MARCIO PAULO PEREIRA

MODIFIED ACTIVITY OF THE GROUND MERISTEM AND TISSUE DIFFERENTIATION IN *Schinus molle* L. UNDER CADMIUM CONTAMINATION

LAVRAS-MG
2016

MARCIO PAULO PEREIRA

MODIFIED ACTIVITY OF THE GROUND MERISTEM AND TISSUE DIFFERENTIATION IN *Schinus molle* L. UNDER CADMIUM CONTAMINATION

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração Botânica, para a obtenção do título de Doutor.

Orientador
Prof. Dr. Fabricio José Pereira

Coorientador
Prof. Dr. Evaristo Mauro de Castro

LAVRAS-MG
2016

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Pereira, Marcio Paulo.

Modified activity of the ground meristem and tissue differentiation in *Schinus molle* L. under cadmiumcontamination / Marcio Paulo Pereira. – Lavras : UFLA, 2016.

51 p. : il.

Tese(doutorado)–Universidade Federal de Lavras, 2016.

Orientador(a): Fabricio José Pereira.

Bibliografia.

1. Cell elongation rate. 2. heavy metal. 3. leaf meristems. 4. leaf primordial. 5. pepper tree. I. Universidade Federal de Lavras. II. Título.

MARCIO PAULO PEREIRA

MODIFIED ACTIVITY OF THE GROUND MERISTEM AND TISSUE DIFFERENTIATION IN *Schinus molle* L. UNDER CADMIUM CONTAMINATION

(MODIFICAÇÃO DA ATIVIDADE DO MERISTEMA FUNDAMENTAL E DIFERENCIAÇÃO DE TECIDOS EM *Schinus molle* L. SOB COMTAMINAÇÃO POR CÁDMIO)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração Botânica, para a obtenção do título de Doutor.

APROVADA em 22/07/2016

Dr. Marcelo Polo	UNIFAL-MG
Dra. Vânia Helena Techio	UFLA-MG
Dr. Jean Marcel Sousa Lira	UFLA-MG
Dr. Manuel Losada Gavilanes	UFLA-MG

_____

Prof. Dr. Fabricio José Pereira

Orientador

Prof. Dr. Evaristo Mauro de Castro

Coorientador

LAVRAS-MG

2016

Aos meus Pais, Paulo Antônio Pereira e Waldelicia do Carmo Pereira.

A Jaqueline Gonçalves da Silva, por estar sempre ao meu lado.

Aos meus amigos de Alfenas, que me ensinaram a sonhar.

Aos meus orientadores, Fabricio José Pereira e Evaristo Mauro de Castro.

Dedico

AGRADECIMENTOS

À Universidade Federal de Lavras (UFLA) e ao Programa de Pós-Graduação em Botânica Aplicada, pela oportunidade concedida para realização do Doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

À Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG)

Aos professores Fabricio José Pereira e Evaristo Mauro de Castro, pela orientação e amizade.

Aos demais professores e técnicos administrativos, pela orientação e suporte técnico.

MUITO OBRIGADO!

RESUMO

Trabalhos anteriores mostram o desenvolvimento de folhas espessas em plantas tolerantes crescendo sob contaminação por cádmio (Cd). Dessa forma, o objetivo desse trabalho foi avaliar o efeito do Cd nos meristemas foliares de *Schinus molle*, uma espécie tolerante ao Cd. Plantas foram cultivadas em solução nutritiva contendo 0, 10 e 50 µM de Cd. Análises anatômicas foram realizadas nos primórdios foliares amostrados em intervalos de tempo regulares. Sob baixos níveis de Cd (10 µM) os primórdios foliares apresentaram aumento da espessura do meristema fundamental, do diâmetro das células, na taxa de alongamento celular e na massa seca das folhas completamente expandidas. Contudo, a concentração de 50 µM de Cd reduziu todas essas variáveis. Além disso, as células do meristema fundamental do primórdio foliar de *S. molle* tornaram-se maiores quando expostos ao Cd. A epiderme, o parênquima paliçádico e os tecidos vasculares desenvolveram primeiro em folhas expostas ao Cd. As modificações encontradas no meristema fundamental podem estar relacionadas ao desenvolvimento de folhas espessas em plantas de *S. molle* expostas a baixos níveis de Cd. Folhas velhas apresentaram maior concentração de Cd em comparação às folhas novas, evitando a toxicidade em órgãos mais funcionais. Assim, baixas concentrações de Cd promovem modificações no meristema fundamental que refletem no desenvolvimento de folhas mais espessas e melhoradas.

Palavras-chave: Aroeira. Meristemas foliares. Metais pesados. Primórdio foliar. Taxa de alongamento celular.

ABSTRACT

Previous works show the development of thicker mature leaves in tolerant plants growing under cadmium (Cd) contamination. The aim of this study was to evaluate the Cd effects in the leaf meristems in the tolerant species *Schinus molle*. Plants were grown in nutrient solution containing 0, 10 and 50 µM of Cd. Anatomical analysis was performed in the leaf primordia sampled at regular time intervals. Under the lowest cadmium level (10 µM) increased ground meristem thickness, diameter of the cells; cell elongation rate and the leaf dry mass were found. However, 50 µM of Cd reduced all these variables. In addition, the cells of the ground meristem of *S. molle* leaf primordia became larger when exposed to cadmium. Epidermis, palisade parenchyma and vascular tissues developed earlier in Cd-exposed leaves. The modifications found in the ground meristem may be related to the development of thicker leaves in *S. molle* plants exposed to low Cd levels. Older leaves showed higher Cd content compared to the younger ones, preventing toxicity to the more functional organs. Thus, low Cd concentrations promote changes in the ground meristem reflecting on the development of thicker and enhanced leaves.

Keywords: Cell elongation rate. heavy metal. leaf meristems. leaf primordial. pepper tree.

SUMMARY

PRIMEIRA PARTE

1 GENERAL INTRODUCTION

Plant adaptation to environment and stress conditions is related to short-term physiological adjustments and long-term anatomical and morpho-physiological changes. These adaptations help plants to reduce the stress and improve resources usage (DICKSON; ISEBRANDS, 1991). Therefore, tolerant plants grow and change their interaction with the environment by favorable structural modifications.

Plants are exposed to heavy metals by natural or anthropogenic origins and non-tolerant species show several physiological and biochemical limitations promoted by heavy metals (SINGH et al., 2016). Cadmium (Cd) can be particularly toxic by biding to sulphydril groups in proteins, causing nutrient uptake disorders and increasing reactive oxygen species production (DELMAIL et al., 2011). However, tolerant plants developed several adaptations to overcome metal toxicity (SINGH et al., 2016).

Cadmium contamination promotes structural changes mainly in roots and leaves. The leaf is a key organ for plant growth and tolerance once it contains the sites for photosynthesis and transpiration. Therefore, favorable leaf structural modifications are important to cope Cd toxicity (PEREIRA et al., 2016a; PÉREZ CHACA et al., 2014; SHI; CAI, 2009; SHI et al., 2014; TSUKAYA, 2006).

Mature leaves of *Schinus molle* L. (Anacardiaceae) exposed to low Cd levels showed improved photosynthesis, increased mesophyll thickness and stomatal density (PEREIRA et al., 2016a). However, the modifications in mature leaves may be related to leaf meristems once they are the source of the mature

tissues. The regulation of leaf development is complex and requires the expression of homeobox KNOX genes, auxin distribution and the establishment of the dorsoventrality (TSUKAYA, 2006). These regulatory events are found mainly in leaf meristems of developing leaves. Likewise, the analysis of the activity of leaf meristems during its ontogeny may explain which cell modifications are related to mature leaf structure under Cd influence. Therefore, this work aimed to evaluate the Cd effects in the activity and structure of leaf meristems during leaf development and how it can be related to anatomical modifications in mature leaves of the tolerant species *Schinus molle*.

2 THEORETICAL BACKGROUND

2.1 Cadmium

Heavy metals are defined as chemical elements classified as metals having with 5 g cm^3 density or higher. Based on their solubility under physiological conditions, 17 heavy metals may be available to living cells and are significant for the plant and animal communities within various ecosystems (HASAN et al., 2009). Cadmium (Cd) is considered to have high toxicity to humans and all other living organisms as it lacks known biological functions (CHEN et al., 2007).

Cadmium is located in the IIB group of the periodic table and its atomic number is 48. It shows chemical similarity with other elements of IIB group especially, with zinc (Zn) and mercury (Hg) (HASAN et al., 2009).

Rocks are the natural sources of Cd for the soil and the food chains throughout the soil formation processes. The average concentration of Cd in the earth's crust is average 0.1 mg kg^{-1} of dry matter and the richest natural sources of Cd are the zinc sulphides (ZnS), wurtzite and phosphatated rocks (MALAVOLTA, 2006)

2.2 Cadmium effects on plants

The rate of Cd uptake from plants is determined both by its concentration in the soil and availability. The latter is influenced by the organic matter, root exudates, mycorrhizae and soil physical and chemical properties such as pH, redox potential, the temperature, and the concentrations of other elements (PÁL et al., 2006). The Cd uptake by roots usually includes the

competition for sites for nutrient absorption (HASAN et al, 2009; SARWAR et al., 2010).

The plant responses to increased Cd levels in soil depend on species capacity to uptake and transport the pollutant. Cadmium is transported within plants in the form of metallo-organic complexes, however, its uptake, translocation and allocation mechanics are complex (EPSTEIN; BLOOM, 2005; HASAN et al., 2009). Most Cd ions remain in the roots as only small amounts are transported to shoots (SALT; SMITH; RASKIN, 1998). The concentration of Cd in plants overall decreases as follows: roots > leaves > fruits > seeds (HASAN et al., 2009).

Heavy metals compete with essential nutrients for uptake pathways thereby disturbing the mineral nutrition of plants; however, once inside plant tissues, it can accumulates in cell compartments or disturbs the overall plant metabolism (HASAN et al., 2009; SANITA DI TOPPI; GABBRIELLI, 1999)

Cadmium can interact with several enzymes and proteins containing sulfhydryl groups. Thus, enzyme activity drops under Cd contamination in many metabolic pathways; particularlym key processes such as chlorophyll synthesis, water photo-oxidation and CO_2 fixation and may be affected (SEREGIN; IVANOV, 2001).

High concentrations of heavy metals may damage plant tissues mainly by promoting oxidative stress. Reactive oxygen species (ROS) can oxidize biomolecules such as DNA, proteins and lipids, causing injuries and damaging plant growth and development (BHADURI; FULEKAR, 2012).

2.3 Leaf ontogeny

Leaves are key organs once they hold the sites of photosynthesis and show several secondary functions with direct effects in plant growth and development. The shoot apical meristem (SAM) is the origin of leaves, therefore modifications in the structure and activity of the SAM must be considered in order to understand leaf modifications under different environments. Leaf evolution produced very different morphologies such as spines and flower parts (sepals, petals, stamens and carpels) (CHRISTENSEN; WEIGEL, 1998; EVERT, 2006) with different roles in plant defense and reproduction.

The leaf primordium is comprised of specific meristems (leaf meristems) which promote leaf growth and differentiation. The leaf meristems are the apical meristem (promotes apical growth or leaf elongation), the marginal meristem (promotes the lateral expansion) and the adaxial meristem (promotes leaf thickening) (CUTTER, 1978). All these leaf meristems cease activity when the organ is fully expanded (mature). The early events in leaf development can be divided into three main processes as follows: the initiation of the leaf primordium, the establishment of dorsiventrality, and the development of a marginal meristem (TSUKAYA, 2013).

Down-regulation of the KNOTTED1 homeobox plant genes provides a molecular marker of leaf initiation in the shoot apical meristem. In Arabidopsis, the WUS, CLV1, CLV2, CLV3, KAPP, and STM genes regulate the fate of the SAM cells that must remain undifferentiated cells or must proceed to the pathway for leaf formation (TSUKAYA, 2002).

The phytohormones, including brassinosteroids have a key role in leaf development. The auxin plays regulates cell proliferation and elongation during leaf primordia differentiation. During leaf development the AXR1 and AXR2

encodes genes that regulate the auxin-dependent proliferation of cells (NAGPAL et al., 2000; TSUKAYA, 2002).

2.4 *Schinus molle* L. (Anacardiaceae)

The natural distribution of *Schinus* genus is limited to South America. However, *Schinus molle* L. is naturally distributed from Mexico to South America and worldwide cultivated. Likewise, this gender spread in temperate regions where several species were introduced as ornamentals (BARKLEY, 1944). The natural distribution of *S. molle* in Brazil is limited to the states of Paraná, Rio Grande do Sul and Santa Catarina (LIM, 2012; SILVA-LUZ; PIRANI, 2013). However, *Schinus molle* can be found cultivated throughout Brazil. This species is popularly known as pepper tree, Peruvian pepper tree or aroeira salsa (in Portuguese). It is known as pepper tree because its fruits are used in cooking as a pepper substitute (ORWA et al., 2009).

S. molle is a tree (3 to 15 m tall), has a short stem and small flowers arranged in panicle-type inflorescences. Fruits are chartaceous drupes with thin exocarp, color from brown to deep red, the mesocarp is resinous and bound to the hard endocarp (BARKLEY, 1944). *S. molle* seeds are positively photoblastic and show physiological dormancy that can be alleviated by acid scarification and dry storage (PEREIRA et al., 2016b).

The *Schinus* molle leaves are compound, alternate with considerable variation in size, shape and number of leaflets. Leaflet adaxial and abaxial epidermis are unisseriated with anomocytic and ciclocytic stomata, isobilateral mesophyll with collateral vascular bundles and secretory channels (PIRES et al., 2015).

The species of the gender *Schinus* are widely used for the restoration of degraded areas (DOGANLAR et al., 2012; IPONGA; MILTON; RICHARDSON, 2008). In addition, *Schinus molle* showed has tolerance to heavy metals such as cadmium (PEREIRA et al., 2013, 2016a). Pereira et al. (2016a) reported thicker mature leaves and increased stomatal density when exposed to low cadmium contractions.

REFERÊNCIAS

BARKLEY, F. A. *Schinus* L. **Brittonia**, Bronx, v. 5, n. 2, p. 160-198, 1944.

BHADURI, A. M.; FULEKAR, M. H. Antioxidant enzyme responses of plants to heavy metal stress. **Reviews in Environmental Science and Biotechnology**, Cham, v. 11, n. 1, p. 55-69, 2012.

CHEN, F. et al. Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. **Chemosphere**, Oxford, v. 67, n. 5, p. 2082-2088, 2007.

CHRISTENSEN, S.; WEIGEL, D. Plant development: The making of a leaf. **Current Biology**, London, v. 8, n. 18, p. 643-645, 1998.

CUTTER, E. G. **Plant anatomy**: cells and tissues. 2nd ed. London: E. Arnold, 1978. 315 p.

DELMAIL, D. et al. Physiological, anatomical and phenotypical effects of a cadmium stress in different-aged chlorophyllian organs of *Myriophyllum alterniflorum* DC (Haloragaceae). **Environmental and Experimental Botany**, Elmsford, v. 72, n. 2, p. 174-181, 2011.

DICKSON, R. E.; ISEBRANDS, J. G. Leaves as regulators of stress response. In: MOONEY, H. A.; WINNER, W. E.; PELL, E. J. (Org.). **Response of plants to multiple stresses**. San Diego: Academic, 1991. p. 3-34.

DOGANLAR, Z. B. et al. Heavy metal pollution and physiological changes in the leaves of some shrub, palm and tree species in urban areas of Adana, Turkey. **Chemical Speciation and Bioavailability**, Surrey, v. 24, n. 2, p. 65-78, 2012.

EPSTEIN, E.; BLOOM, A. J. **Mineral nutrition of plant principles and perspective**. Massachusetts: Sinauer Associates, 2005. 400 p.

EVERT, R. F. **Esau's plant anatomy:** meristems, cells, and tissues of the plant body: their structure, function, and development. New Jersey: J. Wiley, 2006. 601 p.

HASAN, S. A. et al. Cadmium: toxicity and tolerance in plants. **Journal of Environmental Biology**, Lucknow, v. 30, n. 2, p. 165-174, 2009.

IPONGA, D. M.; MILTON, S. J.; RICHARDSON, D. M. Superiority in competition for light: a crucial attribute defining the impact of the invasive alien tree *Schinus molle* (Anacardiaceae) in South African savanna. **Journal of Arid Environments**, London, v. 72, n. 5, p. 612-623, 2008.

LIM, T. K. ***Schinus molle*** **edible medicinal and non-medicinal plants**. Wageningen: Springer Netherlands, 2012. 159 p.

MALAVOLTA, E. **Manual de nutrição mineral de plantas**. São Paulo: Agronômica Ceres, 2006. 638 p.

NAGPAL, P. et al. AXR2 encodes a member of the Aux/IAA protein family. **Plant Physiology**, Rockville, v. 123, n. 2, p. 563-573, 2000.

ORWA, C. et al. **Agroforestree database:** a tree reference and selection guide. Version 4.0. 2009. Disponível em: <http://www.worldagroforestry. org/af/treedb/>. Acesso em: 11 ago. 2016.

PÁL, M. et al. Physiological changes and defense mechanisms induced by cadmium stress in maize. **Journal of Plant Nutrition and Soil Science**, Tharandt, v. 169, n. 2, p. 239-246, 2006.

PEREIRA, M. P. et al. Cadmium tolerance in *Schinus molle* trees is modulated by enhanced leaf anatomy and photosynthesis. **Trees**, Heidelberg, v. 30, n. 3, p. 807-814, 2016a.

PEREIRA, M. P. et al. Lead tolerance during germination and early growth of the Brazilian peppertree and the morpho-physiological modifications. **Revista de Ciências Agrárias**, Belém, v. 56, p. 72-79, 2013. Supl.

PEREIRA, M. P. et al. Seed germination of *Schinus molle* L. (Anacardiaceae) as related to its anatomy and dormancy alleviation. **Seed Science Research**, Cambridge , v. 1, p. 1-11, 2016b.

PÉREZ CHACA, M.V. et al. Effects of cadmium stress on growth, anatomy and hormone contents in *Glycine max* (L.) Merr. **Acta Physiologiae Plantarum**, Krakow, v. 36, n. 10 p. 2815-2826, 2014.

PIRES, M. F. et al. Micromorfometria foliar de *Schinus molle* L. (Anarcadiaceae) em diferentes alturas na copa. **Cerne**, Lavras , v. 21, n. 1, p. 17-25, 2015.

SALT, D. E.; SMITH, R. D.; RASKIN, I. Phytoremediation. **Annual Review of Plant Physiology**, Palo Alto, v. 49, p. 643-668, 1998.

SANITA DI TOPPI, L.; GABBRIELLI, R. Response to cadmium in higher plants. **Environmental and Experimental Botany**, Elmsford, v. 41, n. 2, p. 105-130, 1999.

SARWAR, N. et al. Role of mineral nutrition in minimizing cadmium accumulation by plants. **Journal of the Science of Food and Agriculture**, London, v. 90, n. 6, p. 925-937, 2010.

SEREGIN, I. V.; IVANOV, V. B. Physiological aspects of cadmium and lead toxic effects on higher plants. **Russian Journal of Plant Physiology**, Moscow, v. 48, n. 4, p. 523-544, 2001.

SHI, G.; CAI, Q. Leaf plasticity in peanut (*Arachis hypogaea* L.) in response to heavy metal stress. **Environmental and Experimental Botany**, Elmsford, v. 67, n. 1, p. 112-117, 2009.

SHI, G. et al. Leaf responses to iron nutrition and low cadmium in peanut: anatomical properties in relation to gas exchange. **Plant and soil**, Crawley, v. 375, n. 1, p. 99-111, 2014.

SILVA-LUZ, C. L.; PIRANI, J. R. **Anacardiaceae in lista de espécies da flora do Brasil**. Rio de Janeiro: Jardim Botânico do Rio de Janeiro, 2013. Disponível em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB4398>. Acesso em: 25 jul. 2016.

SINGH, S. et al. Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics and ionomics. **Frontiers in Plant Science**, Lausanne, v. 6, n. 1, p. 1-36, 2016.

TSUKAYA, H. Leaf development. **The Arabidopsis Book**, Rockville, v. 1, n. 1, p. 1-23, 2002.

TSUKAYA, H. Leaf development. **The Arabidopsis Book**,Rockville, v. 11, n. 1, p. 1-21, 2013.

SEGUNDA PARTE

ARTIGO 1: LEAF ONTOGENY OF *Schinus molle* L. PLANTS UNDER CADMIUM CONTAMINATION: THE MERISTEMATIC ORIGIN OF LEAF STRUCTURAL CHANGES

MANUSCRIPT DRAFT

ABSTRACT

Previous works show the development of thicker mature leaves in tolerant plants growing under cadmium (Cd) contamination. The aim of this study was to evaluate the Cd effects in the leaf meristems in the tolerant species *Schinus molle*. Plants were grown in nutrient solution containing 0, 10 and 50 µM of Cd. Anatomical analysis was performed in the leaf primordia sampled at regular time intervals. Under the lowest cadmium level (10 µM) increased ground meristem thickness, diameter of the cells; cell elongation rate and the leaf dry mass were found. However, 50 µM of Cd reduced all these variables. In addition, the cells of the ground meristem of *S. molle* leaf primordia became larger when exposed to cadmium. Epidermis, palisade parenchyma and vascular tissues developed earlier in Cd-exposed leaves. The modifications found in the ground meristem may be related to the development of thicker leaves in *S. molle* plants exposed to low Cd levels. Older leaves showed higher Cd content compared to the younger ones, preventing toxicity to the more functional organs. Thus, low Cd concentrations promote changes in the ground meristem reflecting on the development of thicker and enhanced leaves.

Keywords: Pepper tree, leaf primordia, leaf meristems, cell elongation rate, heavy metal.

INTRODUCTION

Plants face the contamination by heavy metals caused by both natural and anthropogenic sources. Heavy metal exposure causes several physiological and biochemical limitations in non-tolerant species; however, tolerant plants develop several adaptations to cope the metal toxicity (Singh et al. 2016). Cadmium (Cd) is an heavy metal that can bind to the sulfhydryl group in proteins inhibiting its activities, in addition, this metal may cause metabolism disruption, changes in the nutrient uptake, Cd promotes alterations of the cellular redox potential producing reactive-oxygen-species (Delmail et al. 2011).

Cadmium causes anatomical modifications particularly in roots and leaves. Leaves are the main source for important physiological process (namely photosynthesis and transpiration) and changes in the leaf morphology such as increased thickness and stomatal density are key traits in metal tolerance (Tsukaya, 2006; Shi & Cai, 2009; Pérez Chaca et al. 2014; Shi et al. 2014; Pereira et al. 2016). However, heavy metal effects have been investigated only in mature leaves in despite of the key role of meristems in the leaf development.

The regulation of the leaf development is complex and depends on the expression of homeobox KNOX genes, the auxin distribution in the shoot apical meristem and the establishment of dorsoventrality regulated by miRNA-mediated genes (Tsukaya, 2006). All of these regulatory mechanisms are found in leaf meristems. Thus, analyzing the meristematic activity during leaf

ontogeny can set light to the origin of cellular traits (e.g., cell division, cell elongation) which are related to the structural changes in mature leaves.

Fully developed leaves from *Schinus molle* exposed to Cd showed enhanced photosynthesis, thicker leaves and higher stomatal density (Pereira et al. 2016). Therefore, the aim of this study was to evaluate the activity and structure of leaf meristems as related to the anatomical changes on mature leaves of *Schinus molle* exposed to cadmium.

MATERIAL AND METHODS

Plant growth conditions

Schinus molle plants were obtained from seeds collected in a cultivated population in the southern region of Minas Gerais state, Brazil. The plants were grown in plastic bags of 0.35 L containing washed sand and nutrient solution in greenhouse at 25±2°C. The nutrient solution was comprised of the salt concentrations described by Hoagland & Arnon (1950) using the following sources: $NH_4H_2PO_4$, $Ca(NO_3)_2$, $MgSO_4.7H_2O$, KNO_3, H_2BO_3, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $H_2MoO_4 \cdot H_2O$ and $FeSO_4 \cdot 7H_2O$. This solution was replaced at 15-days intervals and the water lost by evapotranspiration refilled daily. These plants remained under these conditions for five months.

Experimental design

Five month old plants were exposed to 0, 10 and 50 µM of Cd using the same Hoagland & Arnon solution described for plant growth and $Cd(NO_3)_2$. Developing leaves were sampled at regular time intervals labeling the leaf primordia to ensure correct determination of age. The experimental design was completely randomized in a factorial 3x4 scheme, considering three

concentrations of Cd and four leaf ages for the analysis of the ground meristem (2, 6, 8 and 10 days) or six ages for the analysis of leaf elongation (6, 8, 10, 14, 20 and 24 days). Sampling of leaf primordia for meristem analysis stopped at 10 days because older leaves were already differentiated. The plants were maintained in these conditions for 30 days. Five replicates were used per treatment both for the analysis of meristematic tissues (n=60) or the analysis of leaf elongation (n=90). Each replicate was constituted of one plant.

Leaf development analysis

Leaves were sampled and fixed in Karnovsky's solution (4% paraformaldehyde and 2.5% glutaraldehyde in sodium cacodylate buffer 0.1M pH 7.2) for 72 h. Further, samples were dried with increasing ethanol concentrations (70%, 90% and 100%) at 2-h intervals and embedded in Historesin according to the manufacturer's instructions (Leica Microsystems, Wetzlar, Germany). Transversal and longitudinal sections were obtained using a semi-automated rotary microtome Yidi YD-335 (Jinhua Yidi Medical Appliance CO., LTD, Zhejiang, China). The sections were stained with toluidine blue 1% (m v^{-1}) and mounted on slides with Entellan (Merck, Darmstadt, Germany). The slides were photographed using a microscope attached to an image capture system (CX31, Olympus, Tokyo, Japan) and quantitative anatomical analysis

was performed using imageJ software. The quantitative analysis of meristem traits was performed only in the ground meristem. The reason for this method was because this meristem is the precursor of the parenchyma cells that comprises most of the mesophyll and leaf thickness. In addition, protodermis is one-layered and the procambium was early changed to xylem and phloem making hard to evaluate cell divisions. *Schinus molle* has compound leaves with 13 to 15 leaflets when mature. We evaluate the development of one leaflet per leaf considering the Cd effects to whole leaf.

Quantitative meristematic traits were evaluated according to equations proposed by Ivanov & Dubrovsky (1997) and the characteristics evaluated were: the cell elongation rate, the number of meristematic cells undergoing mitosis, cell production rate, cell division rate and the cell cycle time. In addition, we evaluated the thickness, diameter of cells and the number of cell layers in the ground meristem.

The leaf growth was evaluated by the following parameters: leaf area, dry mass, marginal growth, length and width. Leaf area was measured scanning the leaves and measuring the area in ImageJ software. Dry mass was measured in a precision scale (AY 220, Shimadzu, Japan) from oven-dried leaves by 72 h at 60 ºC. The marginal growth was measured by tracing the distance between the midrib and the leaflet margin at each time sampled. The leaf length and width was measured at each sampled age with a digital pachymeter. The following

parameters were calculated: the specific leaf area (leaf area/dry mass), and leaf elongation rate (leaf length at the end of experiment - the length at be first day sampled).

Cadmium measurements

At the end of the experiment, the Cd content in young (6 days old) and old (30 days old) leaves was measured. Leaves were dried at 45 °C for 48 h. Dried mass (500 mg) was triturated in small parts and then digested in 10 mL of HNO_3 for 30 min at 150 °C in a block digestion system. Further, 1.0 mL of $HClO_4$ was added, and the temperature elevated to 210 °C for 20 min. The digested material was diluted to 25 mL with distilled water, and the Cd content determined with an atomic absorption spectrometer.

Statistical analysis

Statistical analyzes were performed using the SISVAR 5.0 software (Ferreira, 2011). Prior to parametric analysis, data were tested for a normal distribution using the Shapiro–Wilk test. Further, data were subjected to analysis of variance and means compared by Scott-Knott test at 5% probability.

RESULTS

Cadmium promoted no toxic effects to the shoot apical meristem of *S. molle* (Fig. 1). All samples showed the same overall structure with the shoot apical meristem located at the center and three leaf primordia (Fig. 1). The leaf primordia showed one-layered protodermis, central procambium bundles surrounded by several layers of ground meristem (Fig. 1). Increased leaf primordia size and number of leaflets were found for the Cd exposed plants compared to control (Fig. 1).

The 2 days old leaflets in control plants are comprised only by the midrib with one-layered prododermis, central procambium bundles and several ground meristem layers. The outmost layer of the ground meristem showed partial differentiation to the hypodermis layer found in mature leaves with one layer of darken cells (Fig. 2a and d). The inner parts of the ground meristem are comprised of lighter cells with round shapes and the outer layers are undergoing cell divisions showing smaller cells (Fig. 2a and d). Secretory channels show large spaces surrounded by the one-layered epithelium (Fig. 2a). The innermost part of the leaflet midrib contains pith parenchyma with larger and lighter cells. Procambium can be found surrounding the pith (Fig. 2a). This overall structure was found to all 2 days old leaflets; however, Cd exposed leaflets were larger and showed developed xylem vessels, larger secretory cavities and developed hypodermis (Fig. 2b and e). The 2 days old leaflet morphology is hearth-shaped;

however, Cd strongly increased marginal growth developing expansions at the adaxial side (Fig. 2b and e) this remarkably changed the shape of the leaflet and provided earlier mesophyll development .

The leaflets of control plants started the marginal growth at the 6th day and a few xylem vessel elements can be found at the central part of the structure (Fig. 2g). However, Cd exposed leaflets showed much longer marginal growth and developed protoxylem and protophloem associated to the secretory ducts (Fig. 2h and i). The 8 days old leaves from control plants showed longer marginal growth and first xylem and phloem elements can be found in the midrib and mesophyll regions (Fig. 2j and m). Cadmium enhanced leaflet development and at the 8th day mesophyll was well developed with several vascular bundles, epidermis with first appearance of stomata and the palisade parenchyma at both leaf sides started to show elongated cells (Fig. 2n and o). At the 10th day of development, leaves from all treatments showed full developed midrib and mesophyll regions but those exposed to Cd were thicker (Fig. 2p, q and r). Leaflets older than 10 days were already differentiated.

Cadmium changed the ground meristem activity and structure in *S. molle* leaves (Fig.3). The leaf age and Cd concentration showed significant interaction and combined effects on the ground meristem structure and activity *($P<0.01$). S. molle* leaves exposed to 10 µM Cd showed thicker ground meristem compared to control, however, at 50 µM Cd it was reduced (Fig. 3a).

In addition, leaves showed increased ground meristem thickness with aging (Fig. 3a). The ground meristem cells had larger mean diameter when exposed to 10 µM Cd compared to control and 50 µM Cd treatments (Fig. 3b). In addition, the diameter of the cells from the ground meristem increased over time under Cd levels (Fig. 3b). Interestingly, the number of cell layers in the ground meristem responded to Cd only at the early ages (Fig. 3c). Eight or ten days old leaves showed no differences in the number of cell layers for all treatments. However, leaves under Cd treatments developed quicker achieving the final number of cell layers earlier then those of control treatment (Fig. 3c).

The cell elongation rate of the leaves exposed to 10 µM Cd was always higher compared to control and 50 µM Cd (Fig 4a). In addition, control plants showed higher cell elongation rate on the older leaves (Fig 4a). However, increased cell elongation rate was found in the leaves exposed to 10 µM Cd at early developmental stages (Fig 4a). Furthermore, under 50 µM Cd the cell elongation rate was similar for all leaf ages (Fig 4a).

The number cell division was higher at the intermediary leaf ages (6 and 8 days old leaves) for all treatments (Fig. 4b). However, leaves showed the highest means for this trait at 6 days under Cd treatments although highest were found only at 8 d in control plants (Fig. 4b). The cell production rate was lower in plants exposed to 50 µM of cadmium at all leaf ages (Fig. 4c). However, although 10 days old leaves had reduced cell production rate for from control

and 50 µM treatments, the 10 µM Cd treatment showed similar means for all leaf ages (Fig. 4c). The cell cycle time was longer on 8 days old leaves from the control plants; however, in Cd treated plants this trait showed higher means from 6 days old leaves (Fig. 4d).

Cadmium promoted significant morphological changes in the development of *S. molle* leaves (Fig. 5). Plants exposed to 10 µM Cd developed quicker compared to control and 10 µM Cd (Fig. 5); the 6 days old leaves developed under 10 µM Cd showed leaflets while other treatments had only leaflet primordia (Fig. 5).

The leaf area increased until eight days of development while older leaf ages showed no significant differences (Fig. 6a). Cadmium had no effect in leaf area for all ages assessed (Fig. 6a). However, the leaf dry mass was higher under 10 µM Cd for all leaf ages (Fig. 6b). In addition, the leaf dry mass was particularly reduced in older leaves under 50 µM Cd (Fig. 6b). Likewise, although leaves from control plants showed increasing dry mass during 14 days, this trait only increased by 8 days in Cd exposed plants (Fig. 6b). Leaves developed under 50 µM Cd showed higher specific leaf area compared to other treatments (Fig. 6c). The lowest means for specific leaf area were found for plants exposed to 10 µM Cd (Fig. 6c). The specific leaf area increased from 6 to 8 days of development with no further changes for all treatments (Fig. 6c).The marginal growth increased with time in all treatments achieving the highest

means at 20 days of development (Fig. 6d). However, 10 and 14 days old leaves showed higher marginal growth when exposed to 10 µM Cd (Fig. 6d). The diameter of mature cells in mesophyll was higher in plants exposed to 10 µM Cd (Fig. 6e). The leaf elongation rate was reduced only under 50 µM Cd (Fig. 6f).

Cadmium content increased in both old and young leaves of *Schinus molle* with increasing Cd concentration (Fig. 7). Control plants showed no difference in Cd content old and young leaves. However, Cd treated plants showed higher allocation of the metal in old leaves compared to young ones (Fig. 7).

DISCUSSION

Previous works reported increased chlorenchyma thickness in leaves exposed to heavy metals (Shi & Cai, 2009; Souza et al. 2011; Pereira et al. 2014). Likewise, Pereira et al (2016) reported that low cadmium concentrations promoted thicker chlorenchyma development and enhanced photosynthesis in *Schinus molle* leaves. However, in despite of the effects in mature leaves, the Cd effects in leaf meristems were little investigated.

The ground meristem is the precursor of the parenchyma tissues, including chlorenchyma (Evert, 2006). Therefore, the modifications in mature tissues are meristem driven. Likewise, the ground meristem of *S. molle* leaves showed several modifications when exposed to Cd. This may be related to the thicker leaves in *S. molle* plants grown under Cd contamination.

The Cd effects in meristems are classically toxic causing several problems such as: mitosis inhibition, abnormal microtubule organization and aberrant chromosomes (Shi et al. 2016). The toxic effects of Cd are particularly found in roots where the reduction of meristem size and number of cells are related to the higher nitric oxide production (Yuan & Huang, 2016). Therefore, the effects of Cd in meristematic tissues are mainly reported to the root apical meristems. The mitotic index and the number of cells in root apical meristem decreases depending on the concentration of Cd (Liu et al. 2003; Fusconi et al. 2006). All these effects promoted by Cd in meristems may explain the lower

results to leaf meristematic traits at 50 µM Cd. However, the limited activity found to *S. molle* leaf meristems at 50 µM Cd can't be considered toxic once the mean values are close to those of control plants. Therefore, this corroborates the Cd tolerance of *S. molle* plants as reported by Pereira et al (2016).

Interestingly, *S. molle* plants exposed to 10 µM Cd showed increased leaf ground meristem parameters. However, the anatomical modifications and positive effects on plant growth promoted by low Cd concentrations have been little discussed in literature (Arduini et al. 2004; Pereira et al. 2016). According to Kennedy & Gonsalves (1987), low cadmium levels can hyperpolarize the cell membranes at the root surface, increasing the trans-membrane potential and cation uptake. In addition, low metal concentrations can stimulate enzymatic activity (Sawidis, 2008). Thus, the largest cell elongation rate in *Schinus molle* leaves exposed to 10 µM Cd may be related to cell membrane modifications. Cell walls are very plastic and modifications on its composition and structure are related to cell growth and differentiation (Parrotta et al. 2015). High Cd concentration may reduce the cell wall plastic extensibility causing morphological and structural alterations in pollen grains (Sawidis, 2008). However, previous works have shown that low heavy metal levels may stimulate pollen tube growth (Searcy & Mulcahy 1985; Sawidis & Reiss 1995). Increased cell wall extensibility may be related to the higher cell elongation, diameter and ground meristem thickness found on plants grown at 10 µM Cd. Likewise, the

larger ground meristem cells and thickness may be related to thicker mature leaves under Cd pollution in tolerant plants. This effect was reported in fully developed leaves for different plant species (Shi & Cai, 2009; Souza et al. 2011; Pereira et al. 2016). Therefore, the thicker mature leaves found on tolerant plants under Cd contamination are related to enlargement in ground meristem cells.

Positive effects found in tolerant plants under Cd contamination also include increased growth (Jia et al. 2015), enhanced plastid differentiation in shoot apical meristem (Stoyanova & Tchakalova, 1999), up-regulation of the *AtMRP6* gene transporter (Gaillard et al. 2008), and increased photosynthesis (Jia et al. 2015; Pereira et al. 2016). All these positive modifications, particularly those related to growth and photosynthesis depend on leaf chlorenchyma tissues developed after ground meristem. Therefore, the Cd effects are found early in leaf primordia in tolerant plants such as *S. molle*.

The typical effect of Cd in meristems of non-tolerant plants is the inhibition of mitosis (Fusconi et al. 2006; Siddiqui et al. 2009; Yuan & Huang, 2016). The toxicity of Cd to meristems may be related to abnormal mitosis caused by the disorganization of microtubule, cytoskeleton and tubulin structures (Shi et al. 2016); the repression of auxin production and signaling in meristems (Yuan & Huang, 2016); formation of mitotic aberrations (Fusconi et al. 2006) and the inhibition of biosynthesis of preribosomal RNA precursors (Marcano et al. 2002). However, in tolerant species, positive Cd effects in

meristems can be found (Stoyanova & Tchakalova, 1999). The cell division parameters evaluated in this work were positively affected by 10 µM Cd in *S. molle* leaves. These traits are related to the higher ground meristem activity and associated with the production of larger cells that may explain the thicker mature leaves under Cd effects.

Cadmium toxicity effects in leaves include the reduced area, biomass and elongation in non-tolerant species (Lunáčová et al. 2003; Anjum et al. 2016; Jinadasa et al. 2016). All these leaf trait reductions are very common in non-tolerant species under Cd effects and may be related to the lower meristem activity found in this work. However, this toxicity was found only in *S. molle* plants exposed to 50 µM Cd.

The Cd accumulation in tolerant plants occurs mainly in older leaves. This mechanism of Cd allocation prevents toxicity in younger leaves that are physiologically active (Delmail et al. 2011; De Maria et al. 2013; Xin et al. 2013). In this work, we found that younger leaves (six days old or less) responded differently to Cd compared to older leaves. Thus, this suggests that the cadmium tolerance depends on differential metal allocation in young and old leaves.

CONCLUSION

Cadmium at low concentrations causes cell enlargement and improved mitosis in the ground meristem of *Schinus molle* leaves. Higher cell enlargement under Cd contamination results in thicker ground meristem related to thicker and more functional mature leaves in *S. molle.* The development of thicker leaves in Cd-tolerant species depends on the primary effects on the ground meristem. Older leaves uptake more Cd to protect the younger ones.

Conflict of interest statement: Authors declare that are not conflict of interest regarding this work.

ACKNOWLEDGEMENTS

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico [National Counsel of Technological and Scientific Development]), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [Coordination for the Improvement of Higher Education Personnel]), and FAPEMIG (Fundação de Amparo à Pesquisa do estado de Minas Gerais [Minas Gerais State Research Foundation]) for funding and research grants awarded to complete the present study.

REFERENCES

Anjum S.A., Tanveer M., Hussain S., Ullah E., Wang L., Khan, I.,… Shahzad B. (2016) Morpho-Physiological Growth and yield responses of two contrasting maize cultivars to cadmium exposure. *Clean, Soil, Air, Water* **44**, 29-36.

Arduini, I., Masoni A., Mariotti M. & Ercoli L. Low cadmium application increase miscanthus growth and cadmium translocation. (2004) *Environmental and Experimental Botany* 5**2**, 89-100.

Delmail D., Labrousse P., Hourdin P., Larcher L., Moesch C. & Botineau M. (2011) Physiological, anatomical and phenotypical effects of a cadmium stress in different-aged chlorophyllian organs of *Myriophyllum alterniflorum* DC (Haloragaceae). *Environmental and Experimental Botany* **72**, 174-181.

De Maria S., Puschenreiter M. & Rivelli A.R.(2013) Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. *Plant Soil Environment* **59**, 254-261.

Dickson R.E. & Isebrands J.G. (1991) Leaves as Regulators of Stress Response, In *Response of Plants to Multiple Stresses* (eds H.A. Mooney, W.E. Winner & E.J. Pell), pp 3-34. Academic Press, San Diego.

Evert R.F. (2006) Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development, p 601. John Wiley & Sons, New Jersey.

Ferreira D.F. (2011) Sisvar: a computer statistical analysis system. *Ciência Agrotecnologia* **35**,1039–1042.

Fusconi A., Repetto O., Bona E., Massa N., Gallo C., Dumas-Gaudot E. & Berta G. (2006) Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. Frisson seedlings. *Environmental and Experimental Botany* **58**, 253-260.

Gaillard S., Jacquet H., Vavasseur A., Leonhardt N. & Forestier C. (2008) AtMRP6/AtABCC6, an ATP-Binding Cassette transporter gene expressed during early steps of seedling development and up-regulated by cadmium in *Arabidopsis thaliana*. *BMC Plant Biology* **22**, 1-11.

Hoagland DR, & Arnon DI (1950) The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* **347,**1-39.

Ivanov V.B. & Dubrovsky J.G. (1997) Estimation of the cell-cycle duration in the root apical meristem: a model of linkage between cell-cycle duration, rate of cell production, and rate of root growth. *International Journal of Plant Sciences* **158**, 757-763.

Jia L., Liu Z., Chen W., Ye Y., Yu S. & He X. (2015) Hormesis effects induced by cadmium on growth and photosynthetic performance in a hyper accumulator, *Loonier japonica* Thunb. *Journal of Plant Growth Regulation* **34,** 13-21.

Jinadasa N., Collins D., Holford P., Milham P.J.& Conroy J. P. (2016) Reactions to cadmium stress in a cadmium-tolerant variety of cabbage (*Brassica oleracea* L.): is cadmium tolerance necessarily desirable in food crops?. *Environmental Science and Pollution Research* **23**, 5296–5306.

Kennedy C.D. & Gonsalves F.A.N. (1987) The action of divalent zinc, cadmium, mercury, copper and lead on the trans-root potential and H+ efflux of excised roots. *Journal of Experimental Botany* **38**, 800-817.

Liu D., Jiang W. & Gao X. (2003) Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biologia plantarum* **47**, 79-83.

Lunáčová L., Šottníková A., Masarovičová E., Lux A. & Streŝko V. (2003) Comparison of cadmium effect on willow and poplar in response to different cultivation conditions. *Biologia Plantarum* **47**, 403-411.

Marcano L., Carruyo I., Del Campo A. & Montiel X. (2002) Effect of Cadmium on the Nucleoli of Meristematic Cells of Onion *Allium cepa* L: An Ultrastructural Study, *Environmental Research* **88**, 30-35.

Parrotta L., Guerriero G., Sergeant K., Cai G. & Hausman J.F. (2015) Target or barrier? The cell wall of early-and later-diverging plants vs cadmium toxicity: differences in the response mechanisms. *Frontiers in plant science* **6**, 1-16.

Pereira F.J., Castro E.M., Oliveira C., Pires M.F., Pereira M.P., Ramos S.J. & Faquin V. (2014) Lead tolerance of water hyacinth (*Eichhornia crassipes* Mart.-Pontederiaceae) as defined by anatomical and physiological traits. *Anais da Academia Brasileira de Ciências* **86**,1423-1433.

Pereira, M. P., Rodrigues, L. C. A., Corrêa, F. F., Castro, E. M., Ribeiro, V. E. & Pereira, F. J. (2016) Cadmium tolerance in *Schinus mol*e trees is modulated by enhanced leaf anatomy and photosynthesis. *Trees* **30**, 807-814.

Pérez Chaca M.V., Vigliocco A., Reinoso H., Molina A., Abdala G., Zirulnik F. & Pedranzani H. (2014) Effects of cadmium stress on growth, anatomy and hormone contents in *Glycine max* (L.) Merr. *Acta Physiologiae Plantarum* **36**, 2815-2826.

Sawidis T. (2008) Effect of cadmium on pollen germination and tube growth in *Lilium longiflorum* and *Nicotiana tabacum*. *Protoplasma* **23**, 95-106.

Sawidis T. & Reiss H.R. (1995) Effects of heavy metals on pollen tube growth and ultrastructure. *Protoplasma* **185**, 113-122.

Searcy K.B. & Mulcahy D.L. (1985) The parallel expression of metal tolerance in pollen and sporophytes of *Silene dioica* (L.) Clairv., *S. alba* (Mill.) and *Mimulus guttatus* DC. *Theoretical and Applied Genetics* **69**, 597–602

Shi G., Sun L., Wang X. & Liu C. (2014) Leaf responses to iron nutrition and low cadmium in peanut: anatomical properties in relation to gas exchange. *Plant and soil* **375**, 99-111.

Shi G. & Cai Q. (2009) Leaf plasticity in peanut (*Arachis hypogaea* L.) in response to heavy metal stress. *Environmental and Experimental Botany* **67**, 112-117.

Shi Q., Wang J., Zou J., Jiang Z., Wu H., Wang J.,... Liu D. (2016) Cadmium localization and its toxic effects on root tips of barley. *Zemdirbyste-Agriculture* **103** 151-158.

Siddiqui S., Meghvansi M.K., Wani M.A. & Jabee F. (2009). Evaluating cadmium toxicity in the root meristem of *Pisum sativum* L. *Acta Physiologiae Plantarum* **31**, 531-536.

Singh S., Parihar P., Singh R., Singh V.P. & Prasad S.M. (2016) Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics and ionomics. *Frontiers in Plant Science* **6**, 1-36.

Souza V.L., Almeida A.A.F., Lima S.G., Cascardo J.C.D.M., Silva D.D.C., Mangabeira P.A. & Gomes F.P. (2011) Morphophysiological responses and programmed cell death induced by cadmium in *Genipa americana* L.(Rubiaceae). *Biometals* **24**, 59-71.

Stoyanova D. & Tchakalova E. (1999). Cadmium induces ultrastructural changes in shoot apical meristem of *Elodea canadensis* Rich. *Photosynthetica* **37**, 47-52.

Tsukaya H. (2006) Mechanism of leaf-shape determination. *Annual Review of Plant Biology* **57**, 477-496.

Xin J., Huang B., Yang Z., Yuan J. & Zhang Y. (2013) Comparison of cadmium subcellular distribution in different organs of two water spinach (Ipomoea aquatica Forsk.) cultivars. *Plant and soil* **372**, 431-444.

Yuan H.M. & Huang X. (2016) Inhibition of root meristem growth by cadmium involves nitric oxide-mediated repression of auxin accumulation and signaling in Arabidopsis. *Plant, Cell and Environment* **39**, 120–135.

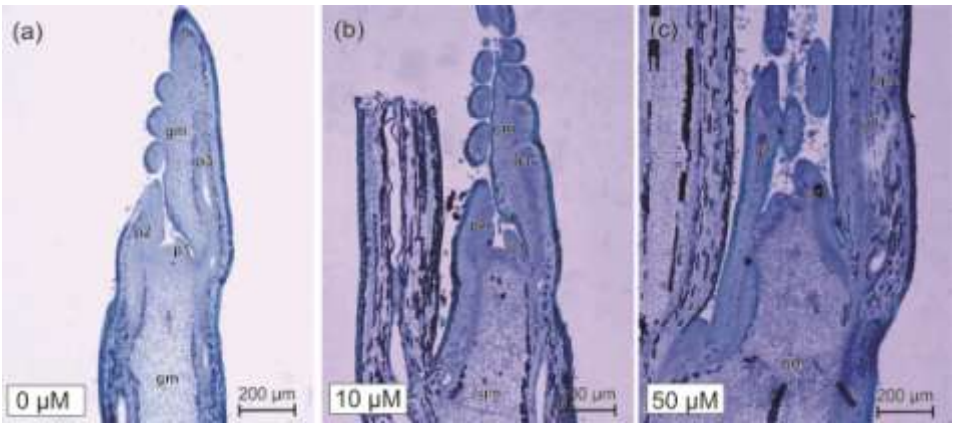

Figure 1. Shoot apex of Schinus molle plants exposed to cadmium. Leaf primordia were labeled from the youngest to oldest as follows: p1= youngest leaf primordium, p2= intermediary leaf primordium and p3 = oldest leaf primordium, gm = ground meristem.

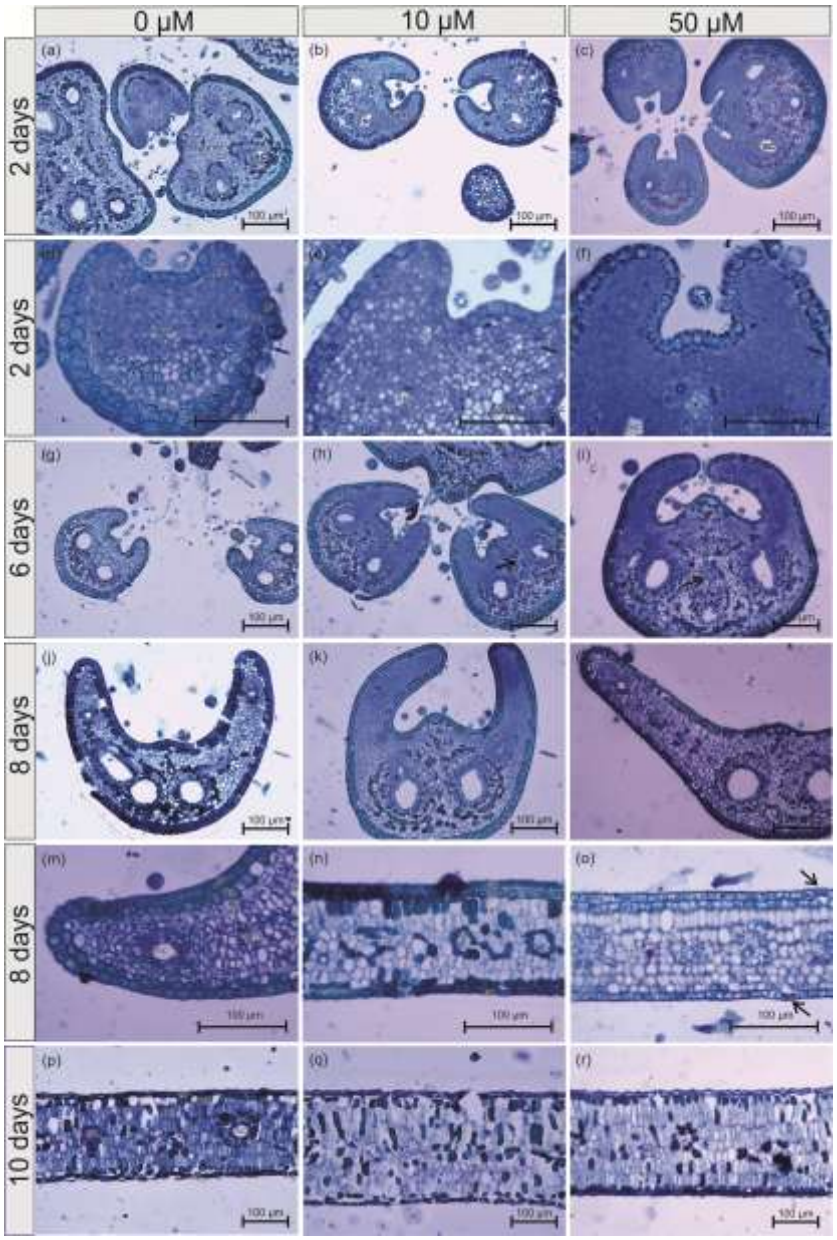

Figure 2. Leaflet development and tissue differentiation of *Schinus molle* plants exposed to Cd. Asterisks in (e) and (f) are indicating xylem vessels and (arrows) are indicating stomata (o). pr = protodermis, hp = hypodermis, gm = ground meristem, sc = secretory channel, vb= vascular bundle, pp = palisade parenchyma, ep = epidermis.

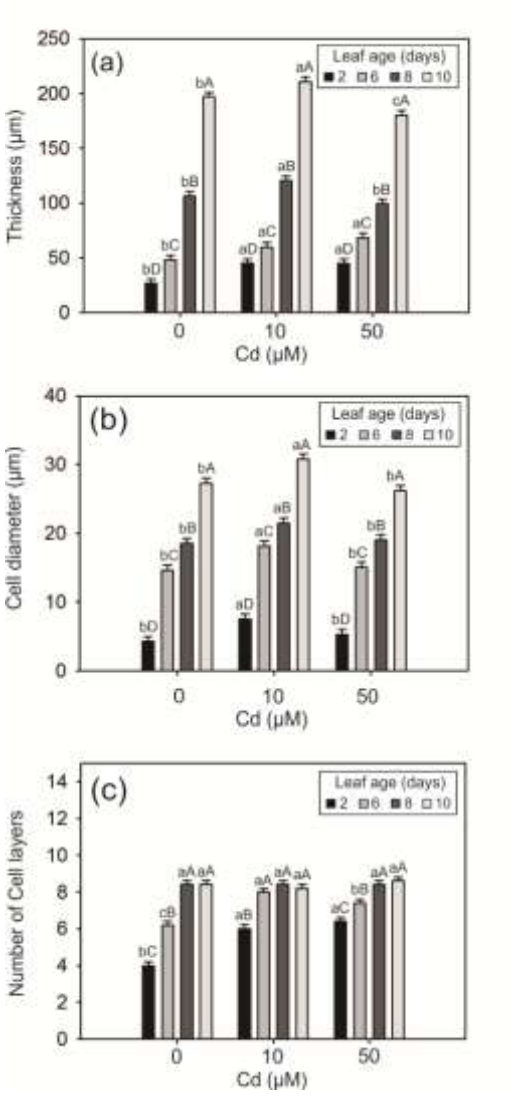

Figure 3. Ground meristem traits of *Schinus molle* leaf primordia exposed to cadmium. The lower case letters compare the Cd concentration at the same leaf age and the uppercase letters compare the leaf age amongst the same Cd concentration. Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level.

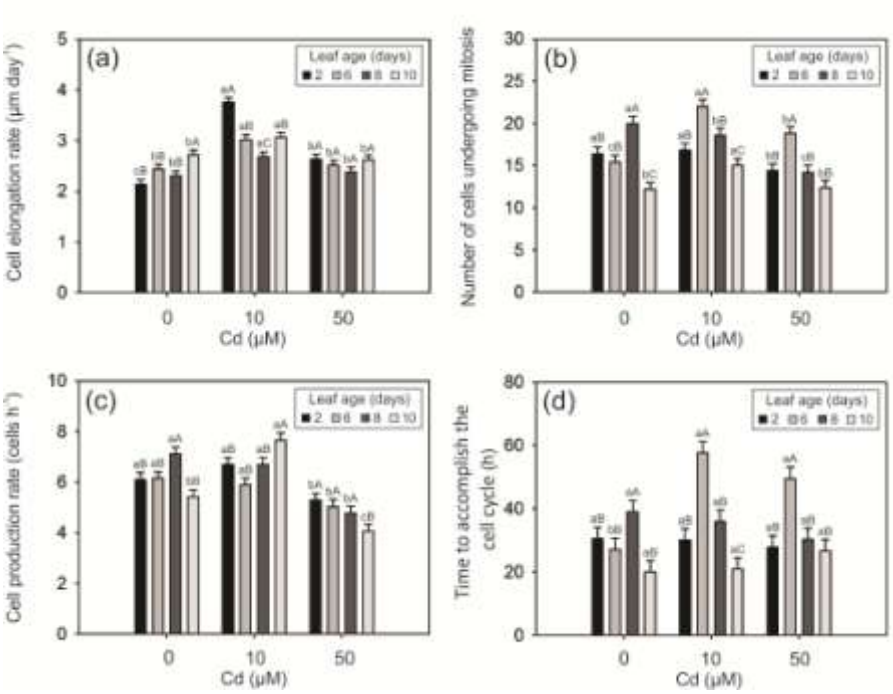

Figure 4. Modifications in cell traits of *Schinus molle* leaf primordia exposed to cadmium. The lower case letters compare the Cd concentration at the same leaf age and the uppercase letters compare the leaf age amongst the same Cd concentration. Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level.

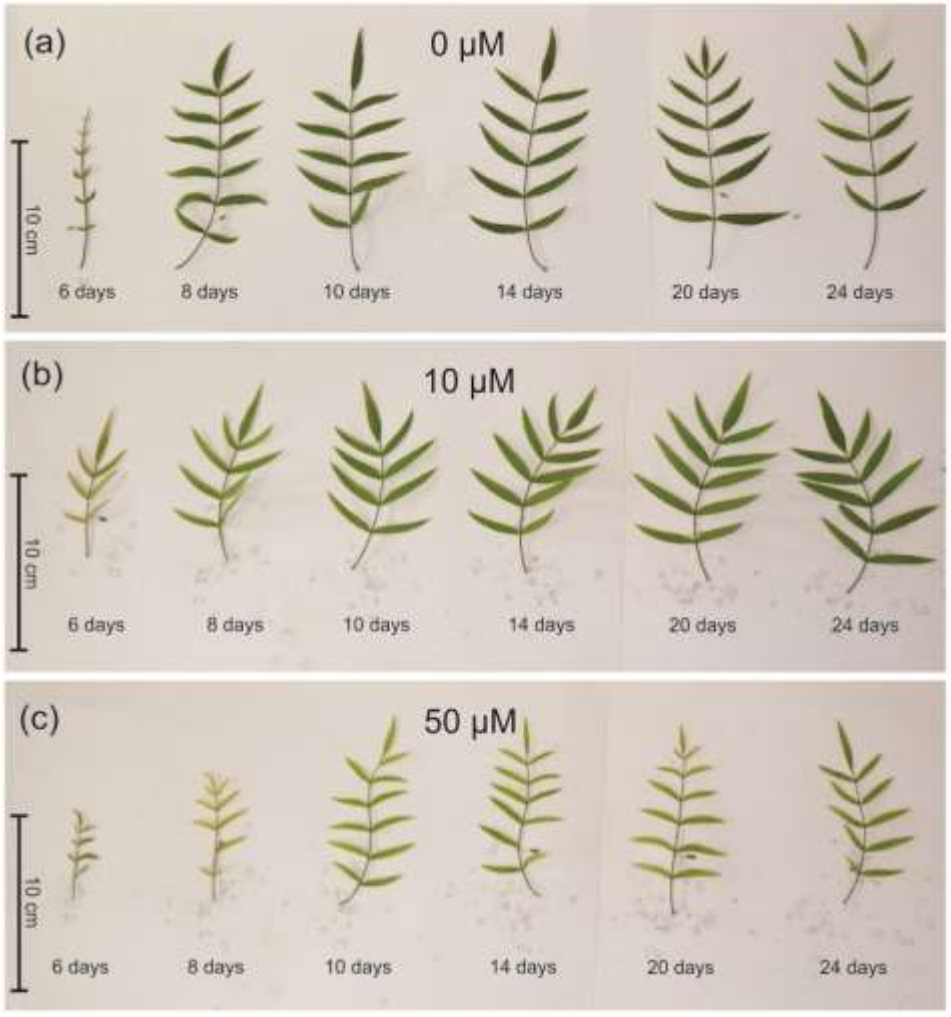

Figure 5. Development of *Schinus molle* leaves exposed to three cadmium concentrations.

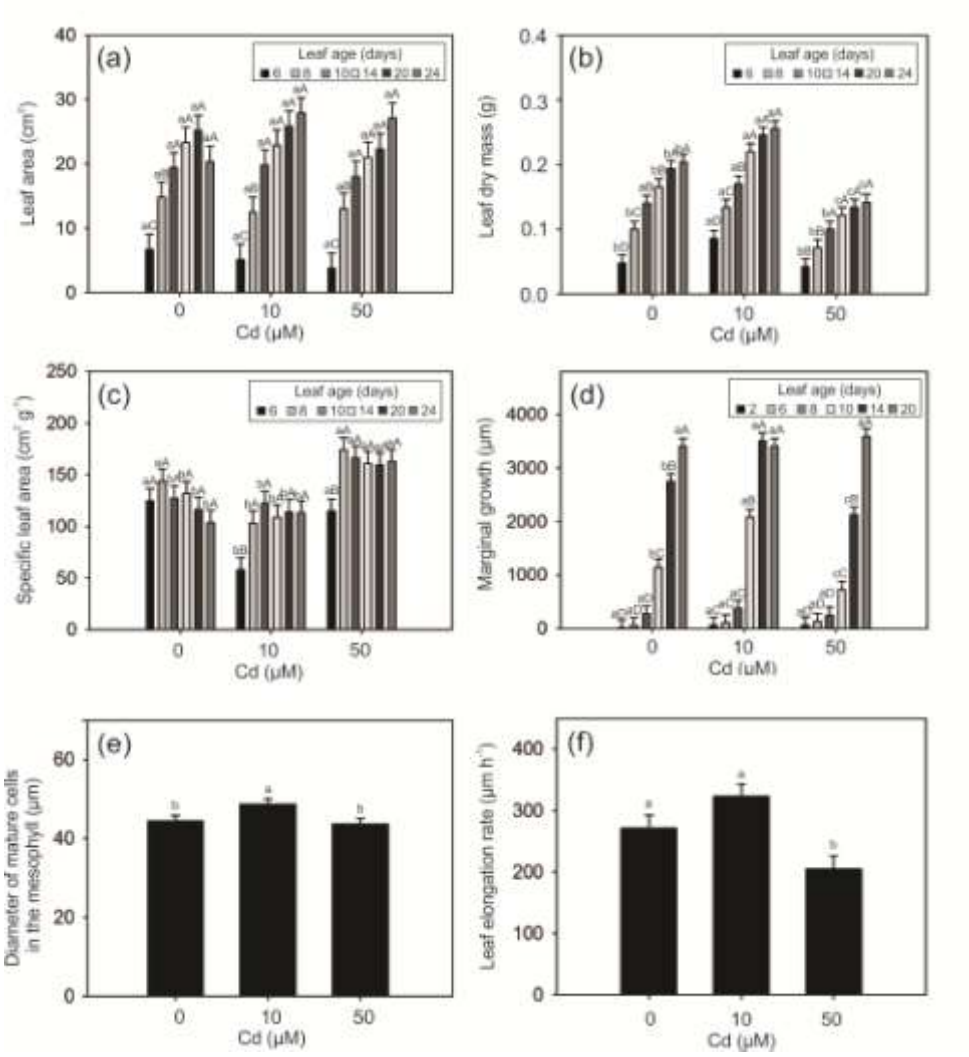

Figure 6. Leaf traits in *Schinus molle* plants exposed to cadmium. The lower case letters compare the Cd concentration at the same leaf age and the uppercase letters compare the leaf age amongst the same Cd concentration. Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level.

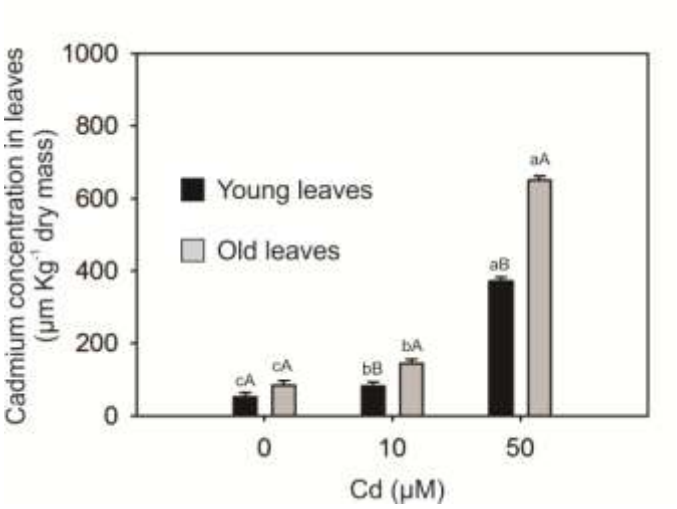

Figure 7. Cadmium content *Schinus molle* leaves at different ages. The lower case letters compare the Cd content at different nutrient solutions and the uppercase letters compare the leaf age amongst the same Cd concentration. Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level.