Involvement of kiwifruit root autotoxicity in its replant problem

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Abstract: As aged kiwifruit trees often reduce the fruit productivity and quality, those aged kiwifruits are replaced with juvenile vigorous plants. However, the productivity and quality of the replaced kiwifruits remain relatively low. In the present research, autotoxicity and allelopathy of kiwifruit roots were evaluated. Aqueous methanol extracts of kiwifruit roots inhibited the growth of cress, lettuce, alfalfa, Lolium multiflorum, Phleum pretense and Echinochloa crus-galli. The extracts of kiwifruit roots also showed an inhibitory effect on the growth of kiwifruits themselves. These results suggest that kiwifruit roots may contain allelopathic and autotoxic substances. The accumulation of autotoxic substances in orchard soil may occur by continuous exudation of autotoxic substances from kiwifruit roots over the long-term cultivations, and by liberation of the substances from the root residues. Accumulated autotoxic substances may suppress the fruit productivity and quality of the replaced kiwifruits. Therefore, autotoxicity may be involved in the replant problem of kiwifruits.

Keywords: allelopathy, autotoxicity, kiwifruit, phytotoxicity, replant problem, root exudation

Abbreviations: IC_{50}, the concentrations required for 50% growth inhibition

Introduction

Plants synthesize various organic compounds and exudate them from their roots into their rhizosphere soil (Bais et al. 2006, Bonanomi et al. 2006). Some of these compounds inhibit the germination and growth of other plant species, which is called as allelopathy (Rice 1984, Belz 2007). If these released compounds inhibit the growth of host plans themselves, the phenomenon is called as autotoxicity (Rice 1984). Thus, autotoxicity is a particular type of allelopathy. Growth of some crop plants such as asparagus, strawberry and cucumber was inhibited by allelopathic substances released by their own roots (Asao et al. 1998, Yeasmin et al. 2014, Li et al. 2016, Kato-Noguchi et al. 2018).

Long-term cultivations of some fruit trees lead a reduction of their vigor and reduce their fruit quality and yield. Even though those aged fruit trees were replaced with juvenile vigorous plants on the same orchards, the productivity and quality of the replaced fruit trees remain relatively low, which is known as a “replant problem” (Bent et al. 2009, Henfrey et al. 2015). The replant problem of fruit trees is well known with peach (Prunus persica (L.) Batsch) and apple (Malus domestica Borkh.) (Mizutani et al. 1988, Henfrey et al. 2015, Yin et al. 2016, He et al. 2019). The replant problems are thought to be caused by a combination of biotic factors such as soil-borne pathogenic fungi and nematodes, and abiotic factors such as deterioration of soil factors (Franke-Whittle et al. 2015, Mahnkopp et al. 2018). However, several fungicide treatments and enough nutrients did not always work to recover significantly crop quality and productivity (Lacy 1979, Rice 1984). The incorporation of charcoal amendments into cultivation soil, which absorb wide range of chemicals, recovered some of crop productivity (Elmer and Pignatello 2011, Ituca and Litus 2015).

Some fruit trees continuously release toxic substances into their rhizosphere soil during long-term cultivation, resulting in the accumulation of the substances in the orchard soil (Rice 1984, Li et al. 2016). Several toxic substances such as phlorizin and
vanillin aldehyde accumulated in the rhizosphere soil of apple trees and those compounds were considered to be involved in the apple replant problem (Yin et al. 2016). Benzoic acid was identified in peach root exudates and its high level of the accumulation was observed in the rhizosphere soil of peach trees (Li et al. 2016). This compound is very toxic and leads to autotoxicity on peach trees (Zhu et al. 2017). In addition, precursors of benzoic acid are abundant in peach roots. Thus, when peach orchards are renewed, large numbers of root residues left in the soil could result in the accumulation of benzoic acid (Zhu et al. 2017, Li et al. 2016). Therefore, autotoxicity of fruit trees may be one of the causes of the replant problem.

Kiwifruit, belongings to Actinidia genus, is originating from central and eastern China, and currently grown in many countries because of its commercial values and tolerance to relatively wide climate conditions (Sanz et al. 2021). Kiwifruit also shows a replant problem (Shinomiya et al. 2020). Therefore, autotoxicity may also be involved in the replant problem of kiwifruits. However, there has been no information available about the autotoxicity of kiwifruit roots. The objective of this study was the investigation of the allelopathic and autotoxic activities of kiwifruit roots.

Materials and Methods

Plant materials

Roots of kiwifruits (Actinidia delicosa (A. Chev.) C.F. Liang et A.R. Fergusson, cv. Hayward) were collected from the experimental field of Faculty of Agriculture, Kagawa University. Dicotyledonous seeds of cress (Lepidum sativum L.), lettuce (Lactuca sativa L.) and alfalfa (Medicago sativa L.) were chosen for bioassay as test plants due to their known seedling growth behavior. Monocotyledonous seeds of Lolium multiflorum Lam., Phleum pretense L. and Echinochloa crus-galli (L.) P.Beauv. were also chosen for the bioassay. Seeds of kiwifruits were collected from the ripe fruits (cv. Hayward), and soaked in 0.5% aqueous solution of fungicide (Homai WP; Nippon Soda, Tokyo, Japan) for 15 min. After stored at 4°C for one month to break seed dormancy, the seeds were used for the determination of autotoxicity of kiwifruits.

Extraction

Kiwifruit roots was cut into small pieces using pruning shears, and the pieces (80 g dry weight) were extracted with 70% (v/v) aqueous methanol (1 L) for 48 h. After filtration of the extract using filter paper (No. 2; Toyo Ltd., Tokyo, Japan), the pieces were extracted again with methanol (1 L) for 48 h and filtered. The two extracts were combined and concentrated under reduced pressure at 40°C.

Determination for allelopathic activity

An aliquot of the extract was added to a sheet of filter paper (No. 2; Toyo Ltd., Tokyo, Japan) in a 2.8 cm Petri dish, and the extract solution of the Petri dish was evaporated in a fume hood. The filter paper was then moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 (Nacalai, Kyoto, Japan). Ten seeds each of cress, lettuce and alfalfa, and 10 germinated seeds each of L. multiflorum, P. pretense and E. crus-galli were placed onto the filter paper in Petri dishes. Before the treatments, seeds of L. multiflorum, P. pretense and E. crus-galli had been germinated on moisten filter paper for 48 h in the dark at 25°C. The length of the roots and hypocotyls/coleoptiles of these plants was measured after incubation for 48 h in the dark at 25°C. The bioassay concentrations of the extracts were 1, 3, 10, 30, 100, and 300 mg dry weight of kiwifruit root equivalent extract mL⁻¹. Control test plants were incubated without the extracts.

Determination for autotoxic activity

Kiwifruit seeds were germinated on moisten paper for 120 h in the dark at 25°C. An aliquot of kiwifruit root extract was added to a sheet of filter paper in a 2.8 cm Petri dish, and the extract solution was evaporated as describe above. After the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20, 10 germinated kiwifruit seeds were arranged onto the filter paper in Petri dishes. The length of the roots and hypocotyls of kiwifruits was measured after incubation for 48 h in the dark at 25°C. The concentrations of the extracts were 1, 3, 10, 30, 100, and 300 mg dry weight of kiwifruit root equivalent extract mL⁻¹. Control test plants were incubated without the extracts.

Statistical analysis

The bioassay was repeated two times using a randomized design with 10 plants for each determination. Significant differences between control and treatment were analyzed using t-test. The concentrations required for 50% growth inhibition ($IC_{50}$ value) of the bioassay plants were determined using a logistic regression function.

Results

Root growth of dicotyledonous plants; cress, lettuce
and alfalfa was significantly inhibited by kiwifruit root extracts at the concentrations greater than 10 mg dry weight equivalent extract per mL, and hypocotyl growth of those plants was significantly inhibited by the extracts at the concentrations greater than 30 mg dry weight equivalent extract per mL (Fig. 1). Increasing the extract concentration resulted in an increase in inhibition. The extract obtained from 30 mg of kiwifruit roots inhibited the root growth of cress, lettuce and alfalfa by 16.3, 22.3 and 44.1% of control growth, respectively, and inhibited the hypocotyl growth of cress, lettuce and alfalfa by 38.7, 57.8 and 53.4% of control growth, respectively. Root growth of cress was significantly promoted by the extracts at the concentration of 1 mg dry weight equivalent extract per mL.

The kiwifruit root extracts significantly inhibited root growth of monocotyledonous plants; *L. multiflorum*, *P. pretense* and *E. crus-galli* at concentrations greater than 10, 1 and 3 mg dry weight equivalent extract per mL, respectively, and their coleoptile growth at the concentrations greater than 30 mg dry weight equivalent extract per mL (Fig. 2). The extract obtained from 30 mg of the extracts inhibited the root growth of *L. multiflorum*, *P. pretense* and *E. crus-galli* by 11.5, 5.5 and 9.5% of control growth, respectively, and inhibited the coleoptile growth of *L. multiflorum*, *P. pretense* and *E. crus-galli* by 28.8, 53.1 and 61.2% of control growth, respectively.

The kiwifruit root extracts also significantly inhibited the root and coleoptile growth of kiwifruit at concentrations greater than 10 and 100 mg dry weight equivalent extract per mL, respectively (Fig. 3). Increasing the extract concentration also resulted in an increase in inhibition of the coleoptiles and roots.

**Discussion**

Some fruit trees continuously release toxic substances such as phlorizin and vanillin aldehyde from apple, and benzoic acid from peach, and those...
Fig. 2. Effects of aqueous methanol extracts of kiwifruit roots on the growth of roots and coleoptiles of *L. multiflorum*, *P. pretense* and *E. crus-galli*. Concentrations of tested samples corresponded to the extracts obtained from 1, 3, 10, 30, 100 and 300 mg dry weight of kiwifruit roots per mL. Means ± SE from two independent experiments with 10 plants for each determination are shown. Asterisks indicate significant differences between control and treatment: *, *P* < 0.05, **, *P* < 0.01, ***, *P* < 0.001.

Compounds accumulated in the orchard soil (Yin et al. 2016, Li et al. 2016). Solubility of those compounds in methanol is much higher than that in water. Therefore, kiwifruit roots were extracted with 70% (v/v) aqueous methanol and methanol for the extraction of both hydrophilic and hydrophobic compounds.

The aqueous methanol extracts of kiwifruit roots showed inhibitory effects on dicotyledonous plant species (cress, lettuce and alfalfa) and monocotyledonous plant species (*L. multiflorum*, *P. pretense* and *E. crus-galli*). The inhibition was concentration dependent (Figs. 1 and 2). IC50 values of kiwifruit root extracts on the roots and hypocotyls/coleoptiles of those plants were determined by a logistic regression analysis based on the concentration response bioassay (Table 1). These IC50 values indicate that the extracts obtained from 2.6–13.2 mg kiwifruit roots inhibited the growth of their roots by 50%, and the extracts obtained from 22.5–124.7 mg kiwifruit roots inhibited the growth of their hypocotyls/coleoptiles by 50%. Comparing IC50 values, root growth of *P. pretense* and hypocotyl growth of cress among them were the most sensitive to the extracts, and root growth of alfalfa and coleoptile growth of *E. crus-galli* were the least sensitive. Thus, the effectiveness of the extracts varied by the target plant species. These results suggest that kiwifruit roots may possess allelopathic activity and contain allelopathic substances. The extract obtained from 1 mg dry weight of kiwifruit root was increased cress root growth (Fig. 1). Very low concentration of particular compounds including allelopathic substances sometimes stimulate the growth of some plant species, which is called homeopathy (Silveira et al. 2010, Jäger et al. 2019). However, it is necessary to analysis further for the evaluation of homeopathy of cress roots by the kiwifruit root extracts.

The aqueous methanol extract of kiwifruit roots also showed an inhibitory effect on the growth of kiwifruit roots and coleoptiles (Fig. 3). The
The inhibition indicates that kiwifruit roots may also possess autotoxic activity and contain autotoxic substances. This is the first report of the presence of autotoxic activity of kiwifruit roots. Plants secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic- derived exudation, and proton-pumping mechanisms (Hawes et al. 2000; Bais et al. 2006, Badri and Vivanco 2009). Long-term cultivations of kiwifruit trees may lead the accumulation of autotoxic substances in the orchard soil like other fruit trees such as apple and peach due to continuous exudation of autotoxic substances from their roots (Yin et al. 2016, Li et al. 2016).

When kiwifruit orchards are replaced with juvenile kiwifruit trees, large numbers of root residues from aged kiwifruits could be left in the orchard soil. Phytotoxic substances in plant residues can be released into the rhizosphere soil by the decomposition processes of the residues, and act as allelopathic and autotoxic substances (Bonanomi et al. 2006, Belz, 2007). Those kiwifruit root residues left in the orchard soil may also release autotoxic substances during their decomposition process.

The present research indicates kiwifruit roots have autotoxic and allelopathic activities. However, it is impossible to separate the causes of allelopathy and autotoxicity distinctly because autotoxicity is a particular type of allelopathy (Rice 1984, Kato-Noguchi et al. 2018). The extracts of kiwifruit leaves also showed allelopathic activity and allelopathic substances, (-)-epicatechin, quercitrin and quercetin were isolated from the leaves. Among them, (-)-epicatechin showed allelopathic and autotoxic activity (Okada et al. 2018, 2019). However, it is not clear if same compounds are involved in both allelopathy and autotoxicity of the kiwifruit roots or different compounds work for allelopathy and autotoxicity. Therefore, it is necessary to isolate and identify the compounds involved in allelopathy and autotoxicity of kiwifruit roots.

**Table 1. IC$_{50}$ values (mg dry weight equivalent extract per mL) of aqueous methanol extracts of kiwifruit roots on the growth of roots and hypocotyls/coleoptiles of bioassay plant species including kiwifruit**

| Plant Species            | Root IC$_{50}$ | Hypocotyl/Coleoptile IC$_{50}$ |
|--------------------------|----------------|---------------------------------|
| Cress                    | 12.9           | 22.5                            |
| Lettuce                  | 10.9           | 32.6                            |
| Alfalfa                  | 13.2           | 25.7                            |
| *Lolium multiflorum*     | 7.6            | 28.0                            |
| *Phleum pretense*        | 2.6            | 32.0                            |
| *Echinochloa crus-galli* | 7.6            | 124.7                           |
| Kiwifruit                | 14.8           | 99.3                            |

The values were determined by the function as described in the text.
As described above, there are at least two possibilities for the accumulation of autotoxic substances in kiwifruit orchard soil: 1) Continuous exudation of autotoxic substances from the roots over the long-term kiwifruit cultivations, 2) Liberation of autotoxic substances from root residues left in the orchard soil. Those accumulated autotoxic substances may suppress the fruit productivity and quality of the replaced kiwifruit trees. Thus, autotoxicity may also be involved in the replant problem of kiwifruits. Replant problems of peach and asparagus were decreased by the incorporation of charcoal amendments (Elmer and Pignatello 2011, Atucha and Litus 2015). Therefore, it is worthwhile to evaluate if the charcoal amendments reduce the replant problem of kiwifruits.

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