https://doi.org/10.26651/allelo.j/2021-52-2-1315 0971-4693/94 Euro 20.00
Allelopathy Journal 52 (2): 181-198 (March, 2021) *International Allelopathy Foundation* 2021
Table: 1, Figs: 3

Allelopathic root inhibition and its mechanisms

K. Šoln[*] and J. Dolenc Koce

Department of Biology
Biotechnical faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia
E. Mail:katarina.soln@bf.uni-lj.si

(Received in revised form: January 25, 2021)

CONTENTS

ABSTRACT

Allelopathy represents a valuable biochemical strategy in plant-plant interactions among different plants, e.g. among crops and weeds. It is an important strategy in the colonization of many invasive alien plants. Allelopathic plants affect the growth of other plants in the vicinity through the release of secondary metabolites (allelochemicals) into the soil. In particular, many allelochemicals suppress the root growth of target plants, but little is known about the mechanism involved in root growth inhibition. In this review, we will highlight the mechanism of root suppression involving: (i) Alterations in auxin homeostasis affecting polar auxin transport and root gravitropism, (ii) Biochemical and physiological processes in inhibited roots associated with oxidative stress due to direct production and accumulation of reactive oxygen species or suppression of antioxidative response and (iii) The ultrastructural modifications in root tip exposed to allelochemicals that drastically suppress the cell division and eventually lead to shorter roots of target plants.

Keywords: Allelochemical, auxin transport, gene expression, oxidative stress, phytotoxicity, reactive oxygen species, root tip, ultrastructure

---

[*] Correspondence author

1. INTRODUCTION

Plants in natural or agricultural communities compete for sunlight, water, space and mineral nutrients(40). Some plant species release allelochemicals into the environment, which negatively affect the growth of neighbouring plants. A reduction in the growth of a recipient plant increases the efficiency of allelochemical producer to exploit the natural resources available and thus increase their growth and fitness(43). This plant-plant interaction is known as allelopathy and was first introduced by Hans Molish in 1937. The term allelopathy comes from the Greek words *allelon* “of each other” and *pathos* “to suffer” (41). Allelopathy usually describes direct or indirect biochemical interactions between the plants with mostly negative effects on the target plant (Fig. 1). The term allelopathy also describe the negative influence of plant allelochemicals on microorganisms and insects living in the rhizosphere (43,51).

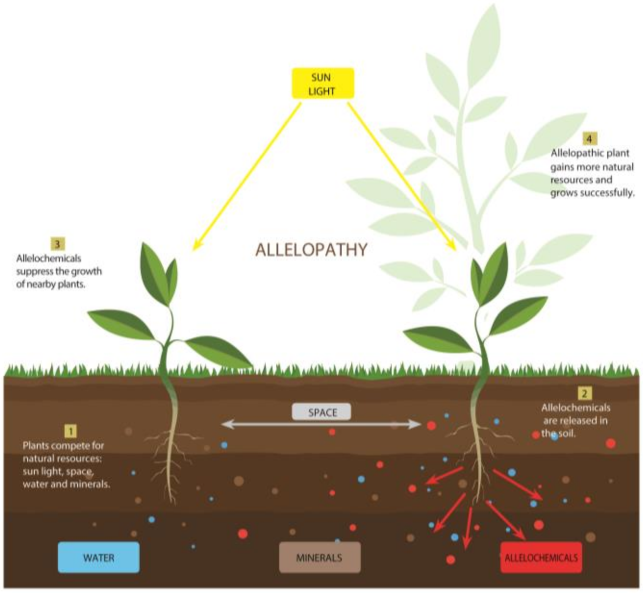

Figure 1. Allelopathy is a chemically mediated interaction between plants, in which allelopathic plants suppress the growth or fitness of nearby plants.

Allelopathic compounds or allelochemicals are secondary metabolites with diverse chemical structure, mainly phenols, terpenoids, glycosteroids and alkaloids (26). Allelochemicals are produced and stored in various plant parts such as roots, bark, leaves, flowers and fruits. They can be released into the environment as root exudates, volatile compounds, leaf leachates or decomposition-derived compounds from plant residues (51). The concentrations of allelochemicals in plants may vary spatially (9,17) and seasonally (9,18). In addition, even taxonomically related species sometimes differ in the released allelochemicals or their concentration, as shown in Myrtaceae family (24). The effects of allelochemicals can strongly vary among the target species. For example, a study of Cruz-Ortega *et al.* (12) showed that the roots of tomato seedlings (*Solanum lycopersicum* L.) are more strongly affected by the extract of beautyberry (*Callicarpa acuminata* Kunth) than bean (*Phaseolus vulgaris* L.) and maize seedlings (*Zea mays* L.). This inhibition was related to the increase in levels of oxidative stress (12). On the other hand, some allelopathic plants have similar negative effects on different target species. The leaf extract of cagaita (*Eugenia dysenterica* DC.) caused a lack of starch grains in the columella of sesame (*Sesamum indicum* L.) and modifies the shape of starch grains in radish (*Raphanus sativus* L.) roots. It also altered the root ultrastructure of both target species e.g. size and shape of root cap cells (38).

Allelopathy is evident in agriculture, as many crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), rye (*Secale cereal* L.), maize and barley (*Hordeum vulgare* L.) release allelopathic compounds in the soil and consequently reduces the growth of other plants in the vicinity (43) or reduces the crop quality due to the soil sickness (23). In the last decade, many researchers also focused on the allelopathy of invasive plant species viz., tree of heaven (*Ailanthus altissima* (Mill.) Swingle) (2), Canadian goldenrod (*Solidago canadensis* L.) (50) or Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.) (32). According to the Novel Weapon Hypothesis, allelopathy is an additional advantage for invasive plants, when they colonize new habitats. Invasive plants quickly become dominant in the introduced range, because native plants did not co-evolve with their novel plant competitors and are therefore more susceptible to their allelochemicals (33). As an example, Fan *et al.* (17) reported that Chinese native plants are more tolerant to allelochemicals of Japanese knotweed than native plants in Europe, where this weed species is invasive plant.

In contrast to mature plants, allelochemicals pose greater threat to the seedlings because they are more sensitive to biotic and abiotic environmental stressors (51). Exposure to the leaf extracts of Japanese knotweed and Bohemian knotweed (*Fallopia* × *bohemica* (Chrtek and Chrtková) J. P. Bailey) suppressed the root growth of radish seedlings, while no negative effect occurred on mature plants. This suggests that adult plants may be more tolerant to such stress (15), partially because their root system is fully differentiated (59). Over the last 20 years, many allelopathic studies have focused on 2-day old (11), 3-day old (17,27), 4-day old (8), 5-day old (38), 7-day old (4,15,44) and 14 day-old seedlings (28,47). Many allelochemicals suppress the germination. As an example, the allelochemical sorgoleone almost completely suppressed the germination of several weeds [white goosefoot (*Chenopodium album* L.), Japanese dock (*Rumex japonicas* Houtt.) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv) (48)]. However once germinated, allelochemicals mainly inhibit the root growth of the target plants. The root is a primary site of action of many allelochemicals, especially in the first days of germination (3). When roots grow intensively, their metabolic activity increases, therefore all potentially phytotoxic compounds can have a severe negative impact on their growth and development, such as modification of auxin transport (57), redox homeostasis (13) and ultrastructural changes in the root tip (8).

This review describes and summarizes the most important morphological, physiological and molecular changes that occur during root inhibition caused by the allelopathic stress. In addition, we outline some possible directions for future research.

2. ALLELOPATHIC EFFECTS ON AUXIN-INDUCED ROOT GROWTH

Plant hormones, especially auxins, play an important role in the growth and development of plants. Indole-3-acetic acid (IAA) is the most important auxin, it regulates root growth by influencing cell division and differentiation, and root elongation (16). Allelochemicals can interfere with IAA homeostasis and therefore, affects the primary root growth (27,28,54,57). The most common allelopathic inhibition signs are presented in Table 1.

2.1. IMPACT ON AUXIN SYNTHESIS AND TRANSPORT

Some allelochemicals increase the level of IAA content in the root tips due to stimulation of auxin biosynthesis. The exposure of *Arabidopsis thaliana* (L.) Heynh. seedlings to benzoic acid upregulated the expression of genes involved in the tryptophan-dependent biosynthetic pathway of auxin (57). In addition, phenols at high concentrations, can reduce auxin degradation and thus increase the auxin concentration (30). The auxin concentration in roots of tomato seedlings increased after 1 day and doubled after 3 days of exposure to the allelochemical cyanamide (44).

In roots, the auxin concentration depends not only on the biosynthesis but also on the distribution of auxin. The auxin transport from the shoot apex to the base of the root is called the basipetal auxin transport. In the root, it provides the maximum auxin at the root tip, while, the auxin level decreases along the apical-basal axis of the root (16).

Auxin is actively transported from cell to cell by the polar auxin transport. Auxin influx and efflux transporters are responsible for the auxin distribution within the cell(16,35,49).The auxin gradient is determined by the pH value. In the cell wall the pH is low, therefore the auxin is protonated and can easily enter the cell through the auxin influx carriers AUXIN1/LIKE AUXIN1 (AUX1/LAX). Once inside the cell, the auxin loses its ability to cross the cell membrane because the pH is higher in the cytosol. Consequently, the auxin is actively pumped out of the cell by auxin efflux transporters known as PIN-FORMED (PIN) proteins (49). Their activity is controlled by phosphorylation and their presence is regulated by the selective endocytosis (35). This enables a high level of PINs on proximal part of the cell and a low level on the distal side. This situation causes auxin transport between cells in only one direction (49). Location and amount of PINs are related to the cellular polarity and depends on the hormone level (35) as well as on the developmental stage of the plant and the cell type. The following PIN pattern was observed in the root tip: in the outer layers of the root tip auxin is transported away from the local auxin maximum in the roots, while in the inner cell layers, auxin is transported towards the local maximum (49).

Auxin concentrations and its transport are unavoidable for normal root growth and development (16,49). However, many allelochemicals can affect the basipetal and polar auxin transport by altering the auxin transporters. Diterpenoid weisiensin B, isolated from the Chinese plant *Isodon weisiensis* (C.Y. Wu) H. Hara reduced the transcription of genes coding for the auxin transporters PIN2, PIN3, PIN4, PIN7 and AUX1 in *Arabidopsis* roots. In addition, weisiensin B also reduced the abundance of auxin transporters in roots and thus increased the auxin concentration in the root apex, which led to the suppression of primary root growth (27). Coumarin modulated the auxin distribution in roots of *Arabidopsis* seedlings by affecting auxin transporters (28). The localization of PIN transport proteins in *Arabidopsis* roots was also modified after treatment with the sesquiterpene farnesene. The auxin transporters PIN4 and PIN7, which are involved in the redistribution of auxin, were particularly affected. When the auxin flow reaches the quiescent centre in the root, it accumulates there instead of redistributing to other parts of the root. Alterations in auxin concentrations at the level of the quiescent centre affected the root apical meristem and was seen as a root phenotype of left-handedness (4). Benzoic acid increased the gene expression in auxin transporters AUX1 and PIN2 in roots of *Arabidopsis* seedlings (57). Both auxin transporters are stress targets and play an important role in the inhibition of root growth in response to other environmental stresses such as alkaline stress (52) or exposure to aluminum toxicity (46).

2.2. IMPACT ON ROOT GRAVITROPISM

The auxin gradient in the root is also important for the root gravitropism, which enables the root to grow in the direction of the Earth´s gravity (58). Due to the high sensitivity of the roots to auxin concentrations, allelochemicals can seriously disturb the root gravitropism. When *Arabidopsis* seedlings were exposed to artemisinin for 48 h, the number of starch grains (statoliths) in the columella cells decreased dramatically in the central region of root cap. Statoliths are at the centre of gravitropic perception, therefore, any change in their level can impair root sensitivity and affect the development of the root system (54). The shape of statoliths was altered in radish and sesame roots treated with leaf extract of a Brazilian tree cagaita (*Eugenia dysenterica* DC.). Furthermore, the statoliths in the treated roots did not keep their regular position within the root cap cells but are scattered around the cytoplasm(38).In the columella cells, there is a special form of the endoplasmic reticulum, called nodal ER, which provides a stable platform for the sedimentation of the statoliths and prevents the deformation of amyloplasts (60). We predict that allelochemicals may also interfere with nodal ER in the columella cells, which may lead to changes in statolith sedimentation, signal transduction pathway and improper gravitropic response (38). The treatment with rye allelochemicals 2(3*H*)-benzoxazolinone (BOA) and 2,4-dihydroxy-1,4(2*H*)-benzoxazin-3-one (DIBOA) reduced (8), number of starch granules in amyloplasts was also reduced in roots of cucumber (*Cucumber sativus* L.).Some allelochemicals, e.g. flavonoids, may also induce asymmetric PIN shifts during gravity stimulus, resulting in unconventional root bending (42).

3. ALLELOCHEMICALS AND OXIDATIVE STRESS IN ROOTS

Allelochemicals also interfere with redox homeostasis in the target plants resulting in excessive production and accumulation of reactive oxygen species (ROS) (Table 1), which leads to oxidative stress (Fig. 2). ROS can oxidize other macromolecules in the cells, especially lipids, proteins and nucleic acids, and thus impair normal cell function(21). On the other hand, in low concentrations of ROS can act as signaling molecules that play crucial role in the regulation of plant ontogenesis, including the development of the root apical meristem (53). They may even alter the defense mechanism and thus increase tolerance to environmental stress, as shown in the study of cucumber seedlings treated with low concentrations of garlic (*Allium sativum* L.) extract(22). However, many studies have reported an imbalance of ROS production in roots due to the phytotoxicity of allelochemicals (6,10,13,29,31,39,45,57).

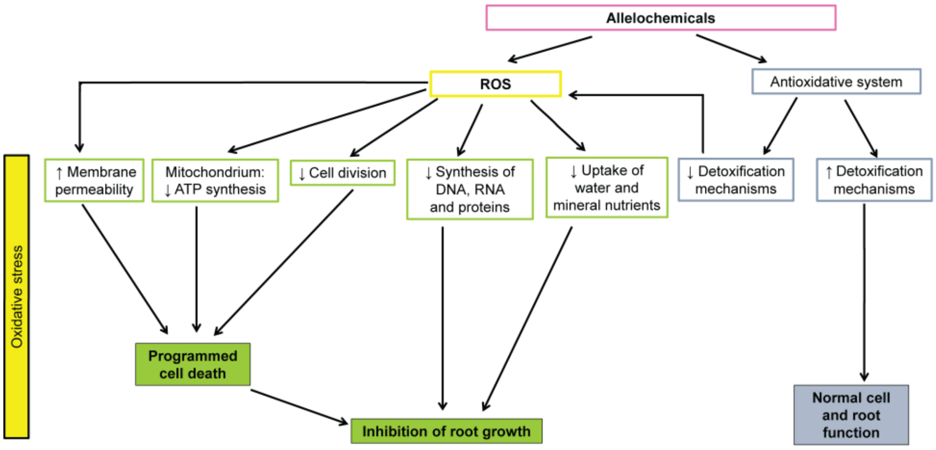

Figure 2. Allelochemicals induce oxidative stress in target plants. This scheme shows example of some changes in roots mediated by reactive oxygen species.

3.1. REACTIVE OXYGEN SPECIES IN ROOTS

The reason for the suppression of primary root growth may be due to the increased production and accumulation of ROS (7). Benzoic acid caused ROS bursting in the roots of *Arabidopsis* seedlings and suppressed the root elongation (57). Similarly, extract from invasive weed nettle leaf goosefoot (*Chenopodium murale* L.) increased the ROS content in wheat seedlings, especially in the root cap (31).

The most common ROS are singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2). These highly reactive molecules can transform into each other (14). Some allelochemicals such as cyanamide induce the accumulation of $^1O_2$ in the root tip, the elongation zone and the differentiation zone of onion roots (*Allium cepa* L.) leading to the suppression of root growth (45). Hydrogen peroxide is more stable than other toxic by-products of aerobic metabolism and can migrate from the site of synthesis to different cell compartments or even to other cells in the vicinity, causing cell damage (19). Treatment with cyanamide induced an over accumulation of H_2O_2 in the roots of onion seedlings, reducing the root viability by 50% (45). Intense accumulation of H_2O_2 in roots was also observed in mung bean (*Phaseolus aureus* Roxb.) seedlings exposed to BOA (6), in cotton (*Gossypium hirsutum* L.) seedlings treated with *p*-hydroxybenzoic acid, phlorogluinol and ferulic acid (56), in several native Brazilian *Tabebuia* species treated with the extract of the invasive herbaceous plant coffee senna [*Senna occidentalis* (L.) Link] (13) and in wheat seedlings after exposure to the extract of the nettle leaf goosefoot. Furthermore, these extracts also induced lipid peroxidation in treated roots (31). Lipid peroxidation is one of the prime ROS effects and is therefore used as one of the most common stress markers in plants. This radical-initiated reaction leads to damage of cell membranes (19). In laboratory experiments, it is often measured as the content of malondialdehyde which is a by-product of lipid decomposition (36). Many studies reported an increase in malondialdehyde content in seedlings exposed to allelopathic stress. Treatments with BOA increased the level of malondialdehyde in mung bean more than 6-folds than untreated roots (6) andextract of coffee senna more than 2-folds in the roots of *Tabebuia roseoalba* (Ridl.) Sandwith (13).

3.2. ANTIOXIDATIVE SYSTEM IN ROOTS

The excessive production of ROS is accompanied by the activation of the antioxidative system, thus maintaining ROS homeostasis. Antioxidants are an important defence mechanism in plants and are responsible for controlling the redox balance (14). Allelopathic stress stimulates ROS-scavenging mechanisms in plants (39). Treatment with the extract of nettle leaf goosefoot more than doubled the antioxidative activity in roots of wheat seedlings (31).

Plants have developed anenzymatic and a non-enzymatic oxidative system. The enzymatic system scavenges ROS and thus minimizes their accumulation in the cells. The most common antioxidative enzymes in plants are superoxide dismutase that transforms$O_2^{\bullet-}$to H_2O_2, and several enzymes responsible for detoxifying H_2O_2.While catalases are active at high concentrations of H_2O_2 in plant cells, various peroxidases(ascorbate peroxidasesand guaiacol peroxidases), participate in the detoxification of H_2O_2 at lower concentrations (19). During allelopathic stress, different H_2O_2 scavenging enzymes are active. Peppermint (*Mentha ×piperita* L.) extract increased the activity of catalase and ascorbate peroxidase in radish seedlings (29). Similarly, the treatment with BOA increased the activity of catalase, ascorbate peroxidase and guaiacol peroxidase in roots of mung bean seedlings. In addition, the activity of glutathione reductase was also increased by BOA stress (6). This antioxidative enzyme reduces the oxidized glutathione with NADPH as reducing agent (19). After 4.0 h exposure to diallyl disulfide, an allelopathic compound in garlic, the expression of genes involved in glutathione metabolism were up regulated in roots of tomato seedlings as well as six peroxidase genes. Consequently, the activity of catalase and peroxidase in the roots increased two days after the treatment (10).

While antioxidant enzymes degrade ROS, non-enzymatic antioxidants are responsible for ROS neutralization. There are several important non-enzymatic antioxidants in roots: ascorbate (vitamin C) detoxifiesH_2O_2, α-tocopherol (vitamin E) is located in membranes and protects membrane lipids from lipid peroxidation, carotenoids quench $O_2^{\bullet-}$ and flavonoids directly scavenge H_2O_2 as well as $OH^{\bullet}$ (14,19). Seven-day exposure to leaf extract of invasive Japanese knotweed and Bohemian knotweed increased the concentration of non-enzymatic antioxidants in roots of radish seedlings (15).

If the scavenging system is altered, the level of ROS increases and consequently inhibits root growth (37). The study of Zhang *et al.* (56) demonstrated that the exposure of cotton seedlings to *p*-hydroxybenzoic acid, phlorogluinol and ferulic acid decreased the antioxidative activity in roots, and the mitochondrial structure was damaged by ROS: the activity of H-ATPase in mitochondrial membrane decreased, the openness of mitochondrial permeability transition pores increased, and the membrane fluidity decreased with increasing phenol concentrations. This led to the increase of mitochondrial cytochrome C in the cytosol and thus disrupted the electron transfer, which favoured the generation of even more ROS and resulted in even stronger inhibition of root growth (56). However, plant species may be differently adapted to the same level of allelopathic stress: the study of Cruz-Ortega *et al.* (12) showed that aqueous leachate of beauty berry (*Callicarpa acuminata* Kunth) increased the catalase activity in the root tips of tomato and bean, whereas it did not affect the root tip of maize. On the other hand, increased lipid peroxidation was observed only in the root tip of tomato, while no such response was observed in the roots of maize or bean seedlings (12) suggesting that these effects are very specific.

4. ALLELOPATHY-INDUCED ULTRASTRUCTURAL CHANGES IN ROOTS

Inhibition of root growth in the presence of allelochemicals is often associated with structural changes in the root tissues (8,20,38). The analysis of root anatomy and root cell ultrastructure after exposure to allelochemicals is rare. Therefore, the studies of Burgos *et al.* (8) and Cruz-Ortega (11) are still of great importance (Table 1). Both studies demonstrated that allelochemicals can alter the internal structure of the roots so severely that it is difficult to even delineate cell types. Four-day exposure of cucumber seedlings to BOA and DIBOA, (allelochemicals from rye), resulted in the delineation of the boundary between root cap and root apical meristem (8). In addition, the root tissues of common bean and bottle gourd (*Cucurbita ficifolia* Bouché) were disorganized after 2-days exposure to leaf extract of cucurbit (*Sicyos deppei* G. Don) (11).A similar reduction of root cap layers was also observed in the recent study of Araniti *et al.* (4), in which *Arabidopsis* seedlings were exposed to the allelochemical farnesene (4). In roots, the most common allelopathic targets are the cells of the root cap (8,38) and the root apical meristem (4,44,45,57) (Fig. 3).

4.1. ULTRASTRUCTURALALTERATIONS INROOT CAP CELLS

The root cap acts as an important protector of the root apical meristem and as a regulator of root growth, therefore any structural changes of its cells can lead to unbalanced root development (25). A leaf extract of cagaita induced an abnormally irregular shape of the root cap in both radish and sesame seedlings. In addition, root cap cells of the treated seedlings were also smaller with more pronounced vacuolization (38). Larger vacuoles were also observed in the root cap cells of cucumber seedlings after exposure to BOA and DIBOA (8), and in common bean and bottle gourd seedlings after exposure to leaf extract of cucurbit (11). An increased vacuolization of root cap cells may be due to their lytic function having a role in the degradation of toxic allelochemicals (20) or damaged cell organelles (11).

The lifespan of root cap cells is short, only a few days. Therefore their formation must be quick and precise (25). As the main cell organizers, the nuclei in root cap cells are among the most affected cell organelles, when the roots are exposed to allelochemicals. After 2 days of exposure to the extract of cucurbit, the change in nucleus shape was observed in bean and bottle guard seedlings. The nuclei were smaller, irregularly shaped and denser, indicating severe disfunction (11).

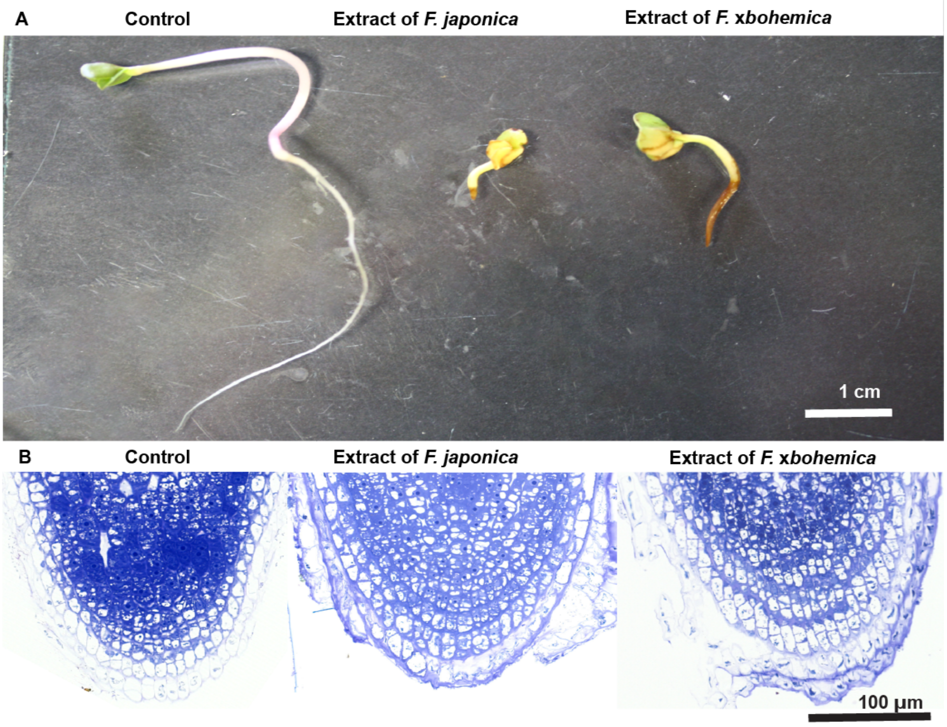

Figure 3**.** Radish (*Raphanus sativus* L.) seedlings exposed for 7 days to the 10% rhizome extract of invasive Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.) and Bohemian knotweed (*Fallopia ×bohemica* (Chrtek and Chrtková) J. P. Bailey) (A) and the root tips of exposed seedlings (B). Photos: Katarina Šoln.

Allelopathic stress can also alter the structure and function of mitochondria in root cap cells. Extract of cucurbit led to the emergence of swollen mitochondria with dilated cristae in root cap cells of common bean (11), while BOA and DIBOA reduced the number of mitochondria in roots of cucumber seedlings (8). Structural anomalies of the mitochondria may be reflected in their function. It has been reported that some allelochemicals interfere with normal respiration. Juglone disrupted mitochondrial membrane potential in the root tip of lettuce seedlings (*Lactuca sativa* L.) (5) and α-pinene inhibited both basal and coupled respiration in isolated mitochondria of maize (1). Other cell structures may also be altered by the allelopathic effects. For example, detachment of the plasma membrane from the cell wall (11), thickening of the cell wall (31), reduction of ribosomes in the cytoplasm and lower density of dictyosomes (8).

4.2. ULTRASTRUCTURAL ALTERATIONSIN ROOT APICAL MERISTEM

Allelochemicals can also affect the root apical meristem by reducing mitosis, which leads to the suppression of primary root growth and reduced root length (20,34).Benzoic acid reduced the size of the meristem zone and the number of meristematic cells in roots of *Arabidopsis* seedlings (57). Similar observations were made during treatment with sesquiterpene farnesene, suggesting that allelopathic stress may force premature differentiation of meristematic cells (4). Thus, visible suppression of root growth may result from alterations in the meristem ultrastructure or cell division (20).

Allelopathic stress often leads to disturbed structure of the cell organelles, especially of the nuclei and mitochondria. The extract of cucurbit altered the shape andpresence of nuclei and nucleoli in meristematic cells of common bean seedlings and promoted the emergence of swollen mitochondria. In addition, allelochemicals can influence the maturation of meristematic cells. The number of vacuoles in meristematic cells of bean seedlings treated with cucurbit increased, while the size of vacuoles in bottle guard seedlings doubled (11). A similarly excessive vacuolization was observed in radish seedlings treated with coumarin (3).

Furthermore, allelopathic stress also affects mitosis. Allelochemical 1,8-cineole reduced the mitotic index as well as suppressed cell proliferation and elongation of BY-2 suspension culture of tobacco (*Nicotiana tabacum* L.) (55). Similar suppression of mitosis was observed in the roots, despite the fact that the meristematic cells are not in the direct contact with allelochemicals (20,34). Cyanamide reduced the root length of onion seedlings by affecting the cell cycle at the G2/M checkpoint and reducing the percentage of dividing meristematic cells. Interestingly, after the treatment with high concentration (10 mM cyanamide), all dividing cells were arrested in the telophase and no other mitotic phases were observed. A lower concentration of cyanamide promoted the shortening and condensation of chromosomes. Cyanamide also modified the arrangement of the cytoskeleton, therefore, the formation of the metaphase plate was altered, which led to a disturbance of cell division (45). Furthermore, cyanamide also reduced the mitotic index in tomato roots, where prophase and metaphase were inhibited by 40 % (44).

Table 1. Allelopathy-mediated root inhibition. Allelochemicals affects auxin homeostasis, induce oxidative stress, and modify root ultrastructure, which suppresses the root growth.

Treatment	**Test plant**	**Changes**	**Effects on roots**	**Reference**
		ALLELOCHEMICALS		
Artemisinin	Arabidopsis (*Arabidopsis thaliana* L.)	Gravitropism	Decreased number of starch grains	4
Benzoic acid	Arabidopsis (*Arabidopsis thaliana* L.)	Auxin homeostasis	Upregulated expression of genes involved in the tryptophan-dependent biosynthetic pathway of auxin and auxin transporters AUX1 and PIN2	5
		Oxidative stress	ROS bursting	
		Root apical meristem	Reduction of the meristem zone size and the number of	

			meristematic cells	
BOA / DIBOA	Cucumber (*Cucumber sativus* L.)	Gravitropism	Decreased number of starch grains	8
		Root tip	Delineation of the boundary between the root cap and root apical meristem	
		Root cap	Larger vacuoles; reduction of the mitochondria number and ribosomes in the cytoplasm; lower density of dictyosomes	
	Mung bean (*Phaseolus aureus* Roxb.)	Oxidative stress	Accumulation of H_2O_2; increased level of malondialdehyde; increased activity of catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase	6
1,8-Cineole	BY-2 cell culture of Tobacco (*Nicotiana tabacum* L.)	Cell division	Reduction of mitotic index, prevention of cell proliferation and elongation	55
Coumarin	Radish (*Raphanus sativus* L.)	Root apical meristem	Excessive vacuolisation	3
	Arabidopsis (*Arabidopsis thaliana* L.)	Auxin homeostasis	Modification of auxin transporters	28
Cyanamide	Onion (*Allium cepa* L.)	Oxidative stress	Increased accumulation of $^1O_2$ and H_2O_2	45
		Root apical meristem	Changed cell cycle at the G2/M checkpoint; percentage of dividing meristematic cells decreased; shorter and more condense chromosomes; modification in the cytoskeleton arrangement; reduction in root viability	
	Tomato (*Solanum lycopersicum* L.)	Auxin homeostasis	Concentration of auxin increased	44
		Root apical meristem	Reduction in mitotic index due to inhibition of prophase and metaphase	
Diallyl disulfide	Tomato (*Solanum lycopersicum* L.)	Oxidative stress	Upregulated expression of genes involved in glutathione metabolism and six peroxidase genes; increased activity of catalase and peroxidase	10

Farnesene	Arabidopsis (*Arabidopsis thaliana* L.)	Auxin homeostasis	Modification in location of auxin transporters PIN4 and PIN7	4
		Root tip	Reduction of the root cap cell layers	
		Root apical meristem	Reduced the size of meristem zone and the number of meristematic cells	
p-Hydroxybenzoic acid; Phloroglucinol; Ferulic acid	Cotton (*Gossypium hirsutum* L.)	Oxidative stress	Increased accumulation of H_2O_2 and O_2^-; decreased activity of superoxide dismutase, catalase and peroxidase; decreased activity of mitochondrial H+-ATPase; decreased mitochondrial membrane fluidity; increased the openness of mitochondria permeability transition pores; decreased the ratio of cytochrome c/a	56
Juglone	Lettuce (*Lactuca sativa* L.)	Root tip	Disrupted mitochondrial membrane potential	5
α-Pinene	Maize (*Zea mays* L.)	Root	Inhibited basal and coupled respiration in isolated mitochondria	1
Weisiensin B	Arabidopsis (*Arabidopsis thaliana* L.)	Auxin homeostasis	Reduction in the promotor activity of the genes for the auxin transporters PIN2, PIN3, PIN4, PIN7 and AUX1; reduction in the abundance of auxin transporters; increased concentration of auxin	27
		PLANTS EXTRACTS		
Beautyberry (*Callicarpa acuminata* Kunth)	Tomato (*Solanum lycopersicum* L.)	Oxidative stress	Increased lipid peroxidation; increased activity of catalase	12
	(*Phaseolus vulgaris* L.)	Oxidative stress	Increased activity of catalase	
Cagaita (*Eugenia dysenterica* DC.)	Radish (*Raphanus sativus* L.), sesame (*Sesamum indicum* L.)	Gravitropism	Alternation in statoliths sedimentation and their shape	38
		Root cap	Smaller cells with abnormal irregular shape; excessive vacuolisation	
Senna (*Senna occidentalis* (L.) Link)	several native Brazilian *Tabebuia* spp	Oxidative stress	Accumulation of H_2O_2; increased the level of malondialdehyde	13
Cucurbit (*Sicyos deppei* G. Don)	Bottle gourd (*Cucurbita ficifolia* Bouché)	Root tip	Disorganized root tissue	11
		Root cap	Larger vacuoles; smaller, dense and irregularly shaped nuclei; detachment of the plasma membrane from the cell wall	

		Root apical meristem	larger vacuoles	
	Common bean (*Phaseolus vulgaris* L.)	Root tip	Disorganized root tissue	
		Root cap	Larger vacuoles; smaller, dense and irregularly shaped nuclei; detachment of the plasma membrane from the cell wall; swollen mitochondria with dilated cristae	
		Root apical meristem	Modified shape and presence of nuclei and nucleoli; swollen mitochondria; numerous larger vacuoles	
Japanese knotweed (*Fallopia japonica)* and Bohemian knotweed (*Fallopia ×bohemica*	Radish (*Raphanus sativus* L.)	Oxidative stress	Increased the concentration of non-enzymatic antioxidants	15
Nettle leaf goosefoot (*Chenopodium murale* L.)	Wheat (*Triticum aestivum* L.)	Oxidative stress	Increased accumulation of H_2O_2; increased lipid peroxidation and antioxidative activity	31
		Root cap	Thickening of the cell wall	
Peppermint (*Mentha ×piperita* L.)	Radish (*Raphanus sativus* L.)	Oxidative stress	Increased the activity of catalase and ascorbate peroxidase	29

5. FUTURE LINES OF WORK

Plants are valuable source of secondary compounds with allelopathic activity. The investigation techniques used to study allelopathy vary, while some studies used extracts or essential oils, others analyzed single isolated compounds from allelopathic plants. The grinded plant material mixed with soil and plants growing together in a pot are common methods to study allelopathy. The plant sample preparation process, as well as the selected test plants parts used, and the origin of plants influence the allelopathic potential and may cause negative effects on recipient plants. Therefore, this should not be ignored, when interpreting the results. Despite a number of studies that have investigated the visible signs of plant-plant allelopathic interactions (seed germination, shoot and root length and plant biomass), there exist a remarkable gap in the current understanding of the cellular mode of actions of allelochemicals.

Future studies should therefore, investigate the following aspects: (i). Structural modifications in cell organelles, (ii). Appearance of new stress-induced structures in root cells of target plants, (iii). Molecular studies using the microarray techniques to investigate the early changes in DNA expression pattern and 2D electrophoresis to detect stress related proteins, (iv). How allelochemicals interact with target plant cells, i.e., (a). whether allelochemicals enter the target plant cells, (b). accumulate in cell walls, (c). where and how they bind, (d). in what type of cell signalling they are involved, and (e). how long they are functional, is still a major mystery. To better understand the allelopathic modes of action, future allelopathic studies should be interdisciplinary and involve a combination of different research methods and techniques.

6. CONCLUSIONS

Allelopathy is an interesting phenomenon in plant-plant interactions that has fascinated people, ever since they started growing plants. Now allelopathy has become even more important, as it is affects both crop production and weed control. In addition, the quest for higher food production has led to massive use of herbicides in fields, which is associated with water and soil pollution. Allelochemicals, for example, may be a good "green" alternative for this problem in the future. Allelopathy is also involved in the success of invasive plants, which pose a serious threat to our native plant diversity with their alien allelochemicals. Therefore, understanding all aspects of allelopathic interactions will be an important tool to manage invasive plants in future. This review highlighted the main reasons for root inhibition after exposure to allelopathic compounds: from auxin homeostasis to over accumulation of ROS and ultrastructural changes in the root tip. Recently molecular techniques have made it possible to detect stress signs in target plants before visible changes occur. However, allelopathy is a complex process influenced by many factors; therefore, it is still a great challenge to find the possible mode of action of allelochemicals. Future research should therefore consider collaboration with experts from different scientific fields from botanists to chemists, soil scientists and molecular biologists to solve the mystery of allelopathy.

ACKNOWLEDGEMENTS

This study was financially supported by the Slovenian Research Agency (grants no. P1-0212). The authors are greatly thankful to Prof. Dr. Marjana Regvar and Prof. Dr. Katarina Vogel Mikuš from Biotechnical faculty, University of Ljubljana, Slovenia, for valuable comments during the manuscript preparation.

7. REFERENCES

1. Abrahim, D., Braguini, W.L., Kelmer-Bracht, A.M. and Ishii-Iwamoto, E.L. (2000). Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. *Journal of Chemical Ecology* **26**: 611-624.
2. Albouchi, F., Hassen, I., Casabianca, H. and Hosni, K. (2013). Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. *South African Journalof Botany* **87**: 164-174.
3. Aliotta, G., Cafiero, G., Fiorentino, A. and Strumia, S. (1993). Inhibition of radish germination and root growth by coumarin and phenylpropanoids. *Journal of Chemical Ecology* **19**: 175-183.

4. Araniti, F., Bruno, L., Sunseri, F., Pacenza, M. and Forgione, I.. (2017). The allelochemical farnesene affects *Arabidopsis thaliana* root meristem altering auxin distribution. *Plant Physiology and Biochemistry* **121**: 14-20.
5. Babula, P., Vaverkova, V., Poborilova, Z., Ballova, L., Masarik, M. and Provaznik, I. (2014). Phytotoxic action of naphthoquinone juglone demonstrated on lettuce seedling roots. *Plant Physiology and Biochemistry* **84**: 78-86.
6. Batish, D.R., Singh, HP., Setia, N., Kaur, S. and Kohli, R.K. (2006). 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). *Plant Physiology and Biochemistry* **44**: 819-827.
7. Bogatek, R. and Gniazdowska, A. (2007). ROS and phytohormones in plant-plant allelopathic interaction. *Plant Signaling & Behavior* **2**: 317-318.
8. Burgos, N.R., Talbert, R.E., Kim, K.S. and Kuk, Y.I. (2004). Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). *Journal of Chemical Ecology* **30**: 671-689.
9. Chen, H., Tuck, T., Ji, X., Zhou, X. and Kelly, G. (2013). Quality assessment of Japanese knotweed (*Fallopia japonica*) grown on Prince Edward Island as a source of resveratrol. *Journal of Agricultural and Food Chemistry* **61**: 6383-6392.
10. Cheng, F., Cheng, Z.H. and Meng, H.W. (2016). Transcriptomic insights into the allelopathic effects of the garlic allelochemical diallyl disulfide on tomato roots. *Scientific Reports* **6**(3): 1-14.
11. Cruz-Ortega, R., Anaya, A.L., Hernández-Bautista, B.E. and Laguna-Hernández, G. (1998). Effects of allelochemical stress produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. *Journal of Chemical Ecology* **24**: 2039-2057.
12. Cruz-Ortega, R., Ayala-Cordero, G. and Anaya, A.L. (2002). Allelochemical stress produced by the aqueous leachate of *Callicarpa acuminata*: Effects on roots of bean, maize, and tomato. *Physiologia Plantarum* **116**: 20-27.
13. da Silva, F. and Vieira, E.A. (2019). Phytotoxic potential of *Senna occidentalis* (L.) Link extracts on seed germination and oxidative stress of Ipê seedlings. *Plant Biology* **21**: 770-779.
14. Das, K. and Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science* **2**: 1-13.
15. Dolenc Koce, J. and Šoln, K. (2018). Phytotoxic effects of *Fallopia japonica* and *F.xbohemica* leaves. *Phyton-Annales Rei Botanicae* **57**(1-2): 47-58.
16. Du, Y. and Scheres, B. (2018). Lateral root formation and the multiple roles of auxin. *Journal of Experimental Botany* **69**: 155–67.
17. Fan, P., Hostettmann, K. and Lou, H. (2010). Allelochemicals of the invasive neophyte *Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae). *Chemoecology* **20**: 223-227.
18. Frantík, T., Kovářová, M., Koblihová, H., Bartůňková, K., Nývltová, Z. and Vosátka, M. (2013). Production of medically valuable stilbenes and emodin in knotweed. *Industrial Crops and Products* **50**: 237-43.
19. Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I. and Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *BioEssays* **28**: 1091-1101.
20. Gniazdowska, A. and Bogatek, R. (2005). Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Physiologiae Plantarum* **27**: 395-407.
21. Gniazdowska, A., Krasuska, U., Andrzejczak, O. and Soltys, D. (2015). Allelopathic compounds as oxidative stress agents: Yes or No. In: *Reactive oxygen and nitrogen species signaling and communication in plants*(Eds., K. J. Gupta, A. U. Igamberdiev) pp.155-176.Springer, Schwitzerland.
22. Hayat, S., Cheng, Z., Ahmad, H., Ali, M., Chen, X. and Wang, M. (2016). Garlic, from remedy to stimulant: Evaluation of antifungal potential reveals diversity in phytoalexin allicin content among garlic cultivars; allicin containing aqueous garlic extracts trigger antioxidants in *Cucumber*. *Frontiers in Plant Science* **7**: 1-15.
23. Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H. and Shi, K. (2013). Plant-soil feedbacks and soil sickness: From mechanisms to application in agriculture. *Journal of Chemical Ecology* **39**: 232-42.
24. Imatomi, M., Novaes, P. and Gualtieri, S.C.J. (2013). Interspecific variation in the allelopathic potential of the family Myrtaceae. *Acta Botanica Brasilica* **27**(1): 54-61.
25. Kumpf, R.P. and Nowack, M.K. (2015). The root cap: A short story of life and death. *Journal of Experimental Botany* **66**(19): 5651-562.

26. Latif, S., Chiapusio, G. and Weston, L.A. (2017). Allelopathy and the role of allelochemicals in plant defence. *Advances in Botanical Research* **82**: 19-54.
27. Li, P., Ding, L., Zhang, L., He, J. and Huan, Z. (2019). Weisiensin B inhibits primary and lateral root development by interfering with polar auxin transport in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry* **139**: 738-745.
28. Lupini, A., Araniti, F., Sunseri, F. and Abenavoli, MR. (2014). Coumarin interacts with auxin polar transport to modify root system architecture in *Arabidopsis thaliana*. *Plant Growth Regulation* **74**: 23-31.
29. Mahdavikia, F., Saharkhiz, M.J. and Karami, A. (2017). Defensive response of radish seedlings to the oxidative stress arising from phenolic compounds in the extract of peppermint (*Mentha* × *piperita* L.). *Scientia Horticulturae*(Amsterdam) **214**: 133-140.
30. Mato, M.C., Méndez, J. and Vázquez, A. (1994). Polypheolic auxin protectors in buds of juvenile and adult chestnut. *Physiologia Plantarum* **91**(1): 23-26.
31. Mitić, N., Stanišić, M., Savić, J., Ćosić T. and Stanisavljević, N. (2018). Physiological and cell ultrastructure disturbances in wheat seedlings generated by *Chenopodium murale* hairy root exudate. *Protoplasma* **255**: 1683-1692.
32. Moravcová, L., Pyšek, P., Jarošik, V. and Zákravský, P. (2011). Potential phytotoxic and shading effects of invasive *Fallopia* (Polygonaceae) taxa on the germination of native dominant species. *NeoBiota* **9**: 31-47.
33. Murrell, C., Gerber, E., Krebs, C., Parepa, M., Schaffner, U. and Bossdorf, O. (2011). Invasive knotweed affects native plants through allelopathy. *American Journal of Botany* **98**: 38-43.
34. Mushtaq, W., Siddiqui, M.B. and Hakeem, KR. (2020). Mechanism of action of allelochemicals. In: *Allelopathy: Potential for Green Agriculture* (Eds., W. Mushtaq, M. B. Siddiqui, K. R. Hakeem) pp. 61-66. Springer, Schwitzerland.
35. Naramoto, S. (2017). Polar transport in plants mediated by membrane transporters: Focus on mechanisms of polar auxin transport. *Current Opinion in Plant Biology* **40**: 8-14.
36. Ortega-Villasante, C., Rellán-Álvarez, R., Del Campo, F.F., Carpena-Ruiz, R.O. and Hernández, L.E. (2005). Cellular damage induced by cadmium and mercury in *Medicago sativa*. *Journal of Experimental Botany* **56**: 2239-2251.
37. Pasternak, T., Potters, G., Caubergs, R. and Jansen, M.A.K. (2005). Complementary interactions between oxidative stress and auxins control plant growth responses at plant, organ and cellular level. *Journal of Experimental Botany* **56**: 1991-2001.
38. Pereira, L.A.R., Pina, G.O., Silveira, C.E.S., Gomes, S.M., Toledo, J.L. and Borghetti, F. (2017). Effects of *Eugenia dysenterica* L. extracts on roots and gravitropism of *Sesamum indicum* L. and *Raphanus sativus* L. *Allelopathy Journal* **42**: 3-19.
39. Pergo Coelho, É.M., Cezar Barbosa, M., Mito, M.S., Mantovanelli, G.C., Oliveira, R.S. and Ishii-Iwamoto, E.L. (2017). The activity of the antioxidant defense system of the weed species *Senna obtusifolia* L. and its resistance to allelochemical stress. *Journal of Chemical Ecology* **43**: 725-738.
40. Pierik, R., Mommer, L. and Voesenek, L. A. (2012). Molecular mechanisms of plant competition: Neighbour detection and response strategies. *Functional Ecology* **27**: 841-853.
41. Rizvi, S.J.H., Haque, H., Singh, V.K. and Rizvi, V. (1992). A discipline called allelopathy. In: *Allelopathy: Basic and Applied Aspects* (Eds., S.J.H. Rizv and, V. Rizvi) pp. 1-10. Chapman & Hall, London, United Kingdom.
42. Santelia, D., Henrichs, S., Vincenzetti, V., Sauer, M. and Bigler, L.(2008). Flavonoids redirect PIN-mediated polar auxin fluxes during root gravitropic responses. *Journal of Biological Chemistry* **283**: 3121-3126.
43. Schandry, N. and Becker, C. (2020). Allelopathic plants: Models for studying plant–interkingdom interactions. *Trends in Plant Science* **25**: 176-185.
44. Soltys, D., Rudzińska-Langwald, A., Gniazdowska, A., Wiśniewska, A. and Bogatek, R. (2012). Inhibition of tomato (*Solanum lycopersicum* L.) root growth by cyanamide is due to altered cell division, phytohormone balance and expansin gene expression. *Planta* **236**: 1629-1638.
45. Soltys, D., Rudzińska-Langwald, A., Kurek, W., Gniazdowska, A., Sliwinska, E. and Bogatek, R. (2011). Cyanamide mode of action during inhibition of onion (*Allium cepa* L.) root growth involves disturbances in cell division and cytoskeleton formation. *Planta* **234**: 609-621.
46. Sun, P., Tian, Q.Y., Chen, J.and Zhang, W.H. (2010). Aluminium-induced inhibition of root elongation in *Arabidopsis* is mediated by ethylene and auxin. *Journal of Experimental Botany* **61**: 347-356.

47. Tucker, S.L. (2016). Comparison of the allelopathic effects and uptake of *Fallopia japonica* phytochemicals by *Raphanus sativus*. *Weed Research* **56**: 97-101.
48. Uddin, M.R., Park, S.U., Dayan, F.E., and Pyon, J.Y. (2014). Herbicidal activity of formulated sorgoleone, a natural product of sorghum root exudate. *Pest Management Science* **70**: 252-257.49.
49. van Berkel, K., de Boer, R. J., Scheres, B. an Tusscher, K. (2013). Polar auxin trsansport: Models and mechanisms. *Development* **140**: 2253-2268.
50. Wang, C., Xiao, H., Zhao, L., Liu, J. and Wang, L. (2016). The allelopathic effects of invasive plant *Solidago canadensis* on seed germination and growth of *Lactuca sativa* enhanced by different types of acid deposition. *Ecotoxicology* **25**: 555-562.
51. Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7**: 472-479.
52. Xu, W., Jia, L., Baluška, F., Ding, G. and Sh,i W. (2012). PIN2 is required for the adaptation of *Arabidopsis* roots to alkaline stress by modulating proton secretion. *Journal of Experimental Botany* **63**: 6105-6114.
53. Yamada, M., Han, X.and Benfey, P.N. (2019). RGF1 controls root meristem size through ROS signalling. *Nature* **577**: 85-88.
54. Yan, Z., Wang, D., Cui, H., Sun, Y. and Yang, X. (2018). Effects of artemisinin on root gravitropic response and root system development in *Arabidopsis thaliana*. *Plant Growth Regulation* **85**: 211-220.
55. Yoshimura, H., Sawai, Y., Tamotsu, S. and Sakai, A. (2011). 1,8-cineole inhibits both proliferation and elongation of BY-2 cultured tobacco cells. *Journal of Chemical Ecology* **37**: 320-328.
56. Zhang, G., Yang, C., Liu, R.and Ni, W. (2019). Effects of three phenolic compounds on mitochondrial function and root vigour of cotton (*Gossypium hirsutum* L.) seedling roots. *Acta Physiologia Plantarum* **41**(60): doi.org/10.1007/s11738-019-2851-8.
57. Zhang, W., Lu, L.Y., Hu, L.Y., Cao, W. and Sun, K. (2018). Evidence for the involvement of auxin, ethylene and ROS signaling during primary root inhibition of *Arabidopsis* by the allelochemical benzoic acid. *Plant and Cell Physiology* **59**: 1889-1904.
58. Zhang, Y. and Friml, J. (2020). Auxin guides roots to avoid obstacles during gravitropic growth. *New Phytologist* **225**: 1049-1052.
59. Zhao, J., Bodner, G., Rewald, B., Leitner, D., Nagel, K.A. and Nakhforoosh, A. (2017). Root architecture simulation improves the inference from seedling root phenotyping towards mature root systems. *Journalof Experimental Botany* **68**: 965-982.
60. Zheng, H.Q. and Staehelin, L.A. (2001). Nodal endoplasmic reticulum, a specialized form of endoplasmic reticulum found in gravity-sensing root tip columella cells. *Plant Physiology* **125**: 252-265.