RESSALVA

Atendendo solicitação do(a) autor(a), o texto completo desta dissertação será disponibilizado somente a partir de 13/10/2023.

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

NICKEL ENHANCES FLAVONOIDS BIOSYNTHESIS AND BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS

Marco Antonio Bosse
Engenheiro Agrônomo

2021

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

NICKEL ENHANCES FLAVONOIDS BIOSYNTHESIS AND BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS

Discente: Marco Antonio Bosse

Orientador: Prof. Dr. André Rodrigues dos Reis

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Agronomia (Produção Vegetal).

2021

B745n

Bosse, Marco Antonio

Nickel enhances flavonoids biosynthesis and biological nitrogen fixation in soybean plants / Marco Antonio Bosse. -- Jaboticabal, 2021

112 p. : il., tabs.

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: André Rodrigues dos Reis

1. Fotossíntese. 2. Urease. 3. Glycine max. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: NICKEL ENHANCES FLAVONOIDS BIOSYNTHESIS AND BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS

AUTOR: MARCO ANTÔNIO BOSSE
ORIENTADOR: ANDRÉ RODRIGUES DOS REIS

Aprovado como parte das exigências para obtenção do Título de Mestre em AGRONOMIA (PRODUÇÃO VEGETAL), pela Comissão Examinadora:

André Rodrigues dos Reis
Prof. Dr. ANDRÉ RODRIGUES DOS REIS (Participaçao Virtual)
Departamento de Engenharia de Biossistemas / Faculdade de Ciencias e Engenharia de Tupa UNESP
André Rodrigues dos Reis
Prof. Dr. PAULO MAZZAFERA (Participaçao Virtual)
Departamento de Fisiologia Vegetal / Universidade Estadual de Campinas UNICAMP - Campinas/SP
André Rodrigues dos Reis
Prof. Dr. FELIPE KLEIN RICACHENEVSKY (Participaçao Virtual)
Universidade Federal do Rio Grande do Sul/UFRGS / Porto Alegre/RS

Jaboticabal, 13 de outubro de 2021

DADOS CURRICULARES DO AUTOR

MARCO ANTONIO BOSSE - Nascido em Três Lagoas, Mato Grosso do Sul, no dia 26 de março de 1993. Em 2017, graduou-se em Engenharia Agronômica pela Universidade Tecnológica Federal do Paraná, Câmpus Pato Branco, PR. Durante a graduação participou do programa Ciências sem Fronteiras, realizando intercâmbio acadêmico na University of Minnesota, nos Estados Unidos da América. Em agosto de 2019, ingressou no Mestrado do Programa de Pós-Graduação em Agronomia (Produção Vegetal), da Universidade Estadual Paulista, Câmpus Jaboticabal, sob a orientação do Prof. Dr. André Rodrigues dos Reis.

DEDICO

Aos meus pais, Antonio e Vilmira Bosse,

e a toda minha família por todo o amor e apoio.

AGRADECIMENTOS

À Deus, agradeço por tudo em minha vida.

Aos meus pais pelo apoio e amor incondicional a mim dispensado. A minha irmã Suellen, por todas as conversar e conselhos.

Ao meu orientador Prof. Dr. André Rodrigues dos Reis, pela oportunidade e por toda a dedicação e conhecimento compartilhados ao longo dessa jornada e que foram essenciais para minha formação.

Aos meus colegas e professores da Pós-graduação pelos conhecimentos compartilhados.

Aos amigos do grupo de estudos em fisiologia agrícola Andressa Mello, Maria Gabriela Dantas, Vinicius Martins Silva, Maycon Araujo, Lara Caroline Alves de Oliveira e a Ana Carolina Roque Cardoso, por toda a ajuda e companheirismo.

A minha cara colega Nandhara Mendes, que foi mais que uma amiga, foi uma mentora e que me ensinou e auxiliou incansavelmente durante a execução dos experimentos.

Ao meu grande amigo Marcelo Pinotti e família que me acolheram e ajudaram durante todo o tempo e que me trouxeram conforto nos momentos de aflição.

Aos meus queridos amigos Mirella e Gilmar, que foram grandes inspirações para mim.

À todos os servidores da UNESP, em especial a Priscilla, Regiane, Yasmin e Jéssica pela atenção e assistência.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo apoio financeiro.

À todos que, de alguma forma, contribuíram para a realização deste trabalho, meus sinceros agradecimentos.

SUMÁRIO

NÍQUEL AUMENTA A SÍNTESE DE FLAVONOIDES E FIXAÇÃO BIOLÓGICA DE NITROGÊNIO NA SOJA

RESUMO – Níquel (Ni) é um elemento essencial para o metabolismo de nitrogênio (N) e pode afetar diversos processos fisiológicos desde a germinação até o crescimento vegetativo, visto que é um componente estrutural das enzimas urease e [NiFe]-hidrogenase, possuindo um papel fundamental na fixação biológica de nitrogênio (FBN) em leguminosas. Portanto, o presente estudo teve como objetivo investigar os efeitos da fertilização com Ni na atividade da enzima urease, no perfil de flavonoides, nodulação, metabolismo de ureídeos e antioxidante, e o seu impacto na germinação e produtividade da planta. Nós testamos três doses de Ni (0.0, 1,5, e 3,0 mg kg^{-1}) e dois genótipos de soja: cultivar comercial TMG 2158, e uma *near-isogenic lines* (NILs), urease-nula (*eu3-a).* Ni aumentou a atividade da urease, convertendo ureia em amônia nas folhas. Ni também aumentou os níveis de daidzeína, genisteína, rutina e kaempferol nas raízes e a nodulação, principalmente para o genótipo TMG 2158. Houve correlação entre os teores de daidzeína, nodulação, ureídeos e produtividade em resposta a fertilização com Ni, exceto para os NILs. Esses resultados sugerem que o Ni também desempenha um papel no metabolismo de flavonoides (principalmente daidzeína), contribuindo para a quimiotaxia dos rizóbios, o que resulta em um aumento de nodulação e metabolismo de ureídeos e maior quantidade de nitrogênio orgânico disponível para as plantas de soja, resultando em maior produtividade. Além disso, a fertilização com Ni aumentou o teor de açúcares totais de todos os genótipos de soja. A fertilização com Ni também levou à diminuição da peroxidação lipídica das membranas celulares do genótipo TMG 2159*,* provavelmente devido ao aumento da atividade de APX, que desempenhou um papel importante na eliminação de H_2O_2. Em relação à reserva de Ni nas sementes, Ni melhorou a germinação e o desenvolvimento das plântulas por meio do aumento da atividade da urease e da remobilização do N, embora esses resultados sejam altamente dependentes do genótipo da soja.

Palavras-chave: atividade da urease, daidzeína, fotossíntese, metabolismo antioxidante, ureídeos.

NICKEL ENHANCES FLAVONOIDS BIOSYNTHESIS AND BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS

ABSTRACT – Nickel (Ni) is an essential element to N metabolism and affects a wide range of physiological processes, from seed germination to vegetative growth, since it is a structural component of urease and [NiFe]-hydrogenase, playing an important role during biological nitrogen fixation (BNF) in legume plants. Therefore, this study aimed to investigate the effects of Ni fertilization on urease activity, flavonoid profile, nodulation, ureide and antioxidant metabolism and its impact on seed germination and plant yield. We tested three Ni doses (0.0, 1.5, and 3.0 mg kg^{-1}) and three soybean genotypes (commercial soybean (TMG 2158), and one soybean near-isogenic lines (NILs), urease-null (*eu3-a*). Ni increased urease activity, breaking down urea into ammonia in the leaves. Ni enhanced daidzein, genistein, rutin and kaempferol in roots and nodulation mainly for TMG 2158 plants. There was a relationship between daidzein, nodulation, ureides and seed yield in response to Ni fertilization, except for NILs. These results suggest that Ni plays a role in flavonoids metabolism (mainly daidzein) inducing chemotaxis between soybean plants and rhizobia, boosting nodulation and ureide metabolism. Enhanced biological nitrogen fixation induced by Ni fertilization promoted higher nodulation generating more organic nitrogen available to soybean plants resulting in a higher number of pods per plant and seed yield. Furthermore, Ni fertilization increased total sugars content of all soybean genotypes. Ni fertilization decreased lipid peroxidation of cell membranes of genotype TMG 2159, probably due to an increase in APX activity, which played an important role in H_2O_2 scavenging. Ni also improved seed germination and seedling development by increasing urease activity and N remobilization, although it is highly dependent on the soybean genotype.

Keywords: antioxidant metabolism, daidzein, photosynthesis, urease activity, ureides.

CHAPTER 1 – General considerations

1. Introduction

Nickel (Ni) is essential for plants, since it is a cofactor that is part of the active site of urease enzyme (Dixon et al., 1975). In addition, Ni is also an essential cofactor of many other enzymes, such as hydrogenase, which is present in diazotrophic bacteria (Bagyinka, 2014) that are directly involved in nitrogen metabolism.

Urease is responsible for catalyzing the hydrolysis of urea, producing ammonia and carbon dioxide (Witte, 2011), while hydrogenase catalyzes the oxidation of molecular hydrogen (H_2), generated during the process of biological nitrogen fixation (BNF) by the enzyme nitrogenase, recovering a certain amount of energy required in this process, as well as reducing the competition between H_2 and nitrogen gas (N_2) from the nitrogenase active site (González-Guerrero et al., 2014), resulting in a more efficient BNF.

Although Ni deficiency in field-grown plants is not observed often, recent studies show latent Ni deficiency in modern varieties of soybean plants, which did not exhibit visual symptoms in leaves. (Freitas et al., 2019). The authors demonstrated that low Ni concentrations benefit N metabolism in soybean plants, resulting in increased seed yield. The authors also found that Ni fertilization caused increased number of nodules; however, the mechanisms involved in this process have not been elucidated.

The first step in nodule formation between plants and diazotrophic bacteria is the formation of a chemical gradient formed by different metabolites exuded by the roots, such as organic acids and amino acid sugars, which attract rhizobia by chemotaxis. Another essential compound for nodulation are flavonoids, which are responsible for inducing the expression of *nod* genes in bacteria, which will result in root infection and nodule formation (Barbour et al., 1991; Kape et al., 1991; Hirsch, 1992). However, there are still few studies investigating the effect of Ni on urease activity in relation to the nodulation process, especially in the metabolism of flavonoids.

Urease is not only involved in the remobilization and assimilation of N in plants, but also in plant defense against pathogens such as fungi and insects. (Carlini and Polacco, 2008; Stanisçuaski and Carlini, 2012; Medeiros-Silva et al., 2014). Furthermore, recent studies have shown that Ni can also act in the antioxidant metabolism of plants by activating the isoform of glyoxylase I, which plays an important role in the degradation of methylglyoxal (MG), a toxic compound naturally produced by cell metabolism (Fabiano et al., 2015). Thus, further investigations are needed in order to elucidate the effects of Ni in beneficial doses on the antioxidant system of plants, since most studies are focused on the toxicity effects of this micronutrient.

This study aimed to evaluate the effects of Ni on urease enzyme activity and its influence on flavonoid profile, photosynthesis, antioxidant metabolism, as well as the effect of Ni contents in soybean grain on germination using mutant seeds for the urease enzyme.

5. Conclusion

Ni fertilization enhance photosynthetic apparaturus, sugar concentration and ROS scavenging systems in soybean plants. Oxidative stress was mitigated by Ni fertilization by decreasing lipid peroxidation governed by boosted APX activity, which played an important role in H_2O_2 scavenging.

Soybean plants fertilized with Ni showed higher number of pods, seed per plants and higher seed yield. Plants treated with Ni showed an enhanced seed germination and seedling development by increasing urease activity and N remobilization, although it is highly dependent on the soybean genotype. These results show important information demonstrating that Ni plays critical roles on soybean photosynthetic and antioxidant system, seed yield and physiological quality and vigour of soybean seeds.

Author contribution

ARR designed the experiment, MAB performed the experiments, biochemical analysis wrote the main text and performed the statistical analysis, NACM performed the biochemical analysis, ARR and NACM revised the manuscript.

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant number 148351/2019-0).

6. References

Alexieva V, Sergiev I, Mapelli S, Karanov E. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment 24: 1337–1344.

Asada K. 1992. Ascorbate peroxidase – a hydrogen peroxide-scavenging enzyme in plants. Physiologia Plantarum 85: 235–241.

Azevedo RA, Alas RM, Smith RJ, Lea PJ. 1998. 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 104: 280–292.

Bagyinka C. 2014. How does the ([NiFe]) hydrogenase enzyme work? International Journal of Hydrogen Energy.

Baral B, Teixeira da Silva JA, Izaguirre-Mayoral ML. 2016. Early signaling, synthesis, transport and metabolism of ureides. Journal of Plant Physiology 193: 97–109.

Barcelos JP de Q, Osório CRW de S, Leal AJF, Alves CZ, Santos EF, Reis HPG, dos Reis AR. 2017. Effects of foliar nickel (Ni) application on mineral nutrition status, urease activity and physiological quality of soybean seeds. Australian Journal of Crop Science 11: 184–192.

Barcelos JPQ, Reis HPG, Godoy C V., Gratão PL, Furlani Junior E, Putti FF, Campos M, Reis AR. 2018. Impact of foliar nickel application on urease activity, antioxidant metabolism and control of powdery mildew (Microsphaera diffusa) in soybean plants. Plant Pathology 67: 1502–1513.

Bieleski R, Turner N. 1966. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. 293: 278–293.

Bosse MA, Silva MB da, Oliveira NGRM de, Araujo MA de, Rodrigues C, Azevedo JP de, Reis AR dos. 2021. Physiological impact of flavonoids on nodulation and ureide metabolism in legume plants. Plant Physiology and Biochemistry 166: 512–521.

Bradford MM. 1976. Rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254.

Chen C, Huang D, Liu J. 2009. Functions and toxicity of nickel in plants: Recent advances and future prospects. Clean - Soil, Air, Water 37: 304–313.

Dixon NE, Gazzola C, Blakeley RL, Zerner B. 1975. Jack Bean Urease (EC 3.5.1.5). A Metalloenzyme. A Simple Biological Role for Nickel? Journal of the American Chemical Society 97: 4131–4133.
Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Biochemistry 28: 350–356.

Fabiano CC, Tezotto T, Favarin J, Polacco JC, Mazzafera P. 2015. Essentiality of nickel in plants: A role in plant stresses. Frontiers in Plant Science 6: 2013–2016.

Farooq MA, Niazi AK, Akhtar J, Saifullah, Farooq M, Souri Z, Karimi N, Rengel Z. 2019. Acquiring control: The evolution of ROS-Induced oxidative stress and redox signaling pathways in plant stress responses. Plant Physiology and Biochemistry 141: 353–369.

Fehr WR, Caviness CE. 1977. 'Stages of soybean development' (1977). Special Report. 80. Iowa State University: 1–12.

Foyer CH, Ruban A V., Noctor G. 2017. Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochemical Journal 474: 877–883.

Freitas DS, Rodak BW, Carneiro MAC, Guilherme LRG. 2019. How does Ni fertilization affect a responsive soybean genotype? A dose study. Plant and Soil 441: 567–586.

Freitas DS, Wurr Rodak B, dos Reis A, de Barros Reis F, de Carvalho T, Schulze J, Carbone Carneiro MA, Guimarães Guilherme LR. 2018. Hidden nickel deficiency? Nickel fertilization via soil improves nitrogen metabolism and grain yield in soybean genotypes. Frontiers in Plant Science 9: 1–16.

Freyermuth SK, Bacanamwo M, Polacco JC. 2000. The soybean Eu3 gene encodes an Ni-binding protein necessary for urease activity. Plant Journal 21: 53–60.

Gajewska E, Skłodowska M, Słaba M, Mazur J. 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biologia Plantarum 50: 653–659.

Giannopolitis CN, Ries SK. 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant physiology 59: 309–14.

Gratão PL, Monteiro CC, Antunes AM, Peres LEP, Azevedo RA. 2008. Acquired tolerance of tomato (Lycopersicon esculentum cv. Micro-Tom) plants to cadmium-induced stress. Annals of Applied Biology 153: 321–333.

Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. Archives of Biochemistry and Biophysics 125: 189–198.

Hogan ME, Swift IE, Done J. 1983. Urease assay and ammonia release from leaf tissues. Phytochemistry 22: 663–667.

Hussein HAA, Darwesh OM, Mekki BB. 2019. Environmentally friendly nano-selenium to improve antioxidant system and growth of groundnut cultivars under sandy soil conditions. Biocatalysis and Agricultural Biotechnology 18: 101080.

Islam MM, Ishibashi Y, Nakagawa ACS, Tomita Y, Iwaya-Inoue M, Arima S, Zheng SH. 2016. Nitrogen redistribution and its relationship with the expression of GmATG8c during seed filling in soybean. Journal of Plant Physiology 192: 71–74.

Kutman BY, Kutman UB, Cakmak I. 2013. Nickel-enriched seed and externally supplied nickel improve growth and alleviate foliar urea damage in soybean. Plant and Soil 363: 61–75.

Kutman BY, Kutman UB, Cakmak I. 2014. Effects of seed nickel reserves or externally supplied nickel on the growth, nitrogenmetabolites and nitrogen use efficiency of urea- or nitrate-fed soybean. Plant and Soil 376: 261–276.

Lavres J, Franco GC, de Sousa Câmara GM. 2016. Soybean seed treatment with nickel improves biological nitrogen fixation and urease activity. Frontiers in Environmental Science 4: 1–11.

Lešková A, Giehl RFH, Hartmann A, Fargašová A, von Wirén N. 2017. Heavy metals induce iron deficiency responses at different hierarchic and regulatory levels. Plant Physiology 174: 1648–1668.

McCullough H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clinica Chimica Acta 17: 297–304.

Parida BK, Chhibba IM, Nayyar VK. 2003. Influence of nickel-contaminated soils on fenugreek (Trigonella corniculata L.) growth and mineral composition. Scientia Horticulturae 98: 113–119.

Piccini DF, Malavolta E. 1992. Effect of nickel on two common bean cultivars. Journal of Plant Nutrition 15: 2343–2350.

Polacco JC, Havir EA. 1979. Comparisons of soybean urease isolated from seed and tissue culture. Journal of Biological Chemistry 254: 1707–1715.

Polacco JC, Krueger RW, Winkler RG. 1985. Structure and Possible Ureide Degrading Function of the Ubiquitous Urease of Soybean. Plant Physiology 79: 794–800.

Polacco JC, Mazzafera P, Tezotto T. 2013. Opinion - Nickel and urease in plants: Still many knowledge gaps. Plant Science 199–200: 79–90.

Polacco JC, Winkler RG. 1984. Soybean Leaf Urease: A Seed Enzyme? Plant Physiology 74: 800–803.

Radin JW. 1973. In vivo Assay of Nitrate Reductase in Cotton Leaf Discs . Effect of Oxygen and Ammonium. Plant Physiology 51: 332–336.

Rainbird RM, Thorne JH, Hardy RWF. 1984. Role of Amides, Amino Acids, and Ureides in the Nutrition of Developing Soybean Seeds. Plant Physiology 74: 329–334.

Rechenmacher C, Wiebke-Strohm B, De Oliveira-Busatto LA, Polacco JC, Carlini CR, Bodanese-Zanettini MH. 2017. Effect of soybean ureases on seed germination and plant development. Genetics and Molecular Biology 40: 209–216.

Reis AR dos, de Queiroz Barcelos JP, de Souza Osório CRW, Santos EF, Lisboa LAM, Santini JMK, dos Santos MJD, Furlani Junior E, Campos M, de Figueiredo PAM, et al. 2017. A glimpse into the physiological, biochemical and nutritional status of soybean plants under Ni-stress conditions. Environmental and Experimental Botany 144: 76–87.

Rodak BW, Freitas DS, Bernardes LF, Lima GJEO, Reis AR, Lavres Junior J, Guimarães Guilherme LR. 2021. Short-term nickel residual effect in field-grown soybeans: nickel-enriched soil acidity amendments promote plant growth and safe soil nickel levels. Archives of Agronomy and Soil Science.

Ruan YL. 2014. Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biology 65: 33–67.

Sachan P, Lal N. 2017. An Overview of Nickel (Ni2+) Essentiality, Toxicity and Tolerance Strategies in Plants. Asian Journal of Biology 2: 1–15.

Silva VM, Rimoldi Tavanti RF, Gratão PL, Alcock TD, Reis AR dos. 2020. Selenate and selenite affect photosynthetic pigments and ROS scavenging through distinct mechanisms in cowpea (Vigna unguiculata (L.) walp) plants. Ecotoxicology and Environmental Safety 201: 110777.

de Souza SCR, Sodek L, Polacco JC, Mazzafera P. 2020. Urease deficiency alters nitrogen metabolism and gene expression in urease-null soybean without affecting growth or productivity under nitrate supply. Acta Physiologiae Plantarum 42: 1–11.

Tegeder M. 2014. Transporters involved in source to sink partitioning of amino acids and ureides: Opportunities for crop improvement. Journal of Experimental Botany 65: 1865–1878.

Tezotto T, Souza SCR, Mihail J, Favarin JL, Mazzafera P, Bilyeu K, Polacco JC. 2016. Deletion of the single UreG urease activation gene in soybean NIL lines: characterization and pleiotropic effects. Theoretical and Experimental Plant Physiology 28: 307–320.

Vogels GD, Van Der Drift C. 1970. Differential analyses of glyoxylate derivatives. Analytical Biochemistry 33: 143–157.

Wiebke-Strohm B, Ligabue-Braun R, Rechenmacher C, De Oliveira-Busatto LA, Carlini CR, Bodanese-Zanettini MH. 2016. Structural and transcriptional characterization of a novel member of the soybean urease gene family. Plant Physiology and Biochemistry 101: 96–104.

Witte CP. 2011. Urea metabolism in plants. Plant Science 180: 431–438.

Yemm EW, Cocking EC, Ricketts RE. 1955. The determination of amino-acids with ninhydrin. The Analyst 80: 209–214.

Zonia LE, Stebbins NE, Polacco JC. 1995. Essential role of urease in germination of nitrogen-limited Arabidopsis thaliana seeds. Plant Physiology 107: 1097–1103.