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**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL**

**NICKEL ENHANCES FLAVONOIDS BIOSYNTHESIS AND
BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS**

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BIOLOGICAL NITROGEN FIXATION IN SOYBEAN PLANTS**

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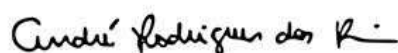
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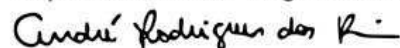
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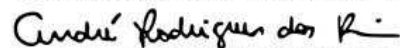
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DEDICO

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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 H₂O₂. 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 H₂O₂ 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 apparatus, 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 H₂O₂ 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.

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