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Benzaldehyde as a new class plant growth regulator on *Brassica campestris*

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Abstract Plant growth regulator is an essential pesticide to date while the available active ingredient is not well understood unlike fungicide, insecticide and herbicide. This study was aimed to evaluate a new chemical class of plant growth regulator, and the total of 92 benzene derivatives were screened for their germination and early stage of the root growth regulation on *Brassica campestris*. Thirty benzaldehydes, nine acids, one amide, and one ester showed potent root growth inhibitory activity (>70 % inhibition) while only salicylaldehyde showed potent germination inhibition (IC_{50} = 81.2 mg/L) suggesting that benzaldehyde was a key module candidate for the growth inhibition. Benzaldehydes were further evaluated for root growth inhibition. 2,3-Dihydroxybenzaldehyde and salicylaldehyde showed IC_{50} values of 8.0 and 83.9 mg/L, respectively. On the other hand, salicylaldehyde, and 2,4,5-trihydroxybenzaldehyde were found to have root growth promotion effects less than 10 mg/L. This result suggests that the benzaldehyde is a new class candidate for plant growth regulator.

Keywords Benzaldehyde · *Brassica campestris* · Root growth inhibition · Structure-activity relationship

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Introduction

Alleopathic interaction with allelochemicals is an important mode of action for development of new classes of growth regulators since this can manage plant communities and crop growth on the manner of sustainable weed management (Takeuchi et al. 2001; Singh et al. 2003; Rani 2008). Many studies on allelochemicals had been performed for the development of herbicide from the extracts and chemicals (Yoshioka et al. 2004; Kim 2005; Reigosa and Pazos-Malvido 2007; Kim et al. 2014), and some alleochemicals from natural sources were evaluated for their mode of action such as sorgoleone as an inhibitor of photosystem II (Gonzalez et al. 1997; Czarnota et al. 2001), (–)-catechin as an inhibitor of the mitochondrial respiration (Weir et al. 2003), and secalonic acid F as an inhibitor of the reactive oxygen species (ROS)-mediated signal transduction (Zeng et al. 2001; Weir et al. 2004). While the herbicidal activity of extracts from biomass had been widely studied for weed growth suppression, studies on their growth regulation of crops are very limited.

Growth regulators are important agrochemicals since they can improve quality and quantity of agricultural produce. Many agrochemicals as growth promoters and inhibitors have been registered in Korea (KCPA 2014). However, the growth regulators account for less than 5 % out of the total registered chemicals at the Korean pesticide markets. Correspondingly, farmers have limited options for appropriate growth regulators. Therefore benzene derivatives derived from natural products as growth inhibitors were investigated since this study will help to understand the difficulties on crop growth regulation and to solve the problems on pesticide residues.

Phenolic compounds have been highlighted for plant growth regulation and antioxidative effect (Inderjit 1996; Yoshioka et al. 2004; Mu et al. 2006; Li et al. 2010). Benzene derivatives (e.g. syringic acid, salicylic acid, juglone, and salicylaldehyde) have been studied for plant growth promotion and inhibition effects due to abundance in various natural resources. In addition, benzoic acid class is currently used as herbicide since it possesses hormone-

like activity (Reigosa and Pazos-Malvido 2007; Lee et al. 2010a, b). For example, salicylic acid is known as a root growth regulator on arabidopsis, barley, and maize and its allelopathy mechanism was considered as the ROS-mediated pathway (Weir et al. 2004; Lee et al. 2010a). Although benzoic acid class is well studied about their herbicidal activity with structure-activity relationship (SAR), plant growth regulation studies like biological activity relationships between structure and antioxidative property are rarely reported. The antioxidative property is considered as an important herbicide screening factor because they can disrupt the electron transporting system in photosynthesis and mitochondrial respiration.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTs) radicals are frequently used for measuring antioxidative property *in vitro*. DPPH has a stable nitrogen free radical molecule, reacting with phenolic compounds (Foti et al. 2008) and HO· (Hristea et al. 2002). ABTs radical is also frequently used for measuring radical scavenging effect and cross-checking the multifaceted antioxidative effect with DPPH. Phenolic compounds are well known due to their antioxidative properties on DPPH and ABTs, but, the relationship between the inhibition effect on root development and antioxidative activity is rarely studied. To date, the SAR of benzene is only reported for the germination regulation on lettuce (Reynolds 1978). In this research, antioxidative effects with two stable free radicals like ABTs and DPPH and the inhibition of root development of benzene derivatives on *Brassica campestris* were examined. Key functional group of benzene was also evaluated for the growth regulation.

Materials and Methods

Chemicals and other materials

All the tested standard benzene derivatives (>98 % purity) were purchased from TCI chemicals (Kawaguchi, Japan). The reagent grade of ABTs diammonium salt, DPPH, potassium persulfate and Tween 20® were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Absolute ethanol and sodium bicarbonate were purchased as ACS grade from Merck (Darmstadt, Germany). Seeds of *B. campestris* were purchased from Asia Seed Co. (Seoul, Korea).

Germination and root development

Germination and root development tests with *B. campestris* were performed by using the method suggested by from Kim et al (2014). Sixty seeds were placed on petri dish containing the sample solution and incubated for 2 d at 23 °C. The control was treated only with 1 % Tween® 20, and the germination ratio was 93±3 %. Thirty germinated seeds were placed on the sample treated petri dish and incubated for 3 d at 23 °C. The inhibition ratio of root development was compared with the control. To reduce the pH effect of the benzoic acids, the inhibition study of the target compound was performed using 0.05 M carbonate buffer (pH 5.5). The hormetic activity on root development was performed with the same condition for the root development but the only difference was that the chemical treatment concentration was adjusted less than 10 mg/L.

ABTs radical scavenging activity

ABTs radical scavenging activity was carried out using the method reported by Lee et al. (2013). ABTs radical stock solution was prepared from the mixture of 7 mM ABTs and 2.45 mM potassium persulphate for 24 h reaction. The stock solution was diluted with ethanol to the absorbance of 0.70 at Abs_{734}. The radical scavenging activity was measured on the mixture of 0.9 mL of the diluted radical and 0.1 mL of sample after 5 min vortexing. The radical scavenging ratio was compared with the control.

DPPH radical scavenging activity

DPPH radical scavenging effect test was performed using the method reported by Alam et al (2013). Sample solution (15 mL) was added to 150 mL DPPH ethanol solution (0.5 mM) and the absorbance at 517 nm was measured after 30 min incubation at 30°C. The radical scavenging ratio was compared with the control.

Results and Discussion

Screening on single benzene derivatives

Benzoic acid class is a known herbicide with the growth hormone activity and salicylic acid is a well-known natural compound with various biological activities including disease resistance and hormonal activity in this class (Raskin 1992; Loake and Grant 2007; Hayat et al. 2010). Thus the preliminary plant growth inhibition tests were designed with salicylic acid derivatives such as salicylalcohol, salicylaldehyde, and salicylamide. From the preliminary test with the four benzoic acid derivatives, salicylaldehyde showed the strongest germination (IC_{50} = 81.2 mg/L) and root development (IC_{50} = 83.9 mg/L) inhibition activities on *B. campestris*. To extend the structure-based study to benzene derivatives, the inhibition activity was preliminarily screened with 46 benzaldehydes, 23 benzoic acids, 8 benzyl alcohols, 9 benzamide, and 6 benzoyl esters on 500 mg/L with the basis of seed germination and root development of seedlings. The inhibition study of germination revealed that 29 benzaldehydes, ethyl-4-hydroxybenzoate, and 2,3,4-trimethoxybenzoic acid exhibited over 70 % of the germination inhibitory activity during screening. However, only salicylaldehyde showed 50 % inhibition at less than 100 mg/L (Tables 1 and 2) among the benzene derivatives. Thus the germination inhibition was not further studied on *B. campestris* due to the poor inhibition potency of the benzene derivatives.

On the root development inhibition study, a total of 92 benzene derivatives were screened for their inhibition potency at 500 mg/

Table 1 Growth inhibition activity of benzaldehydes on *B. campestris* at 500 mg/L

Compound	Growth inhibition*	
	Germination	Root development
2-Hydroxybenzaldehyde	+++	+++
3-Hydroxybenzaldehyde	+	++
4-Hydroxybenzaldehyde	+	++
2-Allyloxybenzaldehyde	+++	+++
2-Benzyloxybenzaldehyde	++	++
2-Ethoxybenzaldehyde	+++	+++
Helicin	+	++
2-Cyanobenzaldehyde	+++	+++
o-Anisaldehyde	+++	+++
o-Tolualdehyde	+++	+++
2-(2-Hydroxyethoxy)benzaldehyde	-	-
2-Chlorobenzaldehyde	+++	+++
3-Chlorobenzaldehyde	+++	+++
4-Chlorobenzaldehyde	+++	+++
2-Nitrobenzaldehyde	+++	+++
3-Nitrobenzaldehyde	+++	+++
4-Nitrobenzaldehyde	+++	+++
2,3-Dihydroxybenzaldehyde	+++	+++
2,4-Dihydroxybenzaldehyde	+++	+++
2,5-Dihydroxybenzaldehyde	+++	+++
3,4-Dihydroxybenzaldehyde	+	++
3,5-Dihydroxybenzaldehyde	+	+
3-Fluoro-4-hydroxybenzaldehyde	++	+++
3-Fluorosalicylaldehyde	+++	+++
2,3-Difluorobenzaldehyde	+++	+++
2,4-Difluorobenzaldehyde	+++	+++
3-Chloro-4-Hydroxybenzaldehyde	+	++
2,3-(Methylenedioxy)benzaldehyde	+++	+++
2-Hydroxy-3-methoxybenzaldehyde	+++	+++
3-Ethoxy-4-hydroxybenzaldehyde	+	+
3-Ethoxysalicylaldehyde	+++	+++
p-Vanillin	+	++
3-Hydroxy-4-methoxybenzaldehyde	+	+
2-Hydroxy-5-nitro-benzaldehyde	+++	+++
4-Hydroxy-3-nitrobenzaldehyde	+++	+++
5-Hydroxy-2-nitrobenzaldehyde	++	+++
3-(Trifluoromethoxy)salicylaldehyde	+++	+++
3-Nitrosalicylaldehyde	+++	+++
2,3,4-Trihydroxybenzaldehyde	+++	+++
2,4,5-Trihydroxybenzaldehyde	+++	+++
2,4,6-Trihydroxybenzaldehyde	+	+
3,4,5-Trihydroxybenzaldehyde	+	++
Syringaldehyde	+	+
3,4-Dimethoxy-5-hydroxybenzaldehyde	+++	+
2-Hydroxy-5-nitro-*m*-anisaldehyde	+++	+++
5-Nitro-*p*-vanillin	+	++

* (-), (+), (++), and (+++) represent average growth inhibition range 0–10, 10–30, 30–70, and over 70 %, respectively

Table 2 Growth inhibition activity of the acid, alcohol, amide and ester derivatives of benzene on *B. campestris* at 500 mg/L

Compound	Growth inhibition*	
	Germination	Root development
Benzoic acid	++	+++
Salicylic acid	++	+++
3-Aminosalicylic acid	-	++
3-Methoxysalicylic acid	-	++
4-Methoxysalicylic acid	++	+++
5-Methoxysalicylic acid	-	+++
6-Methoxysalicylic acid	++	+++
4-Hydroxybenzoic acid	-	++
2,3-Dihydroxybenzoic acid	-°°	+++
2,3-Dimethoxybenzoic acid	-	++
2,4-Dimethoxybenzoic acid	-	++
2,5-Dimethoxybenzoic acid	-	+++
2,5-Dihydroxybenzoic acid	-	++
2,6-Dimethoxybenzoic acid	-	++
2,6-Dihydroxybenzoic acid	-	++
2-Amino-5-hydroxybenzoic acid	-	+++
3,4,5-Trimethoxybenzoic acid	+	-
3,4-Dimethoxybenzoic acid	-	+
3,4-Dihydroxybenzoic acid	+	++
3,5-Dimethoxybenzoic acid	++	++
3-Amino-4-hydroxybenzoic acid	-	++
2,3,4-Trimethoxybenzoic acid	+++	+++
Gallic acid	-	-
Salicylalcohol	-	-
3-Hydroxybenzyl alcohol	-	++
4-Hydroxybenzyl alcohol	-	-
3,5-Dimethoxybenzyl alcohol	++	+
3,5-Dihydroxybenzyl alcohol	-	-
3,4,5-Trimethoxybenzyl alcohol	-	-
Vanillyl alcohol	+	-
Veratryl alcohol	+	-
Salicyl amide	-	+++
4-Methoxysalicyl amide	+	++
4-Aminobenzamide	++	+
4-Methoxybenzamide	++	-
4-Hydroxybenzamide	+	+
2,4-Dihydroxybenzamide	++	++
3,5-Dimethoxybenzamide	+	++
3,5-Dihydroxybenzamide	+	+
3,4,5-Trimethoxybenzamide	++	-
Ethyl 4-hydroxybenzoate	+++	++
Ethyl gallate	-	-
Octyl gallate	-	+
Propyl gallate	-	++
Methyl 4-methoxysalicylate	++	+++
Ethyl salicylate	-	+

* (-), (+), (++), and (+++) represent average growth inhibition range 0–10, 10–30, 30–70, and over 70 %, respectively

Table 3 Antioxidant activity of benzaldehydes

Compound	Antioxidant activity (SC_{50}, mg/L)	
	ABTs	DPPH
Salicylaldehyde	>100	>100
3-Hydroxybenzaldehyde	>100	>100
4-Hydroxybenzaldehyde	>100	>100
2-Allyloxybenzaldehyde	> 100	>100
2-Benzyloxybenzaldehyde	> 100	>100
2-Ethoxybenzaldehyde	> 100	>100
Helicin	> 100	>100
2-Cyanobenzaldehyde	> 100	>100
o-Anisaldehyde	> 100	>100
o-Tolualdehyde	> 100	>100
2-(2-Hydroxyethoxy)benzaldehyde	> 100	>100
2-Chlorobenzaldehyde	> 100	>100
3-Chlorobenzaldehyde	> 100	>100
4-Chlorobenzaldehyde	> 100	>100
2-Nitrobenzaldehyde	>100	>100
3-Nitrobenzaldehyde	>100	>100
4-Nitrobenzaldehyde	>100	>100
2,3-Dihydroxybenzaldehyde	2.7	12.4
2,4-Dihydroxybenzaldehyde	>100	>100
2,5-Dihydroxybenzaldehyde	4.5	15.7
3,4-Dihydroxybenzaldehyde	2.3	10.0
3,5-Dihydroxybenzaldehyde	20.59	>100
3-Fluoro-4-hydroxybenzaldehyde	> 100	>100
3-Fluorosalicylaldehyde	89.6	>100
2,3-Difluorobenzaldehyde	> 100	>100
2,4-Difluorobenzaldehyde	> 100	>100
3-Chloro-4-Hydroxybenzaldehyde	>100	>100
2,3-(Methylenedioxy)benzaldehyde	> 100	>100
2-Hydroxy-3-methoxybenzaldehyde	>100	>100
3-Ethoxy-4-hydroxybenzaldehyde	>100	>100
3-Ethoxysalicylaldehyde	>100	>100
p-Vanillin	>100	>100
3-Hydroxy-4-methoxybenzaldehyde	>100	>100
2-Hydroxy-5-nitro-benzaldehyde	> 100	>100
4-Hydroxy-3-nitrobenzaldehyde	> 100	>100
5-Hydroxy-2-nitrobenzaldehyde	> 100	>100
3-(Trifluoromethoxy)salicylaldehyde	> 100	>100
3-Nitrosalicylaldehyde	> 100	>100
2,3,4-Trihydroxybenzaldehyde	4.79	53.4
2,4,5-Trihydroxybenzaldehyde	3.96	36.1
2,4,6-Trihydroxybenzaldehyde	10.25	>100
3,4,5-Trihydroxybenzaldehyde	4.08	22.4
Syringaldehyde	> 100	>100
3,4-Dimethoxy-5-hydroxybenzaldehyde	>100	>100
2-Hydroxy-5-nitro-*m*-anisaldehyde	> 100	>100
5-Nitro-*p*-vanillin	>100	>100

Table 4 Antioxidant activity of the acid, alcohol, amide and ester derivatives of benzene

Compound	Antioxidant activity (SC_{50}, mg/L)	
	ABTs	DPPH
Benzoic acid	>100	>100
Salicylic acid	>100	>100
3-Aminosalicylic acid	10.7	32.4
3-Methoxysalicylic acid	>100	>100
4-Methoxysalicylic acid	>100	>100
5-Methoxysalicylic acid	>100	>100
6-Methoxysalicylic acid	>100	>100
4-Hydroxybenzoic acid	>100	>100
2,3-Dihydroxybenzoic acid	17.1	2.5
2,3-Dimethoxybenzoic acid	>100	>100
2,4-Dimethoxybenzoic acid	>100	>100
2,5-Dimethoxybenzoic acid	>100	>100
2,5-Dihydroxybenzoic acid	32.8	1.0
2,6-Dimethoxybenzoic acid	>100	>100
2,6-Dihydroxybenzoic acid	>100	>100
2-Amino-5-hydroxybenzoic acid	26.2	28.4
3,4,5-Trimethoxybenzoic acid	>100	>100
3,4-Dimethoxybenzoic acid	>100	>100
3,4-Dihydroxybenzoic acid	17.3	>100
3,5-Dimethoxybenzoic acid	>100	>100
3-Amino-4-hydroxybenzoic acid	26.3	28.4
2,3,4-Trimethoxybenzoic acid	>100	>100
Gallic acid	17.7	11.4
Salicyl alcohol	11.3	>100
3-Hydroxybenzyl alcohol	19.7	>100
4-Hydroxybenzyl alcohol	16.1	>100
3,5-Dimethoxybenzyl alcohol	>100	>100
3,5-Dihydroxybenzyl alcohol	13.2	>100
3,4,5-Trimethoxybenzyl alcohol	>100	>100
Vanillyl alcohol	10.7	>100
Veratryl alcohol	>100	>100
Salicyl amide	>100	>100
4-Methoxysalicylamide	>100	>100
4-Aminobenzamide	>100	>100
4-Methoxybenzamide	>100	>100
4-Hydroxybenzamide	>100	>100
2,4-Dihydroxybenzamide	5.6	>100
3,5-Dimethoxybenzamide	>100	>100
3,5-Dihydroxybenzamide	17.0	>100
3,4,5-Trimethoxybenzamide	>100	>100
Ethyl 4-hydroxybenzoate	>100	>100
Ethyl gallate	23.8	23.8
Octyl gallate	37.4	27.9
Propyl gallate	14.2	26.4
Methyl 4-methoxysalicylate	>100	>100
Ethyl salicylate	>100	>100

L. Thirty different benzaldehydes, nine different benzoic acids, salicylamide, and methyl 4-methoxysalicylate showed over 70 % root development inhibitory activity at the screening level. Furthermore, only benzaldehyde derivatives showed IC_{50} of the root development less than 100 mg/L while the rest of the derivatives showed IC_{50} more than 100 mg/L. Salicylamide (IC_{50} = 270 mg/L) from benzamide group, methyl 4-methoxysalicylate

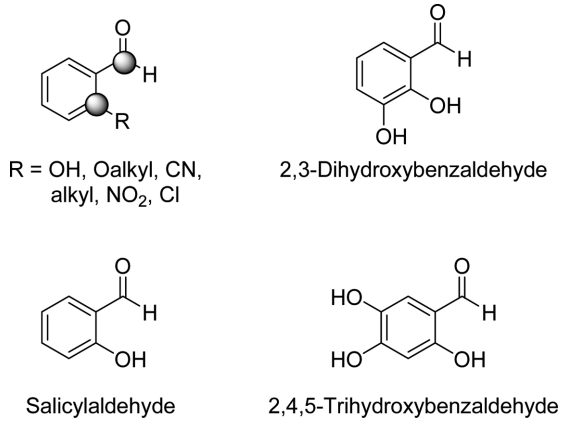

Fig. 1 Chemical structure of plant growth regulators in benzaldehydes

(IC_{50} = 380 mg/L) from benzoyl ester group and 2,3,4-trimethoxybenzoic acid (IC_{50} = 107 mg/L) from the benzoic acid group were the strongest inhibitors in each group. Thus, benzaldehyde group was considered as an important new structure module to be growth inhibitors.

Benzaldehyde derivatives

Root growth inhibitory activity of 46 benzaldehydes was evaluated to find another key structural factor of benzene. C2-functionalized benzaldehydes including salicylaldehyde, 2,3-dihydroxybenzaldehyde, and 2,4,5-trihydroxybenzaldehyde showed stronger inhibition activity than the others. However, the significant difference between the other functional groups at C2-position was not found in this experiment. Compared with electron withdrawing group, 2-nitrobenzaldehyde (IC_{50} = 45 mg/L), and 4-chlorobenzaldehyde (IC_{50} = 50 mg/L) showed their IC_{50}s of less than 100 mg/L, while others ranged from 100 to 250 mg/L.

In this experiment, IC_{50} of three benzaldehydes were less than 50 mg/L; 2,3-dihydroxybenzaldehyde (IC_{50} = 8.0 mg/L), 4-hydroxy-3-nitrobenzaldehyde (IC_{50} = 16.2 mg/L), and 2-nitrobenzaldehyde. The IC_{50} of next seven benzaldehydes ranged from 50 to 100 mg/L like 4-chlorobenzaldehyde, 2-hydroxy-5-nitro-*m*-anisaldehyde (IC_{50} = 51.6 mg/L), 2,3,4-trihydroxybenzaldehyde (IC_{50} = 70.4 mg/L), 2,4-dihydroxybenzaldehyde (IC_{50} = 73.4 mg/L), 2,5-dihydroxybenzaldehyde (IC_{50} = 78.3 mg/L), salicylaldehyde, and 3-ethoxysalicylaldehyde (IC_{50} = 69.9 mg/L). This result indicated that 2-hydroxybenzaldehyde module would give the growth regulatory potentials. In contrast with the previous study for the growth inhibition of lettuce by Reynolds (1978), the 2-hydroxylation of benzaldehyde may hold promise as potential growth inhibitors.

Correlation with radical scavenging effect and root growth inhibition

The hydroxylation of aromatic structure is generally involved with antioxidative activity like radical scavenging activity (Foti et al. 2008). Along with the growth inhibition assay on root length by benzene derivatives, redox effect is generally considered to a key pathway for growth regulation. With the two different radicals, ABTs and DPPH, the scavenging experiment was typical for screening of antioxidative activity. Fifty percent radical scavenging concentration (SC_{50}) was measured with the benzene derivatives and described in Tables 3 and 4. All the nominated growth inhibitor showed their SC_{50}s from 2.7 to >100 mg/L. For example, 2,3-dihydroxybenzaldehyde showed strong radical scavenging activity (SC_{50} 2.7 mg/L on ABTs and 12.4 on DPPH), whereas salicylaldehyde showed no radical scavenging activity (SC_{50} >100 mg/L on the both radicals). Thus, the correlation of radical scavenging effects on ABTs and/or DPPH with the root development inhibition was not found, and another method to measure antioxidative activity should be considered for screening of plant growth regulator.

Hormetic activity of salicylaldehyde derivatives

Hormetic activity is frequently tested to find a new plant growth regulator based on plant hormone activity. To measure its activity of the candidate, benzaldehydes that showed IC_{50} of less than 500 mg/L, were tested for a root growth promotion at low concentration (1–10 mg/L) as the same method for the root development. Salicylaldehyde, and 2,4,5-trihydroxybenzaldehyde exhibited more than 50 % of the root growth promoting activity while the other compounds did not show the activity under the same condition. Therefore, these two natural benzaldehydes may be acceptable as plant growth stimulators.

In conclusion, this SAR study suggests benzaldehyde as a new class candidate for plant growth regulator. To apply benzaldehydes like 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-nitrobenzaldehyde as crop growth regulators into fields, the environmental stability and application manual of the chemicals should be further studied as an active ingredient.

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