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Root responses of contrasting tomato genotypes to cadmium-induced stress

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Thesis presented to obtain the degree of Doctor in  
Science. Area: Genetics and Plant Breeding

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Bachelor of Biological Sciences

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## RESUMO

### **Respostas radiculares de genótipos contrastantes de tomateiro sob estresse induzido por cádmio**

Esta tese apresenta um aprofundamento do conhecimento global sobre as respostas radiculares de genótipos contrastantes de tomateiro ao estresse induzido por cádmio (Cd). Os genótipos cultivados em hidroponia em meio contendo 35 µM de CdCl<sub>2</sub> durante sete dias exibiram acúmulo de metal tanto em raízes como em parte aérea em ambos genótipos, mas com um aumento da alocação de Cd principalmente nas raízes. PR (tolerante) acumulou menores níveis de Cd na parte aérea, exibindo maiores taxas de crescimento e acúmulo maior de MDA comparado ao CR (sensível). No entanto, o genótipo PR parece ter um sistema mais eficiente para lidar com o estresse induzido pelo Cd. Os ensaios enzimáticos revelaram que a presença de Cd alterou o conteúdo de GSH nas raízes de ambos os genótipos, com aumentos nas atividades de APX, GR e GST, que em conjunto podem ser os principais elementos responsivos na defesa contra o estresse oxidativo no genótipo tolerante. Seguindo a caracterização dos sistemas radiculares dos genótipos de tomateiro, o segundo capítulo contém as análises morfológicas. A exposição ao Cd provocou decréscimos nos parâmetros avaliados como comprimento radicular, área superficial e volume, sendo mais severos no genótipo CR. As raízes de tomate expostas ao Cd mostraram alterações na eficiência do uso de nutrientes para vários macro e micronutrientes, sendo menor no CR em comparação com PR. Esses resultados reforçam a explicação de que o fenótipo tolerante observado no genótipo PR pode estar relacionado a um melhor gerenciamento nutricional aliado aos menores danos na morfologia da raiz sob estresse. Finalmente o último capítulo contém dados de proteómica quantitativa, uma abordagem que foi empregada para determinar as alterações provocadas pelo Cd no perfil proteico das raízes de tomateiro. As plantas cresceram em hidroponia e foram expostas ao metal por quatro dias. A contagem espectral revelou um total de 380 proteínas diferencialmente acumuladas (DAP), das quais 62 foram compartilhadas entre os genótipos e apresentaram alterações similares após exposição ao metal. Em geral, CR apresentou maior número de DAP comparado ao PR. DAP foram alteradas em diversas vias, incluindo proteínas envolvidas em parede celular, resposta a estresse e atividades redox. Os resultados obtidos neste estudo contribuíram para aumentar o entendimento da tolerância ao Cd em tomateiro.

**Palavras-chave:** Metais pesados; Antioxidantes; Estresse oxidativo; Eficiência do uso de nutrientes; Proteómica

## ABSTRACT

### **Root responses of contrasting tomato genotypes to cadmium-induced stress**

This thesis presents a more in-depth understanding of global root responses of contrasting tomato genotypes to cadmium-induced stress. Tomato genotypes growing in media containing 35 µM CdCl<sub>2</sub> over seven days showed metal accumulation in roots and shoots of both genotypes, but with increased Cd allocation over time mainly in roots. PR (tolerant) accumulated lower levels of Cd in the shoots, exhibiting higher growth rate and higher levels of MDA in roots compared to CR (sensitive). Therefore, the PR genotype appears to have a more efficient mechanism to cope with Cd-induced stress. Enzymatic analysis revealed that the presence of Cd altered GSH content in roots of both genotypes, whilst increased the activities of APX, GR and GST, which in turn, together may be the main players against oxidative stress in the tolerant genotype. Following the characterization of tomato roots systems against Cd challenge the second chapter brings the root morphology parameters analysis. Cd exposure decreased the root length, the surface area and the volume in both genotypes, being more severe in the CR genotype. Tomato roots exposed to Cd showed NUE for many macro and micronutrients in the CR genotype lower than verified for the PR. These findings reinforce the explanation that the tolerant phenotype observed in PR plants could be related to a better nutrient management and minor damages in root morphology under Cd stress. Finally, the last chapter brings a large scale quantitative proteomic approach employed to determine alterations in the protein profile of tomato roots exposed to Cd. Tomato genotypes were grown in hydroponics and exposed to Cd over four days. The spectral counting revealed a total of 380 differentially accumulated proteins (DAP), which 62 were shared between both genotypes and showed similar alterations after metal exposure. In general, CR genotype presented higher number of DAP compared to PR. DAP showed alterations in diverse pathways, including proteins involved in cell wall, stress response, and redox activities. The results obtained in this study contributed to increase the understanding of Cd-tolerance in tomato plants.

Keywords: Heavy metals; Antioxidants; Oxidative stress; Nutrient use efficiency; Proteomics

## 1. INTRODUCTION

Heavy metals naturally occur in the soils as rare elements, but their excessive amounts in the environment are mainly consequence of anthropogenic activities such as mining, smelting, burning of fossil fuels and intensive use of fertilizers (Candeias *et al.*, 2015). Nowadays, numerous metallic elements are treated as widespread environmental pollutants and a very good example is cadmium (Cd), one of the most highly toxic substances that has been ranked no. 7 among top 20 toxicants (Birke *et al.*, 2017; Gill *et al.*, 2012). Cd is generally considered a non-essential transition metal since to date there is only one documented example of a protein requiring Cd as a cofactor, a Cd-specific carboanhydrase in the marine diatom *Thalassiosira weissflogii* (Lane *et al.*, 2005).

Due to increase of agricultural contaminated area by heavy metals, the problem of Cd toxicity worsens every year, however the amount of plant species with some tolerance degree to this metal is extremely restricted (Choppala *et al.*, 2014). Plants have evolved tolerance mechanisms to deal with heavy metals which means that tolerant plants survive in the presence of high internal metal concentration having, therefore, other cellular mechanisms such as vacuole compartmentalization (Song *et al.*, 2014) to cope with metal induced stress, such mechanisms still unknown to date. Another tolerance strategy is related to ion-excluders and involves decreasing the amount of Cd entering the cell by extracellular precipitation, absorption by cell walls, reduced uptake or increased efflux (Sarwar *et al.*, 2010). According to Choppala *et al.* (2014) tolerance mechanisms include: (i) synthesis of phytochelatins and metallothioneins; (ii) competition between micronutrients and Cd for the same membrane transporters; (iii) alleviation of oxidative stress by antioxidant production; and (iv) restoration of chlorophyll structure damaged by Cd toxicity.

Among cultivated crops the vegetables are more likely to be grown in Cd-contaminated areas, since the disposal of agro-industrial wastes usually occurs around large urban centers (Gallego *et al.*, 2012) where the green belts are located. Among the vegetables, tomato was chosen as a model studies because of many reasons such as the fast life cycle and small size. In addition, the recent publication of the tomato genome (The Tomato Genome Consortium, 2012) have offered the possibility of applying modern molecular approaches to get information on the molecular mechanisms that lead to the accumulation, detoxification and allocation of nutrients and toxic elements such as Cd. The lab team of Dr. Ricardo Antunes de Azevedo has developed researches evaluating the tomato responses to heavy metals and since 1998 has been studying the heavy metals impacts in crops, especially Cd, aiming to evaluate effects on antioxidant metabolism of cells. Since 2004, Azevedo's team adopted Micro-Tom (MT) cultivar as model

studies, and more recently other commercial tomato varieties that have different levels of tolerance to Cd. Several studies have been developed by Azevedo's group with the theme Cd and tomato: Gratão *et al.* (2008), Gratão *et al.* (2009), Monteiro *et al.* (2011), Gratão *et al.* (2012), Dourado *et al.* (2013), Piotto *et al.* (2014), Nogueiro *et al.* (2016), Pompeu *et al.* (2017), however no study comparing contrasting genotypes to Cd-induced stress were performed to date. From the evaluation of a core collection of tomato accessions accomplished by Piotto (2012) it was possible identify plants with a high tolerance degree as well as genotypes extremely sensitive to Cd. Based on these results, the research group has concentrated efforts trying to characterize the screened genotypes. Therefore, the identification of tomato genotypes with contrasting phenotypes and different Cd allocation patterns will allow to explore the possible mechanisms involved in Cd-tolerance.

Once Cd is absorbed by roots this organ plays a significant role in the water and nutrients acquisition from soil solution. However, heavy metal excess in the root medium cause damages in the root functions by reducing its growth and integrity (Lux *et al.*, 2011). Nonetheless this thesis presents a joint approach and tools to characterize the root system of tomato genotypes with differential tolerance to Cd, using enzymatic and non-enzymatic assays, root morphological parameters, nutritional status and proteomics aiming to contribute for understanding the Cd dynamics in roots and to suggest the potential mechanisms involved in Cd-tolerance in tomato plants.

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## 2. FINAL CONSIDERATIONS

In this study it was determined global root responses of contrasting tomato genotypes to Cd-induced stress. In the first chapter, a temporal dynamic of Cd responses was shown. Tomato genotypes growing in media containing 35 µM CdCl<sub>2</sub> over seven days showed metal accumulation in roots and shoots tissues in both genotypes, but with increased Cd allocation over time mainly in roots. PR (tolerant) accumulated lower levels of Cd in the shoots, exhibiting higher growth rate and higher levels of MDA in roots compared to CR (sensitive). Therefore, the PR genotype appears to have a more efficient mechanism to cope with Cd-induced stress. Enzymatic analysis revealed that the presence of Cd altered GSH content in roots of both genotypes, whilst increased the activities of APX, GR and GST, which together may be the main players against oxidative stress in PR genotype exposed to Cd.

Following the characterization of tomato root systems against Cd challenge the second chapter brings the root morphology parameter analysis. In this chapter we tried to establish a relationship between the root morphology and nutrient uptake, finally showing Cd impact on nutrient use efficiency (NUE) of tomato plants. Regarding to morphology root length, surface area and volume decreased in both genotypes exposed to Cd, being more severe in the CR genotype. Tomato roots exposed to Cd showed NUE for many macro and micronutrients in the CR genotype lower than verified for the PR. These findings reinforce the explanation that the tolerant phenotype observed in PR plants could be due to a better nutrient management and minor damages in root morphology under Cd stress. Moreover, under Cd exposure, CR showed more severe symptoms such as decrease in growth, suggesting that intracellular detoxification or defense mechanisms maybe exist or be more efficient in the PR genotype.

Finally, the last chapter brings a large scale quantitative proteomic approach employed to determine alterations in the protein profile of tomato roots exposed to Cd. Tomato genotypes were grown in hydroponics and exposed to Cd over four days. The spectral counting revealed a total of 380 differentially accumulated proteins (DAP), which 62 were shared between both genotypes which showed similar alterations after Cd exposure. In general, CR genotype presented higher number of DAP compared to PR. DAP showed alterations in diverse pathways, including proteins involved in cell wall, stress response and redox activities. Cell wall related proteins such as chitinases, believed to act as second line defense components, showed contrasting responses in tomato genotypes, decreased accumulation in tolerant and increased in sensitive genotype. Regarding to antioxidant responses Cd-induced decreases in antioxidant enzymes such as SOD and CAT, as well as observed in the first chapter. Increased accumulation of GSH and related

proteins mainly GST and TRX were detected in tolerant genotype. Among the changes in root proteome profile of sensitive genotype we can highlight the increased abundance of GSH and GST, thus important decreases in SOD, MDHAR and TRX were detected. Taken together, the present study sheds light on molecular mechanisms involved in Cd tolerance in tomato genotypes and suggests a more active involvement of GSH, GSTs, TRX and peroxidases in Cd-induced stress response.

Summarizing, the tolerant genotype PR accumulated lower levels of Cd in the shoots, exhibited higher growth rate and higher levels of MDA in roots compared to sensitive genotype CR. Also, an initial enhancement of the GSH pool was detected in tolerant roots, which might allow the conjugation of oxidative stress metabolites, together with increased activities of APX, GR and GST, in addition to the higher NUE due to minor damage in root morphology determined the tolerance to Cd in PR plants. Furthermore, protein profile of PR roots altered by Cd exposure reinforces the hypothesis of an SOD and CAT independent defense system active in these plants. In this context, takes place the POD isoenzymes, important in oxidative stress response and it were decreased in sensitive genotype. The next step is to analyze carefully the metabolic pathways altered by the metal, as well as exclusive proteins of tolerant genotype, in the attempt to establish a molecular model of response to Cd in tomato contrasting genotypes. The proteomics opened a new opportunity in the study of Cd-induced stress response providing some biomarkers worth to be exploited.