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BIOCHEMICAL AND STRUCTURAL ALTERATIONS INDUCED BY SELENIUM UNDER CADMIUM STRESS IN TOMATO PLANTS

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TÍTULO DA TESE: BIOCHEMICAL AND STRUCTURAL ALTERATIONS INDUCED BY SELENIUM UNDER CADMIUM STRESS IN TOMATO PLANTS

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AUTHOR'S CURRICULUM INFORMATION

Leticia Rodrigues Alves was born in 1990 at Ribeirão Preto-SP- Brazil. She finished her course in Biological Sciences in 2013 at Federal University of Ouro Preto. During graduation, she was intern of Plant Physiology Laboratory and subsequently realized scientific initiation, under supervision of Alessandra Kozovits. Her interesting in plant physiology had increased, and during the eighth semester of graduation, she participated of Erasmus mundus, a Union European program to incentive education, which supported a scholarship at University of Porto- Portugal. By the way, she studied specifics subjects associated to plant biology, such as plant physiology complementary, plant nutrition and plant development biology. Moreover, she participated of many scientific events and received award and honor during the XIV Brazilian Plant Physiology Congress. In 2016, she concluded mastering in crop production at UNESP / FCAV – Jaboticabal, under supervision of Dra. Priscila Lupino Gratão, in which place she studied antioxidant responses in abiotic stress-conditions of stress perception and signalling in plants. In 2016, Leticia started Phd and she continued her studies in the same research line. In this period, she has ministered lectures and mini courses in universities and conferences, published five research articles and five chapters in books. Therefore, she was co-adviser of four graduation students.

DEDICATÓRIA

Dedico este trabalho a minha orientadora Profa. Dra. Priscila Lupino Gratão que nunca mediu esforços para garantir o melhor para minha formação acadêmica.

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BIOCHEMICAL AND STRUCTURAL ALTERATIONS INDUCED BY SELENIUM UNDER CADMIUM STRESS IN TOMATO PLANTS

ABSTRACT

Cadmium (Cd) contamination is a worldwide concern and one of the most severe causes of abiotic stress in plants, triggering losses in crop production and contamination risks to human health. This heavy metal increased in atmosphere due to human activities. Plants can uptake Cd, causing serious changes in structural, physiological and biochemical processes. Plants developed a complex defence systems including non-enzymatic and enzymatic mechanism to avoid oxidative stress and prevent an uncontrolled oxidation cascade. Some elements, such as selenium (Se), if used in adequate concentration, may induce an improvement in antioxidant system, growth and photosynthetic attributes. It is still unknown the mechanisms of Se in stress responses. The aim of this work was get new insights about the role of selenate and selenite-mediated detoxification strategies, including the evaluation of mineral nutrition, the activity of antioxidant enzymes and non enzymatic compounds, pigments, structural alterations and the role of Se in modulate ethylene, with the use of hormonal mutants as a tool. Our data indicates that Se is an interesting strategy to improve plant metabolism under normal or Cd stressful-condition. Selenium may induce enhancement in antioxidant defence metabolism, probably due to alterations in ethylene signalling. Moreover, under normal condition Se induce structural alterations in cells, which may contribute to plant development. Thus, the information available in this work is a fundamental step towards obtaining a better understanding about Se role in plant metabolism.

Key words: ethylene, microscopy, selenate, selenite, *Solanum lycopersicum,* oxidative stress

ALTERAÇÕES BIOQUÍMICAS E ESTRUTURAIS INDUZIDAS POR SELENIO SOB ESTRESSE POR CADMIO EM TOMATEIROS

RESUMO

As plantas estão expostas a adversidades no ambiente que as circundam, como a contaminação por cádmio (Cd). Este metal pesado tem aumentado na atmosfera devido a atividades humanas. As plantas podem absorver o Cd, causando sérias alterações estruturais, fisiológicas e bioquímicas. As plantas desenvolveram sistemas de defesa complexos, incluindo mecanismos não enzimáticos e enzimáticos para evitar uma cascata de oxidação descontrolada causada pelo estresse oxidativo. Alguns elementos, como o selênio (Se), se utilizados em concentração adequadas, podem induzir uma melhora no sistema antioxidante, no crescimento e nos atributos fotossintéticos. Ainda é pouco conhecido o papel do Se nas respostas das plantas ao estresse. O objetivo deste trabalho foi obter novas informações sobre o papel do selenato e selenito no sistema de desintoxicação das plantas, incluindo a avaliação da nutrição mineral, atividade de enzimas antioxidantes e conteúdo de compostos não enzimáticos, pigmentos, alterações estruturais e o papel do Se na modulação do etileno, com o uso de mutantes hormonais como ferramenta. Nossos dados indicam que o Se é uma estratégia interessante para melhorar o metabolismo da planta sob condições normais ou estressantes. O selênio pode induzir aumento da ação do metabolismo de defesa antioxidante, provavelmente devido a alterações na sinalização do etileno. Além disso, em condições normais, o Se induz alterações estruturais nas células, o que pode contribuir para o desenvolvimento das plantas. Assim, o Se é uma estratégia interessante a ser utilizada na agricultura para melhorar a produção agrícola.

Palavras chave: estresse oxidativo, etileno, microscopia, selenato, selenito, *Solanum lycopersicum*

CHAPTER I – GENERAL CONSIDERATIONS

1. Introduction

Plants are continuously exposed to environmental stresses, which leads to losses in crop production due to changes in cell homeostasis. Cadmium (Cd) is a toxic heavy metal, which have been add in agricultural soil by industrial process, municipal waste and mainly agricultural practices. This metal can be uptake by plants and cause serious changes in structural, physiological and biochemical processes. As a consequence of Cd contamination, occurs an over production of reactive oxygen species (ROS), causing oxidative stress. Plants developed a complex defence systems including non-enzymatic and enzymatic mechanism to avoid oxidative stress and prevent an uncontrolled oxidation cascade. Some elements, such as selenium (Se), if used in adequate concentration, may induce an improvement in antioxidant system, growth and photosynthetic attributes.

In this Thesis, the first chapter consists of a literature review, which addresses important information from relevant articles to support our study. The chapter two, we carried out a study to define adequate Se concentration to tomato plants cv. Micro Tom under Cd stress. Although Se plays an important role in antioxidant metabolism under Cd stress, how this element induces positives responses remains unknown. Through the use of hormonal mutants, our research group have discussed the modulation of ROS signaling, as well as the cellular coordination of components, providing insights about the cross-talk between these pathways and phytohormones (Gratão et al, 2009, Monteiro et al., 2011; Gratão et al., 2012; Gratão et al., 2015; Alves et al., 2017). Thus, in the third chapter we used information obtained in the second chapter to study the role of Se in modulate ethylene responses during Cd stress, using hormonal tomato mutant *epinastic* to add new insights about this mechanism. The data obtained in this work connecting Se-mediated signaling process and detoxification strategies, since hormones are directly involved in such a mechanism.

In the fourth chapter, we decided to investigated if positive responses of Se application could be related with structural changes in root and leaves under Cd

stress. The study of structural changes trigged by Se was possible due to a partnership with Laboratory of Hispatology and Structural Plant Biology – CENA - USP, which contribute to generate an inedited study. These data added an extra element more directly to this thesis related to the role of Se in plant development under normal or Cd stress conditions and contribute in unraveling the impact of Se application in agriculture.

4. Conclusions

Recently, several studies reported Se as an efficient strategy to avoid damages derived from abiotic stresses, including Cd. Although the biochemical responses have been widely reported, structural changes underlying this interplay were not enlightened until now. We found that plants treated with both Se forms used exhibited an improvement in photosynthesis attributes and growth, which are related to structural modifications in leaves. In contrast, Cd stress induced severe damages to chloroplast and others cell structures, which caused decreased photosynthetic rates, affecting growth. The application of Se in plants under stress did not restore cell damages caused by Cd. Although our results did not find that Se may induce ultrastructural and physiological changes (ie, alleviation) during Cd stress, Se plays an important role under non-stress conditions, due to the identification of specific Se-induced structural changes which could promote benefits to plant development. We conclude that the novelty information provided by this study had not yet been described in plant anatomical studies, being a fundamental step towards obtaining a

better understanding of structural changes, caused by Se and the elucidation of its role in plant metabolism.

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