, v. 6, n. 1, p. 9, 4 dez. 2015.

KONNERUP, D. et al. Waterlogging tolerance, tissue nitrogen and oxygen transport in the forage legume Melilotus siculus: a comparison of nodulated and nitrate-fed plants. **Annals of Botany**, v. 121, p. 699–709, 2018.

KOPSELL, D. E. et al. Ratio of calcium to magnesium influences biomass,

elemental accumulations, and pigment concentrations in kale. **Journal of Plant Nutrition**, v. 36, n. 14, p. 2154–2165, 6 dez. 2013.

KREUZWIESER, J.; RENNENBERG, H. Molecular and physiological responses of trees to waterlogging stress. **Plant, Cell & Environment**, v. 37, n. 10, p. n/a-n/a, 1 maio 2014.

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, 30 abr. 2013a.

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, 30 abr. 2013b.

KUMUTHA, D. et al. Effect of waterlogging on carbohydrate metabolism in pigeon pea (Cajanus cajan L.): Upregulation of sucrose synthase and alcohol dehydrogenase. **Plant Science**, v. 175, n. 5, p. 706–716, 2008.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017a.

LÄMKE, J.; BÄURLE, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. **Genome Biology**, v. 18, n. 1, p. 124, 27 dez. 2017b.

LASA, B. et al. Role of glutamate dehydrogenase and phosphoenolpyruvate carboxylase activity in ammonium nutrition tolerance in roots. **Plant Physiology and Biochemistry**, v. 40, n. 11, p. 969–976, 1 nov. 2002.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011a.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011b.

LI, C. et al. Waterlogging pretreatment during vegetative growth improves tolerance to waterlogging after anthesis in wheat. **Plant Science**, v. 180, n. 5, p. 672–678, maio 2011c.

LI, X. et al. Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. **Plant and Soil**, v. 352, n. 1–2, p. 377–387, 14 mar. 2012.

LI, X.; LIU, F. Drought Stress Memory and Drought Stress Tolerance in Plants: Biochemical and Molecular Basis. In: **Drought Stress Tolerance in Plants, Vol 1**. Cham: Springer International Publishing, 2016. p. 17–44.

LICAUSI, F.; PERATA, P. Low Oxygen Signaling and Tolerance in Plants. In: **Advances in Botanical Research**. [s.l: s.n.]. v. 50p. 139–198.

LIMAMI, A. M.; DIAB, H.; LOTHIER, J. Nitrogen metabolism in plants under low oxygen stress. **Planta**, v. 239, n. 3, p. 531–541, 27 mar. 2014.

LIN, K.-H. R. et al. Study of the root antioxidative system of tomatoes and eggplants under waterlogged conditions. **Plant Science**, v. 167, n. 2, p. 355–365, ago. 2004.

LINKEMER, G.; BOARD, J. E.; MUSGRAVE, M. E. Waterlogging effects on growth and yield components in late-planted soybean. **Crop Science**, v. 38, n. 6, p. 1576–1584, 1998.

LIU, B.; RENNENBERG, H.; KREUZWIESER, J. Hypoxia induces stem and leaf nitric oxide (NO) emission from poplar seedlings. **Planta**, v. 241, n. 3, p. 579–589, 2014.

LIU, Z. J.; GUO, Y. K.; BAI, J. G. Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. **Journal of Plant Growth Regulation**, v. 29, n. 2, p. 171–183, 23 jun. 2010.

LOGAN, B. A.; ADAMS, W. W.; DEMMIG-ADAMS, B. Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. **Functional Plant Biology**, v. 34, n. 9, p. 853, 24 set. 2007.

LORETI, E.; PERATA, P. The Many Facets of Hypoxia in Plants. **Plants**, v. 9, n. 6, p. 745, 12 jun. 2020.

LORETI, E.; STRIKER, G. G. Plant Responses to Hypoxia: Signaling and Adaptation. **Plants**, v. 9, n. 12, p. 1704, 3 dez. 2020.

LORETI, E.; VAN VEEN, H.; PERATA, P. **Plant responses to flooding stressCurrent Opinion in Plant Biology**Elsevier Ltd, , 1 out. 2016.

MAIA, L. B.; MOURA, J. J. G. How Biology Handles Nitrite. **Chemical Reviews**, v. 114, n. 10, p. 5273–5357, 28 maio 2014.

MANRIQUE-GIL, I. et al. Nitric oxide function during oxygen deprivation in physiological and stress processes. **Journal of Experimental Botany**, 25 out. 2020.

MARTINEZ-MEDINA, A. et al. Recognizing Plant Defense Priming. **Trends in Plant Science**, v. 21, n. 10, p. 818–822, out. 2016.

MAUREL, C.; VERDOUCQ, L.; RODRIGUES, O. Aquaporins and plant transpiration. **Plant Cell and Environment**, v. 39, n. 11, p. 2580–2587, 2016.

MELZER, J. M.; KLEINHOFS, A.; WARNER, R. L. Nitrate reductase regulation: Effects of nitrate and light on nitrate reductase mRNA accumulation. **MGG Molecular & General Genetics**, v. 217, n. 2–3, p. 341–346, jun. 1989.

MEN, S. et al. Effects of supplemental nitrogen application on physiological characteristics, dry matter and nitrogen accumulation of winter rapeseed (Brassica napus L.) under waterlogging stress. **Scientific Reports**, v. 10, n. 1, p. 10201, 23 dez. 2020.

MILLER, A. J. et al. Amino acids and nitrate as signals for the regulation of nitrogen acquisition. **Journal of Experimental Botany**, v. 59, n. 1, p. 111–119,

18 dez. 2007.

MIRA, M.; HILL, R. D.; STASOLLA, C. Regulation of programmed cell death by phytoglobins. **Journal of Experimental Botany**, v. 67, n. 20, p. 5901–5908, 1 out. 2016.

MITTLER, R. ROS Are Good. **Trends in Plant Science**, v. 22, n. 1, p. 11–19, 1 jan. 2017.

MITTLER, R.; BLUMWALD, E. The roles of ROS and ABA in systemic acquired acclimation. **Plant Cell**, v. 27, n. 1, p. 64–70, 2015.

MIYASHITA, Y. et al. Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. **The Plant Journal**, v. 49, n. 6, p. 1108–1121, 22 fev. 2007.

MODOLO, L. V. et al. Nitrite as the major source of nitric oxide production by Arabidopsis thaliana in response to Pseudomonas syringae. **FEBS Letters**, v. 579, n. 17, p. 3814–3820, 4 jul. 2005.

MOHN, M.; THAQI, B.; FISCHER-SCHRADER, K. Isoform-Specific NO Synthesis by Arabidopsis thaliana Nitrate Reductase. **Plants**, v. 8, n. 3, p. 67, 16 mar. 2019.

MORALES-OLMEDO, M.; ORTIZ, M.; SELLÉS, G. Effects of transient soil waterlogging and its importance for rootstock selection. **Chilean journal of agricultural research**, v. 75, p. 45–56, 1 ago. 2015.

MORARD, P. et al. Nitrate uptake and nitrite release by tomato roots in response to anoxia. **Journal of Plant Physiology**, v. 161, n. 7, p. 855–865, jul. 2004.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, dez. 2008a.

MURSHED, R.; LOPEZ-LAURI, F.; SALLANON, H. Microplate quantification of enzymes of the plant ascorbate–glutathione cycle. **Analytical Biochemistry**, v. 383, n. 2, p. 320–322, 15 dez. 2008b.

MUSTAFA, G.; KOMATSU, S. Quantitative proteomics reveals the effect of protein glycosylation in soybean root under flooding stress. **Frontiers in Plant Science**, v. 5, n. NOV, p. 627, 18 nov. 2014.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 2015a.

MUTAVA, R. N. et al. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. **Plant Physiology and Biochemistry**, v. 86, p. 109–120, 1 jan. 2015b.

NAKANO, Y.; ASADA, K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. **Plant and Cell Physiology**, v. 22,

n. May, p. 867–880, 1981.

NEILL, S. J.; DESIKAN, R.; HANCOCK, J. T. Nitric oxide signalling in plants. **New Phytologist**, v. 159, n. 1, p. 11–35, 1 jul. 2003.

NUNES MENOLLI LANZA, L.; FERREIRA LANZA, D. C.; SODEK, L. Utilization of 15NO3- by nodulated soybean plants under conditions of root hypoxia. **Physiology and Molecular Biology of Plants**, v. 20, n. 3, p. 287–293, 2014.

OLIVEIRA, H. C.; FRESCHI, L.; SODEK, L. Nitrogen metabolism and translocation in soybean plants subjected to root oxygen deficiency. **Plant Physiology and Biochemistry**, v. 66, p. 141–149, maio 2013.

OLIVEIRA, H. C.; SODEK, L. Effect of oxygen deficiency on nitrogen assimilation and amino acid metabolism of soybean root segments. **Amino Acids**, v. 44, n. 2, p. 743–755, 19 fev. 2013.

OOSTERHUIS, D. M. et al. Physiological responses of two soybean [Glycine max (L.) Merr] cultivars to short-term flooding. **Environmental and Experimental Botany**, v. 30, n. 1, p. 85–92, 1 jan. 1990.

PARRA, M. et al. Increasing plant vigour and tomato fruit yield under salinity by inducing plant adaptation at the earliest seedling stage. **Environmental and Experimental Botany**, v. 60, n. 1, p. 77–85, 1 maio 2007.

PEDÓ, T. et al. Physiological attributes, growth and expression of vigor in soybean seeds under soil waterlogging. **African Journal of Agricultural Research**, v. 10, n. 39, p. 3791–3797, 2015.

PERAZZOLLI, M. et al. Arabidopsis Nonsymbiotic Hemoglobin AHb1 Modulates Nitric Oxide Bioactivity. **The Plant Cell**, v. 16, n. 10, p. 2785–2794, 1 out. 2004.

PETERMAN, E. J. et al. Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. **Biophysical Journal**, v. 69, n. 6, p. 2670–2678, 1995.

PHUKAN, U. J.; MISHRA, S.; SHUKLA, R. K. Waterlogging and submergence stress: affects and acclimation. **Critical Reviews in Biotechnology**, v. 36, n. 5, p. 956–966, 2 set. 2016.

PLANCHET, E. et al. Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. **The Plant Journal**, v. 41, n. 5, p. 732–743, 2 fev. 2005.

PODSĘDEK, A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. **LWT - Food Science and Technology**, v. 40, n. 1, p. 1–11, 1 jan. 2007.

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. 212, 1 dez. 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. 117, 18 jul. 2020.

PUCCIARIELLO, C. et al. Plant responses to flooding. **Frontiers in Plant Science**, v. 5, n. MAY, p. 226, 23 maio 2014.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. **Plant, Cell & Environment**, v. 40, n. 4, p. 473–482, 13 abr. 2017a.

PUCCIARIELLO, C.; PERATA, P. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. . 13 abr. 2017 b, p. 473–482.

PUYANG, X. et al. Antioxidant responses to waterlogging stress and subsequent recovery in two Kentucky bluegrass (Poa pratensis L.) cultivars. **Acta Physiologiae Plantarum**, v. 37, n. 10, p. 197, 8 out. 2015.

R CORE TEAM. **A Language and Environment for Statistical ComputingR Foundation for Statistical Computing**Vienna, AustriaR Foundation for Statistical Computing, , 2020. Disponível em: <https://www.r-project.org/>. Acesso em: 27 dez. 2020

RAHANTANIAINA, M.-S. et al. Glutathione oxidation in response to intracellular H 2 O 2 : Key but overlapping roles for dehydroascorbate reductases. **Plant Signaling & Behavior**, v. 12, n. 8, p. e1356531, 3 ago. 2017.

REDDY, A. S. N. et al. Coping with Stresses: Roles of Calcium- and Calcium/Calmodulin-Regulated Gene Expression. **The Plant Cell**, v. 23, n. 6, p. 2010–2032, 1 jun. 2011.

REGGIANI, R. et al. Accumulation and Interconversion of Amino Acids in Rice Roots under Anoxia. **Plant and Cell Physiology**, v. 29, n. 6, p. 981–987, 1 set. 1988.

REGGIANI, R.; BERTINI, F.; MATTANA, M. Incorporation of nitrate nitrogen in rice seedlings transferred to anaerobic conditions. **Amino Acids**, v. 13, n. 2, p. 183–188, jun. 1997.

RHINE, M. D. et al. Yield and nutritional responses to waterlogging of soybean cultivars. **Irrigation Science**, v. 28, n. 2, p. 135–142, 8 jan. 2010.

RICOULT, C.; CLIQUET, J.-B.; LIMAMI, A. M. Stimulation of alanine amino transferase (AlaAT) gene expression and alanine accumulation in embryo axis of the model legume Medicago truncatula contribute to anoxia stress tolerance. **Physiologia Plantarum**, v. 123, n. 1, p. 30–39, 1 jan. 2005.

ROBERTS, J. K. et al. Mechanisms of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. **Proceedings of the National Academy of Sciences of the United States of America**, v. 81, n. 11, p. 3379–3383, 1 jun. 1984.

ROCHA, M. et al. Glycolysis and the Tricarboxylic Acid Cycle Are Linked by Alanine Aminotransferase during Hypoxia Induced by Waterlogging of Lotus japonicus. **Plant Physiology**, v. 152, n. 3, p. 1501–1513, mar. 2010a.

ROCHA, M. et al. Analysis of alanine aminotransferase in various organs of soybean (Glycine max) and in dependence of different nitrogen fertilisers during hypoxic stress. **Amino Acids**, v. 39, n. 4, p. 1043–1053, 23 out. 2010b.

ROLLAND, F.; BAENA-GONZALEZ, E.; SHEEN, J. SUGAR SENSING AND SIGNALING IN PLANTS: Conserved and Novel Mechanisms. **Annual Review of Plant Biology**, v. 57, n. 1, p. 675–709, jun. 2006.

SAHA, R. et al. Physiological and biochemical changes in waterlog tolerant sesame genotypes. **SAARC Journal of Agriculture**, v. 14, n. 2, p. 31–45, 23 jan. 2017.

SAIRAM, R. K. et al. Physiology and biochemistry of waterlogging tolerance in plants. **Biologia plantarum**, v. 52, n. 3, p. 401–412, 1 set. 2008.

SAIRAM, R. K. et al. Waterlogging-induced increase in sugar mobilization, fermentation, and related gene expression in the roots of mung bean (Vigna radiata). **Journal of Plant Physiology**, v. 166, n. 6, p. 602–616, 1 abr. 2009.

SARKER, U.; OBA, S. Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected Amaranthus tricolor under salinity stress. **Scientific Reports**, v. 8, n. 1, 1 dez. 2018.

SASIDHARAN, R.; VOESENEK, L. A. C. J. Ethylene-Mediated Acclimations to Flooding Stress 1. v. 169, n. September, p. 3–12, 2015.

SCHÖFFEL, E. R. et al. EXCESSO HÍDRICO SOBRE OS COMPONENTES DO RENDIMENTO DA CULTURA DA SOJA. **Ciência Rural**, v. 31, n. 1, p. 7–12, 2001.

SHARMA, P. et al. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. **Journal of Botany**, v. 2012, p. 1–26, 24 abr. 2012.

SHARMA, R. et al. Recent Advances in Dissecting Stress-Regulatory Crosstalk in Rice. **Molecular Plant**, v. 6, n. 2, p. 250–260, 1 mar. 2013.

SILVA, T. A. DA et al. Condicionamento fisiológico de sementes de soja, componentes de produção e produtividade. **Ciência Rural**, v. 46, n. 2, p. 227–232, fev. 2016.

SINGH, N.; BHATLA, S. C. Hemoglobin as a probe for estimation of nitric oxide emission from plant tissues. **Plant Methods**, v. 15, n. 1, p. 39, 23 dez. 2019.

SINGH, S. K.; RAJA REDDY, K. Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (Vigna unguiculata [L.] Walp.) under drought. **Journal of Photochemistry and Photobiology B: Biology**, v. 105, n. 1, p. 40–50, 5 out. 2011.

SOARES, M. M. et al. Estresse hídrico e salino em sementes de soja classificadas em diferentes tamanhos. **Pesquisa Agropecuaria Tropical**, 2015.

SOJKA, R.; SCOTT, H. Aeration Measurement. In: **Encyclopedia of Soil Science, Second Edition**. [s.l.] CRC Press, 2005.

SOSBAI, S. S.-B. D. A. I. **ARROZ IRRIGADO: Recomendações Técnicas da Pesquisa para o Sul do Brasil**. XXXII REUNIÃO TÉCNICA DA CULTURA DO ARROZ IRRIGADO. **Anais**...Farroupilha- RS- Brasil: 2018Disponível em:

<http://www.sosbai.com.br/>. Acesso em: 15 abr. 2020

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002a.

SOUSA, C. A. F. DE; SODEK, L. The metabolic response of plants to oxygen deficiency. **Brazilian Journal of Plant Physiology**, v. 14, n. 2, p. 83–94, ago. 2002b.

SOUZA, R. P. et al. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. **Environmental and Experimental Botany**, v. 51, n. 1, p. 45–56, fev. 2004.

SOUZA, S. C. R.; MAZZAFERA, P.; SODEK, L. Flooding of the root system in soybean: biochemical and molecular aspects of N metabolism in the nodule during stress and recovery. **Amino Acids**, v. 48, n. 5, p. 1285–1295, 29 maio 2016.

SPRINGER, B. et al. The Shrunken gene on chromosome 9 of Zea mays L is expressed in various plant tissues and encodes an anaerobic protein. **MGG Molecular & General Genetics**, v. 205, n. 3, p. 461–468, dez. 1986.

STOIMENOVA, M. et al. The role of nitrate reduction in the anoxic metabolism of roots II. Anoxic metabolism of tobacco roots with or without nitrate reductase activity. **Plant and Soil**, v. 253, n. 1, p. 155–167, jun. 2003.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, 6 jun. 2007a.

STOIMENOVA, M. et al. Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. **Planta**, v. 226, n. 2, p. 465–474, jul. 2007b.

SULLIVAN, M. et al. Evaluating On-Farm Flooding Impacts on Soybean. **Crop Science**, v. 41, n. 1, p. 93–100, 1 jan. 2001.

SUZUKI, N. et al. Abiotic and biotic stress combinations. **New Phytologist**, 2014.

SWEETLOVE, L. J. et al. Lactate metabolism in potato tubers deficient in lactate dehydrogenase activity. **Plant, Cell & Environment**, v. 23, n. 8, p. 873–881, 25 ago. 2000.

TAIZ, L.; ZEIGER, E. **Plant Physiology. 3rd edn.** [s.l: s.n.]. v. 3

TAKAHAMA, U. Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: Physiological significance of the oxidation reactions. **Phytochemistry Reviews**, v. 3, n. 1–2, p. 207–219, jan. 2004.

TAMANG, B.; FUKAO, T. Plant Adaptation to Multiple Stresses during Submergence and Following Desubmergence. **International Journal of Molecular Sciences**, v. 16, n. 12, p. 30164–30180, 17 dez. 2015.

TAN, W. et al. Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. **Photosynthetica**, v. 46, n. 1, p. 21–27, mar. 2008.

TANG, B. et al. Changes of Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Waterlogging-Tolerant and Waterlogging-Sensitive Maize Genotypes at Seedling Stage. **Agricultural Sciences in China**, v. 9, n. 5, p. 651–661, 1 maio 2010.

TESNIERE, C. et al. Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. **Journal of Experimental Botany**, v. 57, n. 1, p. 91–99, 2006.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016a. v. 2p. 23–40.

TEWARI, S.; ARORA, N. K. Soybean Production Under Flooding Stress and Its Mitigation Using Plant Growth-Promoting Microbes. In: **Environmental Stresses in Soybean Production**. [s.l.] Elsevier, 2016b. v. 2p. 23–40.

TEWARI, S.; MISHRA, A. Flooding Stress in Plants and Approaches to Overcome. In: **Plant Metabolites and Regulation Under Environmental Stress**. [s.l.] Elsevier, 2018. p. 355–366.

THOMAS, A. L.; GUERREIRO, S. M. C.; SODEK, L. Aerenchyma Formation and Recovery from Hypoxia of the Flooded Root System of Nodulated Soybean. **Annals of Botany**, v. 96, n. 7, p. 1191–1198, 1 dez. 2005.

THOMAS, A. L.; SODEK, L. Amino acid and ureide transport in the xylem of symbiotic soybean plants during short-term flooding of the root system in the presence of different sources of nitrogen. **Brazilian Journal of Plant Physiology**, v. 18, n. 2, p. 333–339, jun. 2006.

THOMASHOW, M. F. Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. **Plant Physiology**, v. 154, n. 2, p. 571–577, 2010.

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, 1 jun. 2019a.

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, 1 jun. 2019b.

TIMILSINA, A. et al. Potential Pathway of Nitrous Oxide Formation in Plants. **Frontiers in Plant Science**, v. 11, 31 jul. 2020.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012a.

TOUGOU, M. et al. Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. **Plant Biotechnology**, v. 29, n. 3, p. 301–305, 2012b.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003a.

TOURNAIRE-ROUX, C. et al. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. **Nature**, v. 425, n. 6956, p. 393–397, 25 set. 2003b.

TREWAVAS, A. Aspects of Plant Intelligence. **Annals of Botany**, v. 92, n. 1, p. 1–20, 9 maio 2003.

TREWAVAS, A. Green plants as intelligent organisms. **Trends in Plant Science**, v. 10, n. 9, p. 413–419, 1 set. 2005.

TRIPATHI, B. N.; MÜLLER, M. **Stress Responses in Plants**. Cham: Springer International Publishing, 2015.

UDVARDI, M.; POOLE, P. S. Transport and Metabolism in Legume-Rhizobia Symbioses. **Annual Review of Plant Biology**, v. 64, n. 1, p. 781–805, 29 abr. 2013.

UNGER, P. W.; KASPAR, T. C. Soil Compaction and Root Growth: A Review. **Agronomy Journal**, v. 86, n. 5, p. 759–766, 1 set. 1994.

UNITED STATES DEPARTMENT OF AGRICULTURE. **World Agricultural Production**. [s.l: s.n.].

VAN AMERONGEN, H.; CHMELIOV, J. Instantaneous switching between different modes of non-photochemical quenching in plants. Consequences for increasing biomass production. **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, v. 1861, n. 4, p. 148119, 1 abr. 2020.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015a.

VAN DONGEN, J. T.; LICAUSI, F. Oxygen Sensing and Signaling. **Annual Review of Plant Biology**, v. 66, n. 1, p. 345–367, 29 abr. 2015b.

VAN HANDEL, E. Direct microdetermination of sucrose. **Analytical Biochemistry**, v. 22, n. 2, p. 280–283, 1 fev. 1968.

VAN NGUYEN, L. et al. Mapping quantitative trait loci for root development under hypoxia conditions in soybean (Glycine max L. Merr.). **Theoretical and Applied Genetics**, v. 130, n. 4, p. 743–755, 1 abr. 2017.

VANDOORNE, B. et al. Long term intermittent flooding stress affects plant growth and inulin synthesis of Cichorium intybus (var. sativum). **Plant and Soil**, v. 376, n. 1, p. 291–305, 29 nov. 2014.

VELIKOVA, V.; YORDANOV, I.; EDREVA, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. **Plant Science**, v. 151, n. 1, p. 59–66, 2002.

VITOR, S. C.; SODEK, L. Products of anaerobic metabolism in waterlogged roots of soybean are exported in the xylem. **Plant Science**, v. 284, p. 82–90, 1 jul. 2019.

VOESENEK, L. Flooding tolerance : O 2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flooding tolerance: O2 sensing and survival strategies. **Current Opinion in Plant Biology**, v. 16, n. 5, p. 647–653, 2013.

VOESENEK, L. A. C. J.; BAILEY-SERRES, J. Flood adaptive traits and processes: an overview. **New Phytologist**, v. 206, n. 1, p. 57–73, 7 abr. 2015.

VRIET, C.; HENNIG, L.; LALOI, C. Stress-induced chromatin changes in plants: of memories, metabolites and crop improvement. **Cellular and Molecular Life Sciences**, v. 72, n. 7, p. 1261–1273, 13 abr. 2015.

WALTER, J. et al. Do plants remember drought? Hints towards a drought-memory in grasses. **Environmental and Experimental Botany**, v. 71, n. 1, p. 34–40, 1 abr. 2011.

WALTER, J. et al. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. **Environmental and Experimental Botany**, v. 94, p. 3–8, 1 out. 2013.

WANG, X. et al. Physiological and proteomic mechanisms of waterlogging priming improves tolerance to waterlogging stress in wheat ( Triticum aestivum L.). **Environmental and Experimental Botany**, v. 132, p. 175–182, dez. 2016.

WANG, X.; LIU, F.; JIANG, D. Priming: A promising strategy for crop production in response to future climate. **Journal of Integrative Agriculture**, v. 16, n. 12, p. 2709–2716, dez. 2017.

WANY, A. et al. Nitrate nutrition influences multiple factors in order to increase energy efficiency under hypoxia in Arabidopsis. **Annals of Botany**, v. 123, n. 4, p. 691–705, 14 mar. 2019.

WANY, A.; FOYER, C. H.; GUPTA, K. J. Nitrate, NO and ROS Signaling in Stem Cell Homeostasis. **Trends in Plant Science**, v. 23, n. 12, p. 1041–1044, 1 dez. 2018.

WANY, A.; KUMARI, A.; GUPTA, K. J. Nitric oxide is essential for the development of aerenchyma in wheat roots under hypoxic stress. **Plant Cell and Environment**, v. 40, n. 12, p. 3002–3017, 1 dez. 2017.

WEI, W. et al. Morpho-anatomical and physiological responses to waterlogging of sesame (Sesamum indicum L.). **Plant Science**, v. 208, p. 102–111, 1 jul. 2013.

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, n. 3, p. 307–313, 1994.

YEMM, E. W.; COCKING, E. C.; RICKETTS, R. E. The determination of amino-acids with ninhydrin. **The Analyst**, v. 80, n. 948, p. 209–214, 1 jan. 1955.

YETISIR, H.; MEHMET, E. C. Some physiological and growth responses of watermelon [ C itrullus lanatus ( Thunb .) Matsum . and Nakai ] grafted onto

Lagenaria siceraria to flooding. **Environmental and Experimental Botany**, v. 58, p. 1–8, 2006.

YIU, J.-C. et al. Changes in antioxidant properties and their relationship to paclobutrazol- induced flooding tolerance in Welsh onion. **Journal of the Science of Food and Agriculture**, v. 88, n. 7, p. 1222–1230, maio 2008.

YORDANOVA, R. Y.; CHRISTOV, K. N.; POPOVA, L. P. Antioxidative enzymes in barley plants subjected to soil flooding. **Environmental and Experimental Botany**, v. 51, n. 2, p. 93–101, 2004.

YU, F. et al. Comparative proteomic analysis revealing the complex network associated with waterlogging stress in maize ( *Zea mays* L.) seedling root cells. **PROTEOMICS**, v. 15, n. 1, p. 135–147, 1 jan. 2015.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, fev. 2009a.

ZABALZA, A. et al. Regulation of Respiration and Fermentation to Control the Plant Internal Oxygen Concentration. **Plant Physiology**, v. 149, n. 2, p. 1087–1098, 1 fev. 2009b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, p. 290–298, 2019a.

ZHANG, J. et al. Sedum mexicanum ‘Gold Mound’ exhibits better adaptive characters in contrast to S. spurium ‘Coccineum’ when subjugated to sustained waterlogging stress. **Acta Horticulturae**, v. 1263, n. 1263, p. 141–148, nov. 2019b.

ZHANG, J. et al. Modulation of Morphological and Several Physiological Parameters in Sedum under Waterlogging and Subsequent Drainage. **Russian Journal of Plant Physiology**, v. 66, n. 2, p. 290–298, 14 dez. 2019c.

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, 2017.

ZHANG, X. et al. Physiological and transcriptional analyses of induced post-anthesis thermo-tolerance by heat-shock pretreatment on germinating seeds of winter wheat. **Environmental and Experimental Botany**, v. 131, p. 181–189, nov. 2016.

ZHOU, W. et al. Plant waterlogging/flooding stress responses: From seed germination to maturation. **Plant Physiology and Biochemistry**, v. 148, p. 228–236, 1 mar. 2020.

## 4.7. Appendix A. Supplementary data

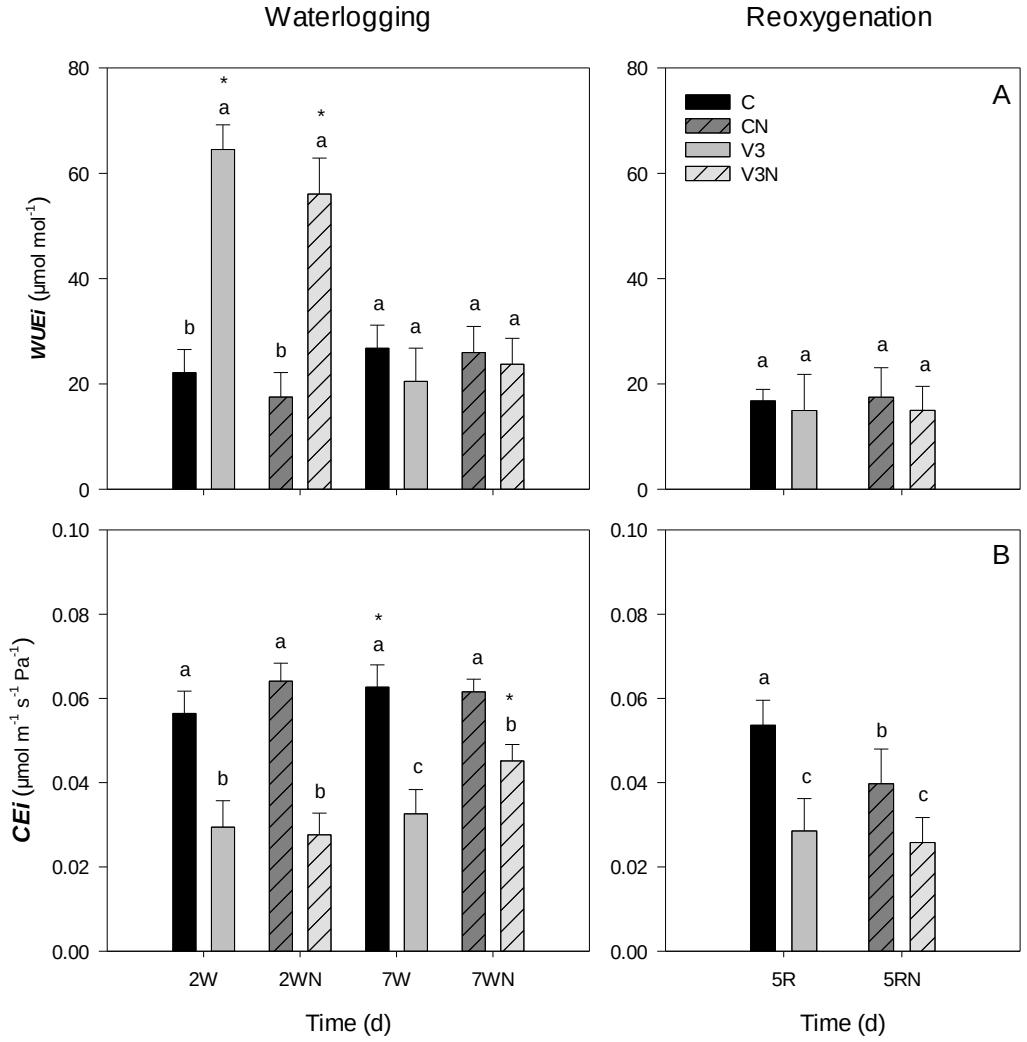


Figure S1. Intrinsic water use efficiency (WUEi) (A), and instantaneous carboxylation efficiency (CEi) (B) in leaves of soybean plants stage V3 in response to waterlogging and reoxygenation without and with nitrate feeding submitted. Non-waterlogged plants were used as a control to the waterlogged condition, while plants under waterlogging were used as a control to the reoxygenation condition. Data are mean values ± SD; n = 7. The distinguishing lowercase letters within the same time (W-WN) and asterisks indicate significant differences between two-day and seven-day stressed plants without nitrate and with nitrate feeding submitted by Tukey test ($P < 0.05$).

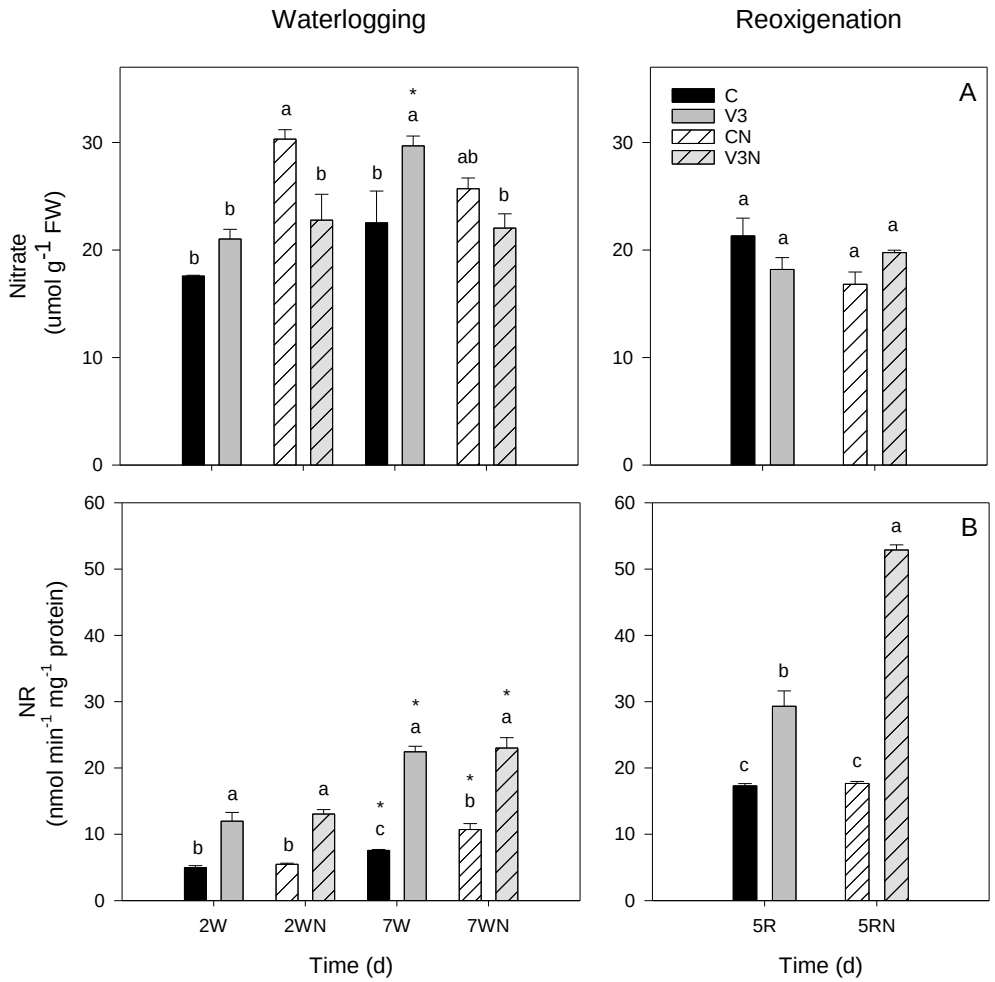


Figure S2. Levels of nitrate (A) and nitrate reductase (NR) activity (B) in leaves of soybean plants stage V3 in response to waterlogging and reoxygenation without and with nitrate feeding submitted. Non-waterlogged plants with or without nitrate feeding were used as controls. Data are mean values ± SD; n = 4. The distinct letters within the same time of waterlogging (W and WN) or re-oxygenation (R and RN) indicate significant differences between treatments by Tukey test (P=0.05) and asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t test; P < 0.05).

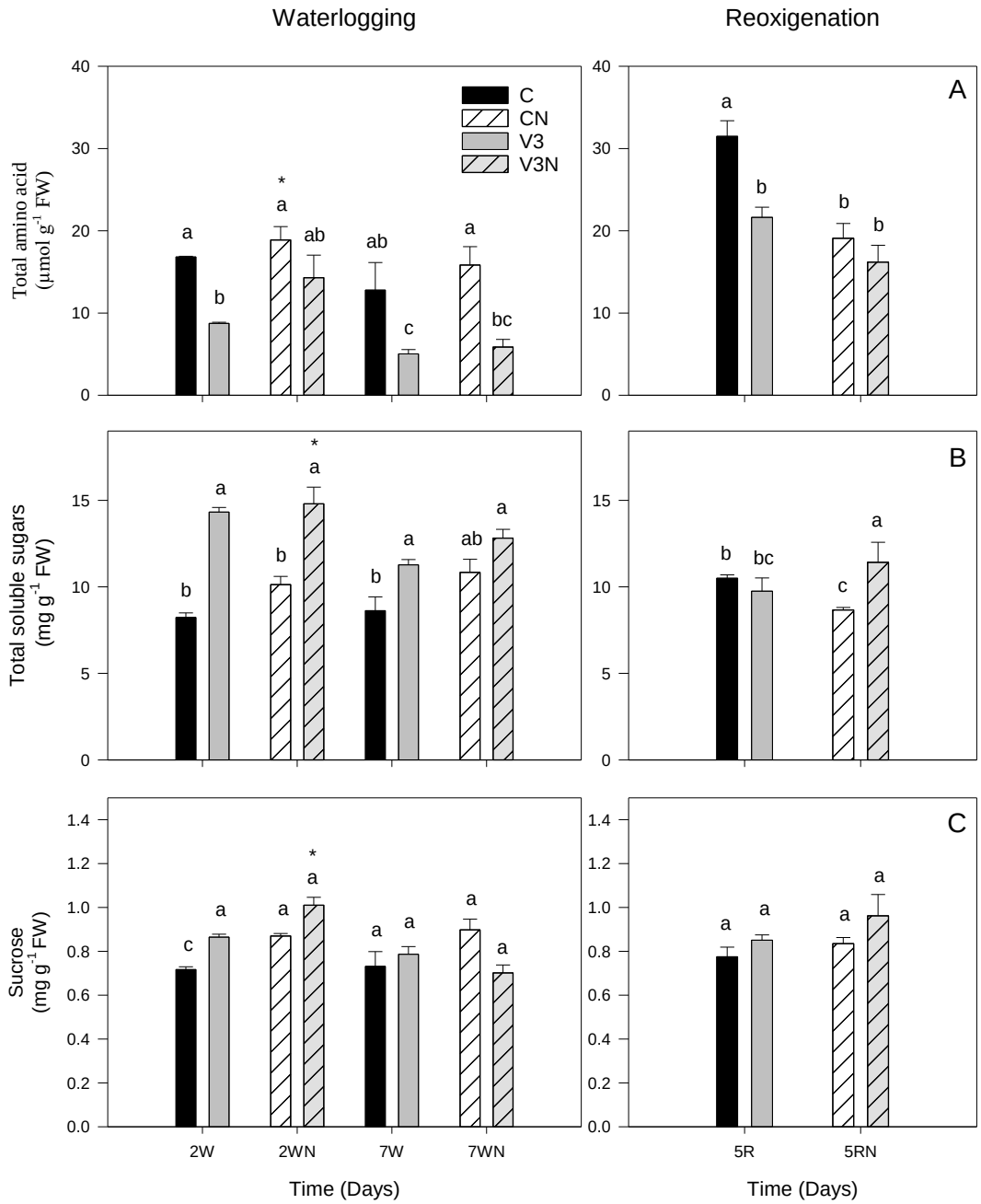


Figure S3. Levels of total amino acids (A), total soluble sugars (B) and sucrose (C) in leaves of soybean plants stage V3 in response to waterlogging and reoxygenation without and with nitrate feeding submitted. Non-waterlogged plants with or without nitrate feeding were used as controls. Data are mean values ± SD; n = 4. The distinct letters within the same time of waterlogging (W and WN) or re-oxygenation (R and RN) indicate significant differences between treatments by Tukey test (P=0.05) and asterisks indicate significant differences between two days and five days for each treatment during waterlogging (t-test; P < 0.05).

## 5. Final considerations

Under field conditions soybean plants subjected to waterlogging stress at vegetative growth stage V3 (priming treatment) and subsequently at R2 stage (V3R2) under hypoxic condition and recovery, show changes in physiological and biochemical mechanisms of hypoxia tolerance.

In soybean plants of V3R2 groups by virtue of adaptive response, yield components are increased compared to R2 plants, suggesting that priming response to initial water stress (V3 stage) caused by physiological conditioning substantially favors yield compared to non-conditioned plants although they are lower than in non-waterlogged plants.

The metabolic responses to the memory of waterlogging stress in soybean plants associated with the supply of nitrate, improves the response to transient environmental stimuli (recurrent waterlogging) being also a factor to be considered to trigger tolerance to waterlogging when applied alone.

The V3R2 and V3R2N groups showed that both recurrent stress priming and nitrate application improved hypoxia tolerance in relation to gas exchange parameters, antioxidant enzyme activity, hypoxic metabolism, allocation of carbohydrates and nitrogenous compounds.

A broad and integrated understanding of the biochemical and molecular mechanisms of soybean (Glycine max L. Merr) plants in response to waterlogging stress will ultimately allow us to improve stress tolerance in this crop by applying them in new practices. One possibility could be to exploit stress priming mechanisms to induce a constitutive state, thereby increasing the ability of a crop to tolerate stress and disease without incurring penalties for biomass accumulation and yield.