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Effects of *Eugenia dysenterica* L. extracts on roots and gravitropism of *Sesamum indicum* L. and *Raphanus sativus* L.

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ABSTRACT

We tested the effects of allelochemicals phytotoxicity from *Eugenia dysenterica* leaf extracts on the gravitropism in radish and sesame plants. We also investigated the morpho-anatomical, ultrastructural aspects of sesame and radish seedlings exposed to *E. dysenterica* extracts. The roots underwent structural modifications with severe structural abnormalities when exposed to *E. dysenterica* extracts. Hence, these roots bended upwards, the hypocotyl thickened, root tip malformations and showed early lateral root development. Internally, tissue oxidation, faster damage to xylem and root caps. Furthermore, the gravitropic response of roots was altered due to abnormalities in columella cells ultrastructure. Thus, allelochemicals may interfere with the differentiation and positioning of statoliths, which is a determining factor for a proper gravitropic response in plants.

Key Words: Allelopathy, Cerrado, columella, *Eugenia dysenterica*, extracts, gravitropism, phytotoxicity, radish, *Raphanus sativus* L., roots, sesame, *Sesamum indicum* L., statocytes

INTRODUCTION

Allelopathy is plant-to-plant, plant-to-microorganism or microorganism-to-microorganism, interactions phenomenon mediated by secondary metabolites that affects various physiological processes and biological systems. Allelopathy is negative or positive effects that one plant can exert on another through release of chemical compounds into the environment (1,2). These compounds, termed as allelochemicals are present in plant leaves, stems, roots, fruits, seeds and flowers (2,3,4). The interest in allelopathic compounds has enormously increased due to their effects on seeds germination, plant growth and development (5). This phenomenon has great potential to provide environmental friendly compounds for weed control, these can be used as bioherbicides, which, compared with regular herbicides, would minimize the damages caused to the environment (6,7,8,9,10). For instance, the allelopathic potential of berberine extracted from *Coptis chinensis* inhibited the growth and development of invasive weeds *Bidens pilosa* and *Arabidopsis* CYCB1: GUS line (11).

Majority of the phytotoxic and allelopathic studies are based on descriptive parameters and quantifies the effects of either plant extracts or isolated molecules on physiological phenomena such as germination, seedling growth, changes in biomass and/or, and less frequently, on morphological aspects, which include overgrowth of plant

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parts, root morphology and anatomy and changes in plant tropism (2,9,12,13,14,15,16). Recently the importance of research on allelochemical impacts on plant biochemistry and cell functions have increased (16,17). The study of effects of plant extracts or products on growth of other organisms are essential to quantify the phytotoxicity of such compounds, however, the studies regarding the effects of allelochemicals on plant cell metabolism and differentiation are rare. This investigation opens new perspectives for understanding the effects of phytotoxic and allelopathic agents on plant development (2). Therefore, studies that investigate the effects of phytotoxic and allelopathic compounds on anatomy and cell ultrastructure of plant tissues are an important step to better understand the phytotoxicity and allelopathic phenomena in plant metabolism. Moreover, the mechanism of these phytotoxic processes opens a possibility for the use of these substances of organic nature as an alternative to herbicides and synthetic growth regulators (10,18).

There are no systematic allelopathic studies to investigate the effects of Brazilian flora plant extracts or products on metabolism, plant growth and differentiation (2). Now the interest in phytotoxic and allelopathic studies involving Cerrado species is growing rapidly and the Myrtaceae family has been studied most (3).

Eugenia dysenterica DC. (Myrtaceae), phytotoxic effects were investigated due to its several uses [source of tannins, ornamental plant, fruits to make jellies, sweets, popsicles, and ice cream. Besides its use in folk medicine against diarrhea (leaves) and constipation (fruits) (19,20,21)]. Its phytotoxic potential has been fully demonstrated (3,22,23). Previous research has shown that *E. dysenterica* leaf extracts affected the seedling development of sesame and radish (15). Roots were more sensitive to the extracts than shoots. In both target species, the extracts reduced root growth, promoted root darkening and early lateral root and root hairs differentiation. Interestingly, it was also seen that theses aqueous extracts altered the root gravitropic response in both target species, whose roots grew upwards, a pattern of response frequently observed in allelopathic studies (2).

This work aimed to investigate the effects of aqueous extracts of *E. dysenterica* leaves on morpho-anatomical and ultrastructural changes in roots of *Sesamum indicum* L. (Pedaliaceae - sesame) and *Raphanus sativus* L. (Brassicaceae - radish).

MATERIAL AND METHODS

Eugenia dysenterica L. leaves were collected from Cerrado area trees, in our Brasilia University Campus (Latitude 15º 46’11” S; longitude 47º 52’ 6” W at 1038 m altitude). The leaf extracts were prepared as per Pina *et al.* (2009). The samples, assays and microscopy analyses for this research were done between July 2008 and July 2009.

Sesame (*Sesamum indicum*, var. ‘Negra’) and radish (*Raphanus sativus*, var. ‘Vip Crimson-Feltrin’) seedlings were grown in Petri dishes for five days with *E. dysenterica* aqueous leaf extracts of 3%, 1% and 0% (control) concentrations. The growth conditions of each target species were the same as those described by Pina *et al.* (2009). Therefore, seeds of both target-species were grown in BOD chambers (Marconi MA 403), 12 h-photoperiod, light intensity of 400 μmol.m^{-2}.s^{-1}) and temperatures set at 25 and 30 ^{o}C for sesame and radish, respectively.

One-day, 3-day and 5-day old seedlings of each treatment were fixed in 0.05 M calcium cacodylate buffer, 2% glutaraldehyde, 4% paraformaldehyde, pH 7.0 under vacuum at RT for 1 h and overnight at 4°C (13).

For scanning electron microscopy (SEM) the plant material was fixed as described above, dehydrated in graded acetone series, dried to the critical point and coated with gold (Balzers CPD 30 dryer). The samples were analysed and photographed in a Jeol JEM-840A scanning electron microscope.

For light microscopy, the fixed tissue samples were dehydrated in an ethanol series and then infiltrated and embedded in paraffin. The root samples were sequentially sectioned to 10 µm thickness (both transversely and longitudinally), laid as ribbons on microscope slides and allowed to dry on a hot plate for 48 h at 45 ^{o}C. The slides were dewaxed in xylene, re-hydrated and then stained with Toluidine Blue (TBO). Subsequently, the microscope slides were dehydrated, and mounted in Entellan® (Merck), analysed and photographed on a Zeiss Axioskop light microscope. The transverse sections were compared approximately at the same distance from the root tip to standardize the analysis.

Ferric chloride histochemical test using fresh hand-sectioned samples were done to detect the phenolic compounds (21). The ferric chloride test yielded a dark brown colour in presence of tannins and other phenols.

For transmission electron microscopy (TEM), the samples were post-fixed with 2% osmium tetroxide diluted with 0.05 M calcium cacodylate buffer (1:1 v/v) for 1h, in the dark. The samples were also block stained with 0.5% uranyl acetate solution overnight, and then dehydrated in crescent alcoholic series, infiltrated and embedded in Spurr resin. Subsequently, the tissue was sectioned to a thickness of 80-100 nm with a Reichert Supernova – Leica ultramicrotome, extended and fished with platinum grids (300 meshes). The sections were post-stained with 0.5% uranyl acetate and 0.4% lead citrate (w/v) for 40 min and 10 min, respectively. The sections were analysed and photographed in a transmission electron microscope Jeol 1011 TEM.

RESULTS AND DISCUSSION

The presence of allelochemicals in *E. dysenterica* has been reported in previous studies (15,24), which have revealed great potential of this plant as source of bioherbicides. Giotto *et al.* (2008) working with *Lactuca sativa* and Pina *et al.* (2009) with sesame and radish, showed that aqueous *E. dysenterica* extracts impaired the growth of target plants, due to the adverse effects on root system.

As mentioned, *E. dysenterica* extracts adversely affected the plant growth and development, but little is known about the morpho-anatomical and cellular effects of allelopathic compounds in plants. Our results have shown that substances present in *E. dysenterica* leaf extracts induced several root abnormalities, in both external and internal structure, as wells as in physiological processes such as the gravitropic response of these plants.

Leaf extracts induced an abnormal development of root as well as the differentiation of columella cells, specifically of the statocytes in the target species, which may directly affect the gravitropic response of the root. The following sections will describe and discuss, all these morpho-anatomical and ultrastructural changes observed in target plants.

Morphological changes in sesame and radish seedlings.

Figure 1 (A to F) shows the effects of *E. dysenterica* leaf extracts (1% and 3% dilutions) on the growth and development of 5-day old sesame and radish seedlings compared with respective controls. In target species, the leaf extracts inhibited the shoot and root growth in comparison to respective controls (Figure 1). Nevertheless, the root growth was more affected by *E. dysenterica* extract than shoots. Moreover, *E. dysenterica* leaf extracts induced an upward root bending in both species (Figure 1 B, C and E, F), and sometimes this bending tended to roll up the tip. Both extract concentrations (1% and 3%) reduced the root growth, but 3% extract was more inhibitory than 1%. Therefore, for roots, the phytotoxic response to *E. dysenterica* extracts followed a dose-dependent fashion (Figure 1 A and B). Usually, root apices were also damaged by the extract, and necrosis occurred in the root region (Figure 1 B, C and E, F).

Figure 1. Effects of *Eugenia dysenterica* leaf extract on the growth and development of *Sesamum indicum* (A, B, C) and *Raphanus sativus* (D, E, F) seedlings. (A) Sesame seedling grown in water (control). (B) Sesame seedlings treated with 1%. (C) Sesame treated with 3% extract. (D) Radish control. (E) Radish grown in 1% extracts (F) Effect of 3% *E. dysenterica* leaf extracts on radish seedlings. Note that root growth of target plants was substantially reduced and tended to grow upwards. Bar = 1 cm.

E. dysenterica leaf extracts directly affected the seedlings development of sesame and radish, with dramatic effects on root morphology. Figure 2 (A to D) depicts root external morphology of control treatments, as well as sesame and radish seedlings grown in *E. dysenterica* leaf extracts. Compared with the controls, extract treated seedlings had roots with thinner and pointy apices (Figure 2 A to D), which usually bended upwards (Figure 1 A and B). Moreover, these plants showed an early development of lateral roots (Figure 2 D to F), and root hairs differentiation closer to tips (Figure 2 D, E, and F). Additionally, signs of tissue oxidation were frequently observed in seedlings treated with

E. dysenterica extracts, mainly on the side in direct contact with this solution (Figure 3). Histochemical tests indicated the presence of phenolic compounds in root oxidized areas (Figure 4).

In some plants, the presence of the extracts also induced development of thicker hypocotyls (Figure 2 C and E). The *E. dysenterica* leaf extract stimulated the hypocotyl overgrowth in most plants.

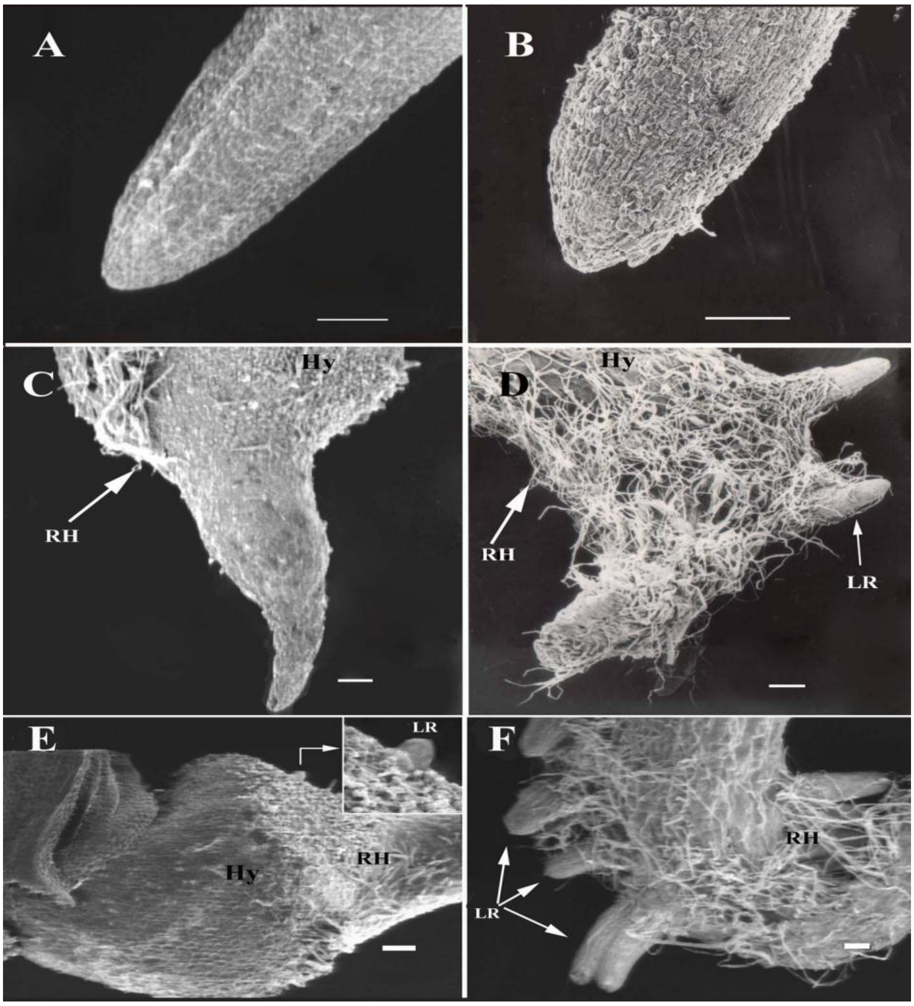

Figure 2. Scanning electron micrographs of *Sesamum indicum* and *Raphanus sativus* roots. (A) Sesame: root tip from control treatment. (B) Radish: root tip from control. (C)): Root from 5-day old sesame seedling grown in 3% leaf extract. Note the thick hypocotyl. (D) Root from radish seedling grown in 3% leaf extract for five days. Besides a thick hypocotyl region, it can be noticed several lateral roots near the tip. (E) Hypocotyl region of sesame roots from a extract treated seedling. It can be seen a differentiation of a lateral root (Inset). (F) Radish root grown in *E. dysenterica* extract with several lateral roots. Hy: hypocotyl; LR: lateral root; RH: root hair. Bar = 100 µm.

The results showed that sesame and radish root morphology was severely affected by the exposure to the allelochemicals of *E. dysenterica* extracts. Reports on the sensitivity of roots to allelochemicals are relatively frequent. For instance, root emergence was dramatically affected when seeds were germinated with lemon grass essential oils (12). Wheat growth was also inhibited by lemon grass essential oil and the root system was more sensitive to allelochemicals than shoots (12). Although, not a ubiquitous phenomenon, root systems growth shows the presence of allelochemicals (4,17,25). For instance, root length of *P. tabuliformis* was also diminished due to long exposure to allelochemicals present in leachates of *Quercus variabilis* (26). Thus, this is consistent with the fact that root growth and development of sesame and radish had been more affected than the shoots by *E. dysenterica* leaf extracts.

Allelopathic effects on the morphology and anatomy of target plants have not yet been the object of a detailed investigation. There have been few reports that deal with the morphology of roots subjected to the presence of allelochemicals. In *Alnus crispa* var. mollis (green alder), poplar balsam induced root tips necrosis and low density of root hairs (24). Furthermore, similar changes were seen in alfalfa roots as a result of autotoxicity (26). Additionally, Oliveira *et al.* (2004) reported a decrease in sesame root hairs, in seedlings treated with *Solanum lycocarpum* leaf extracts.

Plant hormones have been associated with several plant responses to biotic and abiotic stresses, which include allelopathic response (28). Both laboratory bioassays and soil grown plants treated with *E. dysenterica* extracts showed similar signs of root depleted growth in the target plants (15). Therefore, there is very little doubt that sesame and radish seedlings growing in presence of *E. dysenterica* extract are under stress. Furthermore, it has been stated that allelochemicals may induce imbalance and changes in phytohormone contents (28). Based on the morpho-anatomical changes seen in target plants, ethylene may play a role on how these plants react to allelochemicals.

In fact, root length is directly affected by ethylene, which suppresses cell elongation in roots (29). Besides stunted roots, sesame and radish seedlings also showed early formation of lateral roots, which can be related to ethylene production in exposed plants as in *Arabidopsis* (30). *Lotus japonicus* transgenic ethylene insensitive lines produced fewer lateral roots (31), which demonstrates a positive correlation between ethylene and lateral roots formation. Root growth inhibition has been reported due to allelochemical exposure. It is interesting to note that along with the changes in root size and lateral roots formation, hypocotyl hypertrophy was relatively frequent and has not yet been described in other species. It appears that root cap cells of extract grown seedlings easily sloughed off, which was consistent for all seedlings exposed to leaf extracts.

Besides lateral root differentiation, hypocotyl thickness and root elongation have also been associated with ethylene levels. In *Arabidopsis* mutant *alh1*, an ethylene overproducing plant, seedlings developed thicker hypocotyls (32). In the case of hypocotyl thickness, ethylene and auxins act synergically in roots exposed to light, which results in higher elongation and thinner hypocotyls. Thereby, the presence of allelochemicals may induce ethylene production, and possibly other plant hormones related to the process of morphogenesis and root architecture (33).

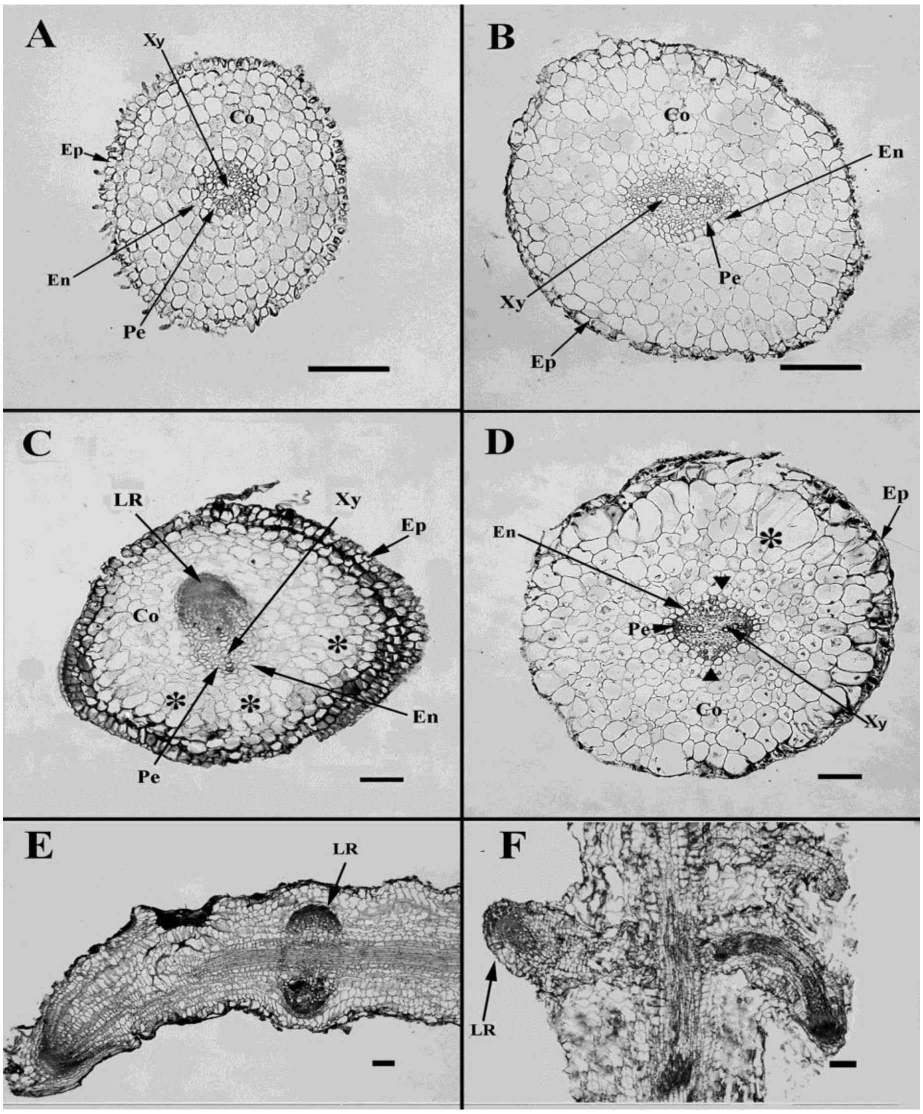

Figure 3. Transverse and longitudinal sections of *Sesamum indicum* and *Raphanus sativus* roots. (A) Sesame control. (B): Radish control. (C) Transverse section of roots from 5-day old sesame seedling grown in 3% leaf extract. (D) Cross section from 5-day old radish roots treated with 3% leaf extract. Note that, compared with respective controls, the outer cell layers were darker due to tissue oxidation, mainly in the region in contact with allelochemicals (*). Moreover, the cross sections also show the initial differentiation of lateral root (arrow heads). (E) Root longitudinal sections from sesame extract treated roots showing the differentiation of lateral roots close to the tip. (F) Longitudinal radish root section grown in leaf extract with lateral roots. Co: cortex; En: endodermis; Ep: epidermis; LR: lateral roots; Pe: pericycle; Xy: xylem. Bar = 100 µm.

Root anatomical and developmental modifications observed in extract grown seedlings.

With respect to the anatomy of sesame and radish roots, *E. dysenterica* leaf extract induced several structural modifications when compared with the controls (Figure 3 A-F). Transverse sections showed that roots of control treatments of both target species had simple epidermis, no distinguishable exodermis, parenchymatous cortex, endodermis, one-layered pericycle, as well as diarch vascular system (Figure 3 A and B). The contact with extract of *E. dysenterica* leaves resulted in substantial changes in the epidermis, cortex and vascular system (Figure 3 C and D). Moreover, the epidermal cells were severely damaged and turned brownish (Figure 3 C and D). Furthermore, cells from the outer layers of the cortex also darkened in the presence of leaf extracts, which progressed to the inner area of the cortex. Compared with respective controls, cortical cell hypertrophy was seen on the root side in contact with the extract, mainly in cells near the epidermis. These overgrown cells were radially longer in contrast with those of the control treatments (Figure 3 C and D).

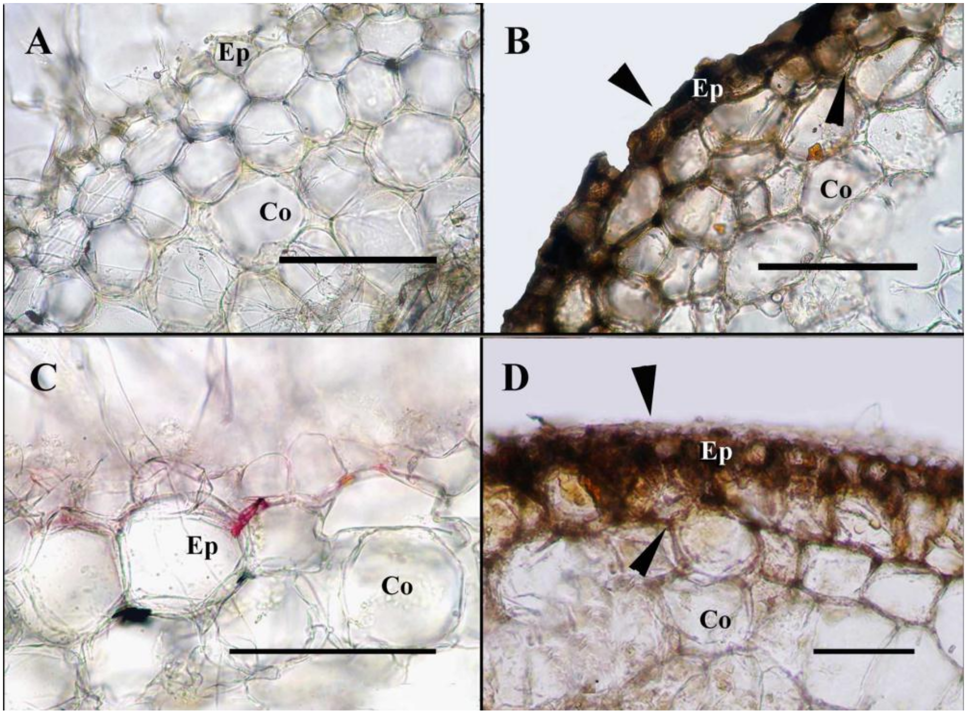

Figure 4. Histochemical test with ferric chloride reagent for phenolic compounds detection in the roots of *Sesamum indicum* and *Raphanus sativus* seedlings. A) Transverse section of sesame root grown in water. There was no color reaction. B) Sesame root grown in 3 % *E. dysenterica* leaf extract tested with ferric chloride. The dark coloration (arrows) indicates the presence of phenols. C) Micrograph of radish root grown in water and tested with ferric chloride. There was no positive reaction for the presence of phenols. D) Radish root grown in 3% leaf extract. The brown staining (arrows) indicates phenol presence. Bar = 100 µm. Co: cortex; Ep: epidermis.

Concerning to the vascular system, both plants were diarch and the main differences between treated plants and controls were: the precocious development of lateral roots as well as xylem differentiation in treated seedlings (Figure 3 C to F). A one-layered pericycle could be observed in all plants. In those seedlings exposed to the extracts, lateral roots started to show up even before the differentiation of metaxylem had been completed (Figure 3 C and D). While, in sesame, the lateral roots arose from the pericycle area near the protoxylem pole, in radish these roots emerged in protoxylem poles as well as in other pericycle regions (Figure 3 C and D).

Although, externally the root apices were substantially different from those of controls (Figure 2 A-D), internally, root longitudinal sections did not show striking differences in the organization of root apical meristem between plants grown in *E. dysenterica* extracts and respective controls (Figure 5 A-D). As shown in the Figure 2, externally, the differences reside mainly in the root shape and root cap of extract treated plants. On the other hand, it appears that the root meristems of extract treated plants were reasonably organized, and no clear abnormalities were seen in the promeristem. Nevertheless, an important difference could be noticed in the meristematic regions of root elongation zone. Tissue differentiation started earlier in plants grown in the extracts than those of control treatments (Figure 5 A-D). Note that, in the control seedlings, root apical cells retained their meristematic features such as dense cytoplasm, prominent nuclei, and small vacuoles (Figure 5 A-C) much longer than those of roots exposed to the extracts (Figure 5 B-D). For both target species, in the apical regions of these roots, the vacuolization and tissue differentiation may be seen closer to the apex compared with the controls (Figure 5 A-D).

Conversely, the extracts had a severe effect on the root caps and induced an abnormal development of these structures (Figure 5 A-D). While control seedlings had a regular conical root cap shape (Figure 5 A and C), in the extract grown plants the root caps were smaller (Figure 5 B) and frequently of irregular shape (Figure 5 D). It is noteworthy that these plants consistently presented an altered gravitropic response. Roots of both target species reacted to the extract by showing a negative gravitropism.

Also, it was noticed that the columella of such root caps was frequently less conspicuous than those of the control. Nonetheless, the presence of statoliths could be seen in all columella types. Therefore, the ultrastructure of the columella cells was investigated to determine whether or not there was any abnormality that could interfere with the gravitropic response of the roots of extract treated seedlings.

As mentioned, roots from extract grown sesame and radish seedlings presented dramatic anatomical defects. Tissue darkening (34) and necrosis (35, 2) are common to plants grown in the presence of allelochemicals. Plant tissue oxidation has been associated with an increase phenol accumulation in plant tissues (36, 37). In addition, phenols were detected in roots of seedlings exposed to *E. dysenterica* extract, which correlates the oxidation process and allelochemicals (38).

The change in the curvature of the root in both targeted species grown with leaf extract is definitely correlated with the overgrowth of cells on one side of the root. The contact with allelochemicals induced a radial cell elongation and resulted in an upward bending of roots. This suggests that sesame and radish seedlings reacted to minimize the contact with the compounds in *E. dysenterica* leaf extract.

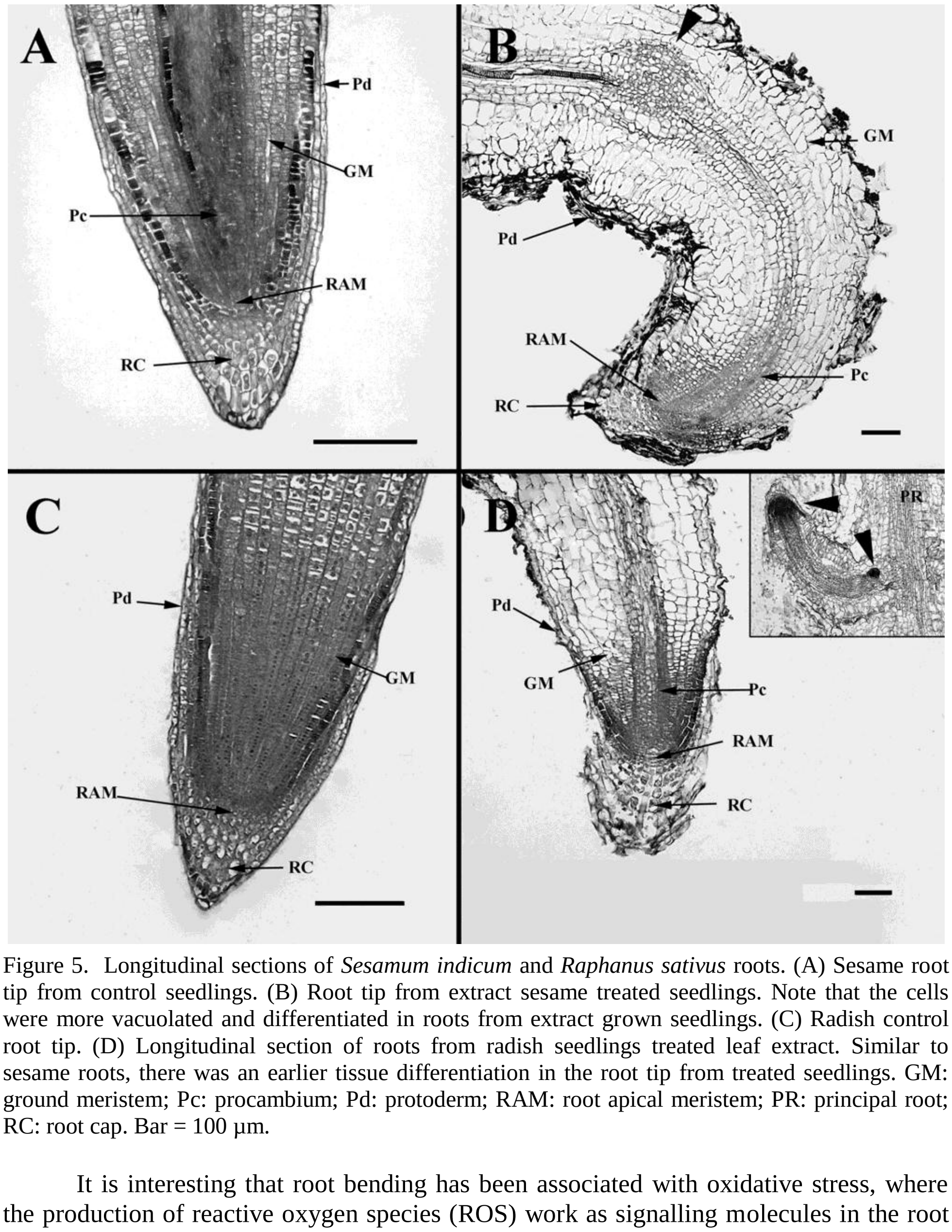

Figure 5. Longitudinal sections of *Sesamum indicum* and *Raphanus sativus* roots. (A) Sesame root tip from control seedlings. (B) Root tip from extract sesame treated seedlings. Note that the cells were more vacuolated and differentiated in roots from extract grown seedlings. (C) Radish control root tip. (D) Longitudinal section of roots from radish seedlings treated leaf extract. Similar to sesame roots, there was an earlier tissue differentiation in the root tip from treated seedlings. GM: ground meristem; Pc: procambium; Pd: protoderm; RAM: root apical meristem; PR: principal root; RC: root cap. Bar = 100 µm.

It is interesting that root bending has been associated with oxidative stress, where the production of reactive oxygen species (ROS) work as signalling molecules in the root growth orientation process (39, 40). Additionally, a disruption in normal ROS distribution led to an altered root gravitropic response in *Arabidopsis* roots (40). It appears that auxin regulates ROS distribution in *Arabidopsis* roots, whose action restored a normal gravitropic response in these roots (39).

Therefore, it appears that auxin and oxidative stress are necessary for proper gravitropic response in plants (39, 40). In addition, it has been suggested a positive correlation between allelochemicals and synthesis/accumulation of ROS (6, 41). It is very reasonable to suppose that similar response mechanism may have taken place in sesame and radish roots grown in *E. dysenterica* leaf extracts. Nonetheless, these facts may not be the only components in this phenomenon, as root caps were also dramatically affected by *E. dysenterica* leaf extract. This disruption in root cap structure certainly plays a role in the altered gravitropism seen in these target plants.

Another intriguing change in root anatomy was the earlier cell differentiation in root apices of extract treated seedlings. Changes in xylem differentiation due to allelochemicals has already been reported. Gatti *et al.* (2010) reported that, in sesame roots, aqueous extracts of *Aristolochia esperanzae* O. Kuntze resulted in 50% reduction in the size of xylem cells. Plant hormones such as auxins and ethylene are crucial for xylem differentiation in pea roots (43, 44). Therefore, plant hormones, either ethylene alone or synergically with auxin, appear to be related to the anatomical modification seen in *E. dysenterica* and other plant species (33).

Finally, these morpho-anatomical changes can certainly impair root functionality. Roots grow continually to explore the soil to obtain nutrients and water. In case of disruption of this growth process the root capacity to uptake nutrients would be dramatically compromised. Pina *et al.* (2009) observed that the root system of sesame and radish plants grown in soil and watered with *E. dysenterica* leaf extracts was severely affected. Additionally, for now, the role and consequences of plant hormone in the morpho-anatomical changes can only be just suggested. Hormone measurements in extract treated and non-treated plants must be done to investigate the function of plant hormones in the allelopathic responses.

Ultrastructure of columella cells

The most striking difference between columella cells of control and extract treated plants resided in the statoliths. These statoliths differed structurally as well as their location and distribution in columella cells. The statoliths of control sesame and radish seedlings had multiple starch grains and were larger than those seen in the respective extract grown plants (Figures 6 A and B; 7 A and B). Besides the size difference, the statoliths of these seedlings had fewer and smaller starch grains than those of controls (Figures 6 and 7). In sesame seedlings grown in *E. dysenterica* leaf extracts the statoliths had either tiny starch grains or none at all (Figure 6 A to D). In treated radish, these organelles had several forms, most of which were smaller, and sometimes amoeboid (Figure 7 B and D). Nonetheless, in radish was observed the presence of rare statoliths whose shapes resembled those of control plants. The statoliths of treated plants did not keep their regular cellular place within the columella cells as seen in samples of the controls, whose statoliths were regularly seen at the bottom side of the statocytes (Figures 6 and 7). Thus, the micrographs of extract treated plants revealed that the statoliths were scattered in the cytoplasm of columella cells (Figures 6 A and C; 7 A and C). Additionally, it was seen that the statocytes of extract grown seedlings were more vacuolated than those of the controls (Figures 6 and 7). Although, this feature was more noticeable in sesame seedlings (Figure 6 A and C), in radish, it was also a consistent characteristic (Figure 7 A and C).

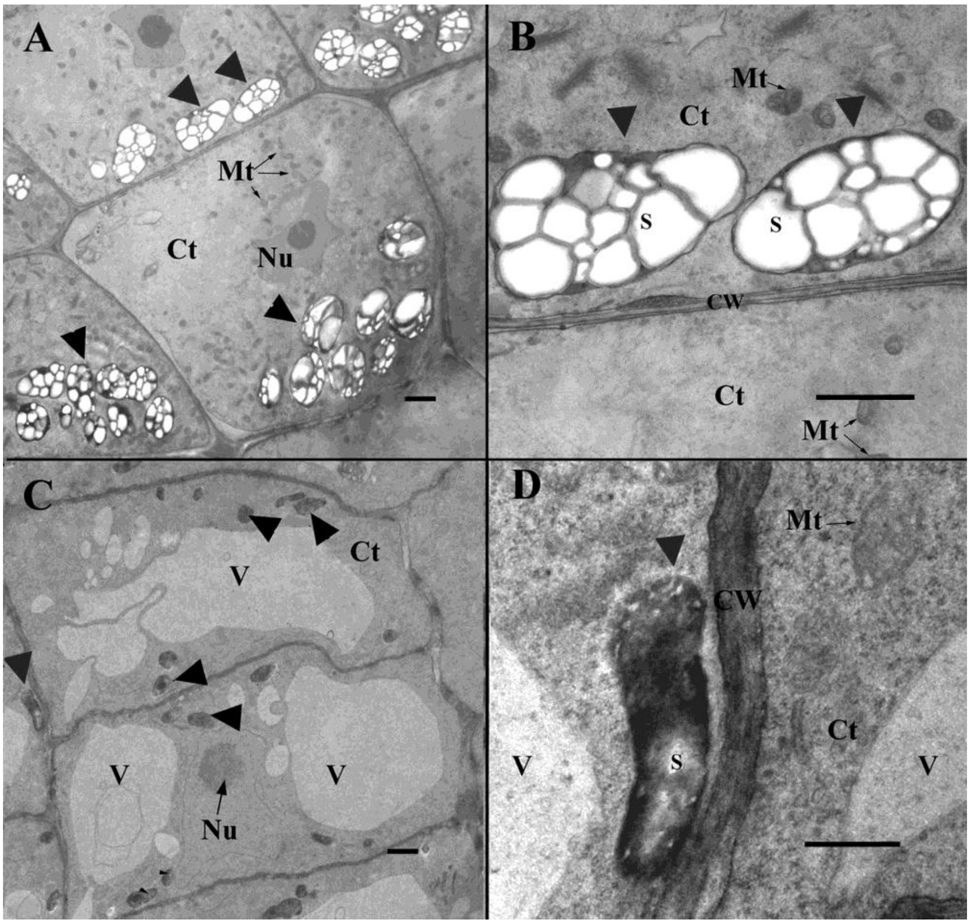

Figure 6. Transmission electron micrographs of columella cells from *Sesamum indicum* roots. (A) Control treatment: lower magnification view of columella cells showing statocytes with statoliths (arrow heads) on the lower cell wall. (B) Detail of statoliths from control root caps (arrow heads). (C) Columella cells from sesame treated (3%) roots. Note that the statoliths (arrow heads) were small and scattered in the cytoplasm. (D) Close view of statoliths (arrow head) from sesame treated plants, which were abnormal and had little amount of starch. Ct: cytoplasm; CW: cell wall; Mt: mitochondria; Nu: nucleus; S: starch; V: vacuole. Bar = 5 µm.

There have been reports on the effects of allelochemicals on root caps. For instance, rye allelochemicals (BOA: 2(3H)-benzoxazolinone; and DIBOA: 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one) induced several structural changes in root statoliths of cucumber seedlings (44). The authors observed that cucumber amyloplasts exposed to allelochemical had fewer and smaller starch grains. Moreover, cucumber root cap cells from roots of extract treated plants were more vacuolated. All these features are consistent with the findings in the present study for sesame and radish columella amyloplasts of extract treated plants (Figures 5, 6, 7).

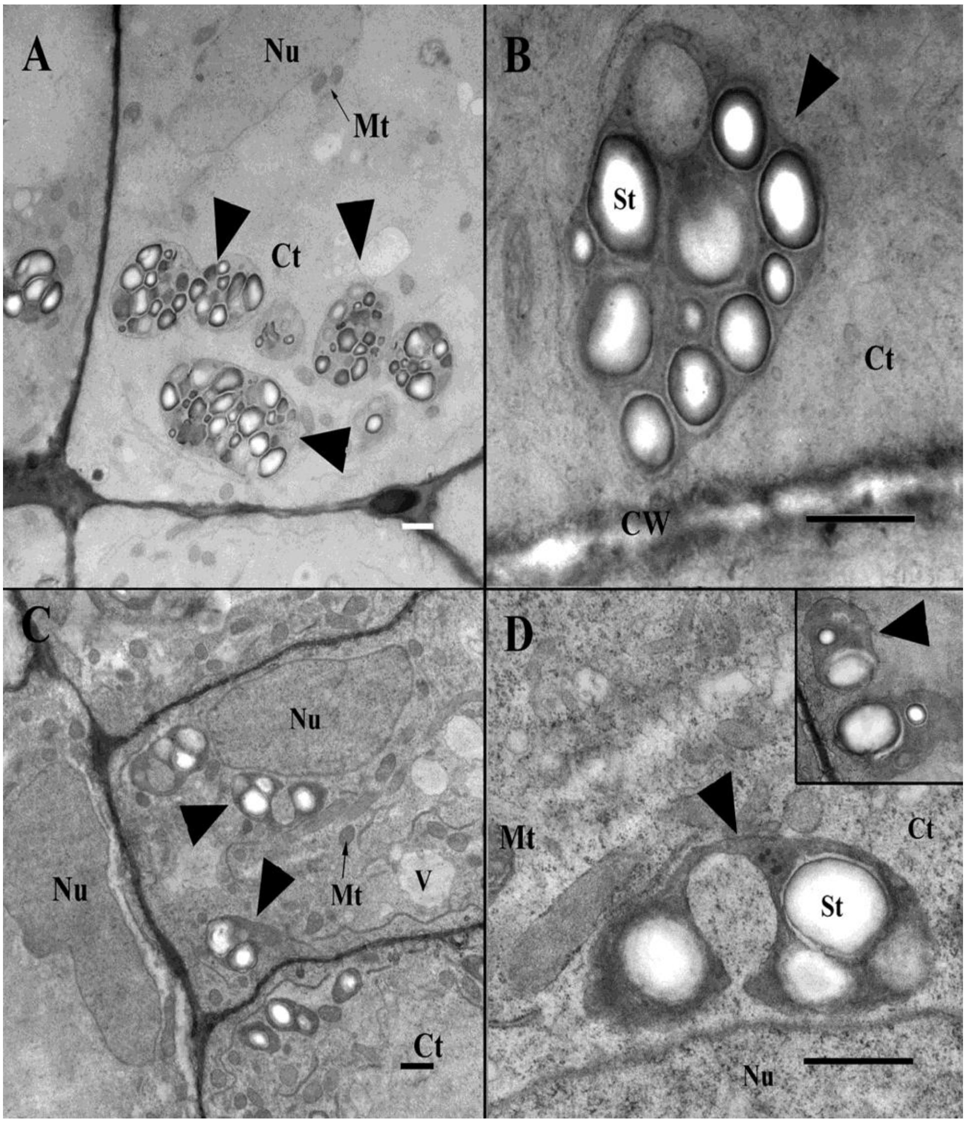

Figure 7. Transmission electron micrographs of columella cells from *Raphanus sativus*. (A) Control treatment: lower magnification view showing statocytes with statoliths (arrow heads) grouped on the lower cell wall. (B) Statoliths from control roots (arrow heads). (C) Columella cells from radish extract treated roots (3%). Observe in the cytoplasm of smaller and scattered statoliths (arrow heads). (D) Close view of statolith (arrow head) from treated plants with abnormal statoliths and low amount of starch. Ct: cytoplasm; CW: cell wall; Mt: mitochondria; Nu: nucleus; S: starch; V: vacuole. Bar = 1 µm.

The actual reasons for these abnormalities in statoliths and columella cells exposed to allelochemicals plants are not thoroughly comprehended. Nonetheless, starch synthesis is another biochemical pathway influenced by stress and plant hormone such as ethylene (45). Plants under stress have their photosynthetic capability compromised, which negatively affects starch synthesis and accumulation (45,46,47). Therefore, the stress induced by allelochemicals can diminish photoassimilate production and decrease starch production, which will have a negative impact on statolith formation.

Additionally, *ETR2* (Ethylene response Gene 2) overexpressing rice lines, which have reduced sensitivity to ethylene, showed higher starch accumulation in the stems (48). Hence, ethylene may negatively affect starch synthesis. As ethylene release and stress are often associated, the synthesis of starch could be hindered in statocytes from roots exposed to allelochemicals. This data is consistent with smaller starch grains seen in the statoliths of sesame and radish roots exposed to *E. dysenterica* allelochemicals.

Besides the structural abnormalities, sesame and radish statoliths did not keep their regular place in columella cells and were scattered in the cytoplasm. Statolith position and sedimentation have been associated with graviperception and signal transduction pathway that results in a proper gravitropic response, and statoliths displacement is capable of inducing root curvature (48,49,50). Despite the fact that the role of statoliths in gravitropic response has been under scrutiny, and some authors considered that these structures are not essential to this phenomenon (51, 52). Nonetheless, no one disregards the importance of statoliths on gravitropism, and the starch–statolith hypothesis is still considered the best explanation for root gravity perception (53).

Statolith location in the columella cells depends on the gravitational stimulus as well as the tension force exerted by cytoskeletal proteins that supposedly counteracts the sedimentation process (30). Therefore, the starch content and size of statoliths possibly are important during plant gravitropic response, as these organelles have to overcome the cytoskeleton tension force to sediment. Furthermore, the small statoliths of *E. dysenterica* treated plants may not be structurally fit to subjugate the cytoskeletal opposition and sediment. Therefore, it could result in cytoplasmic statolith scattering as they may be too light to assume the regular place in response to the gravitational stimulus.

Allelochemicals do affect root development as observed in sorghum, where hydroalcoholic extracts of leaf extracts from *Myrcia guianensis* were inhibitory on root development as well as supressed the expression of several genes (25). Consequently, it is not unusual to correlate the root cap abnormalities to allelochemicals. Therefore, the structural defects seen in root caps of target plants is likely a result of the toxicity of *E. dysenterica* extracts.

CONCLUSIONS

The allelochemicals in leaf extracts of *E. dysenterica* disorganized the columella structure of sesame and radish plants at cellular level. The statoliths in target plants were severely affected, whose shape and position within the statocytes were dramatically altered, which directly interfered in the gravitropic response of sesame and radish roots.

Additionally, it appears that stress induced by allelochemicals can unlock several physiological events, which indicates the involvement of plant hormones. Therefore, it is necessary to investigate the role of plant hormone, mainly ethylene in these events. Such studies will contribute to understand the allelopathic phenomenon, as well as will help to address its effectiveness in weed control.

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