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Suppression of Morningglory (*Ipomoea Hederacea*) Growth by Rhizobacteria and IAA-3-ACETIC Acid

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Indole-3-acetic acid (IAA) biosynthesis by bacteria occurs widely in rhizospheres. Bacterial species able to synthesize IAAmay be exploited for beneficial interactions in crop management systems. The objective of this study was to determine the response of ivyleaf morningglory (*Ipomoea hederacea*) seedlings to IAA and to an IAA-producing rhizobacterum, *Bradyrhizobium japonicum* isolate GD3. IAA solution and isolate GD3 suppression of seedling growth measured as radicle length and biomass depended on IAA concentration. Seedling radicle length was significantly reduced by ca. 29% with more than 1.0 μM of IAA solution, compared to the control, 48 h after application. The cell concentration at 50% growth reduction (GR_{50}) of the seedling radicle was IAA production by isolate GD3 at $10^{4.82}$ cfu, the cell concentration for 50% growth reduction (GR_{50}) of seedling radicle was 0.24 iM, which was much lower than the IAA solution concentration (117.48 μM) required for GR_{50}. Therefore, excess IAA production by isolate GD3 may be more detrimental to morningglory radicle growth than standard IAA solution. Results confirmed involvement of IAA in suppressive effects of isolate GD3 on morningglory seedlings grown in a hydroponic system.

Key words : *indole-3-acetic acid, phytohormones, Bradyrhizobium japonicu, ivyleaf morningglory (Ipomoea hederacea), deleterious rhizobacteria*

I. Introduction

Ivyleaf morningglory (*Ipomoea hederacea*) is a noxious weed and is one of the most competitive weed species in soybean production (Dowler, 1992). Morningglory species are not greatly susceptible to glyphosate and may become increasingly tolerant as widespread use of glyphosate-resistant (GR) crops continues (Shaner, 2000; Shaw and Arnold, 2002). Frequently,

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tolerance of annual weed species to glyphosate leads to increased application rates of glyphosate or applications of other herbicides for residual weed control in glyphosate-resistant soybean (*Glycine max*). These additional herbicide applications may injure crops through decreased chlorophyll contents (Johnson et al., 2002) and reduced nodulation (King et al., 2001) under especially adverse environmental conditions such as temperature, water, and nutritional stresses and result in potential yield decreases (Reddy et al., 2000).

The rhizosphere generally provides microenvironments supporting microbe-plant interactions due to abundant and diverse nutrients released in root exudates, resulting in larger microbial populations and greater activity than in the bulk soil (Kennedy, 2005; Kremer, 2006). A beneficial rhizosphere interaction that might be exploited is the biological control of weed seedlings by phytotoxic metabolites synthesized by deleterious rhizobacteria (DRB). A biological control approach may provide potential alternatives to herbicides or may supplement herbicides such as glyphosate to improve control of herbicide-tolerant weeds. The effect of DRB on host seedlings varies depending on root-colonizing ability, specific phytotoxin production, and resistance to antibiotics produced by other rhizosphere microorganisms (Kremer, 2006).

Numerous rhizobacteria isolated from rhzospheres of both GR and non-transgenic soybean synthesized various levels of auxin-like compounds (Kim, 2006). The colonization and growth inhibition of morningglory roots by selected DRB was further demonstrated to be related to production of high amounts of indole-3-acetic acid, IAA (Kim and Kremer, 2005). Growth-suppressive activity based on endogenous and exogenous production of high amounts of IAA has been previously described for several DRB (Kremer and Sarwar, 1995; Barazani and Friedman, 1999). Kremer and Sarwar (1995) reported microbial production of IAA by a number of rhizobacteria isolates and that IAA produced from L-tryptophan substrate by *Enterobacter taylorae* isolate 3.8.12.7 inhibited growth of a diverse array of weed seedlings including velvetleaf (*Abutilon theophrasti*), morningglory, redroot pigweed (*Amaranthus retroflexus*), and green foxtail (*Setaria viridis*) in agar bioassays. In the rhizosphere, exogenous tryptophan may be released in root exudates in sufficient amounts to induce significant IAA biosynthesis of rhizobacteria.

IAA biosynthesis in bacteria (*Agrobacterium, Pseudomonas, Bradyrhizobium*, etc.) can occur by multiple pathways, which are classified based on the pathway intermediates including: indole-3-acetamide, indole-3-pyruvate, tryptophan side chain, tryptamine, and indole-3-acetonitrile (Gaudin et al., 1994 Patten and Glick, 1996). Bacteria involved in IAA biosynthesis may comprise up to 80% of the bacterial population of rhizospheres and rhizoplanes, depending on environmental conditions and the plant species (Leinhos and Vacek 1994). IAA and other

auxin-like compounds produced by bacteria greatly affect host plants by stimulating plant growth and through phytopathogenesis. At physiological concentrations, auxins control plant cell differentiation, structural organization, and the shift from vegetative to reproductive growth. However, excess concentrations of exogenous IAA in the rhizosphere lead to cell-wall loosening by stimulating cellulase activity, and membrane leakage, resulting in loss of water and nutrients (Cohen et al., 2002).

Few experiments have been conducted to investigate the effect of auxin compounds on morningglory. The population thresholds of IAA-producing bacteria that are required to affect morningglory seedling growth have not been quantified. Characterization of morningglory response to IAA and IAA-producing rhizobacteria may be important in providing a management option for controlling populations of this weed that are tolerant to glyphosate. The objectives of this study were to determine the response of morningglory to IAA and to quantify levels of an IAA-producing rhizobacterium that suppress growth of morningglory.

Ⅱ. Methods and Materials

1. Seedling growth and response of morningglory seedlings to IAA solution

Ivyleaf morningglory seeds were surface sterilized by immersing in 70% ethanol for 2 min, rinsing in sterile water, immersing in 1.25% sodium hypochlorite for 4 min, rinsing 4~6 times with sterile water and blotting on autoclaved filter paper. The surface-sterilized seeds were germinated in petri dishes containing 1.5% agar. Petri dishes were wrapped with parafilm and incubated at 27℃ overnight. Ten pre-germinated seeds were transferred aseptically into a petri plate with agar (1.5%) amended with 1.0 ml of the appropriate IAA concentration in 5 replicates. For the control, sterilized distilled water (1 mL) was used to amend the agar.

2. Bacteria culture conditions for IAA assays and bacteria identification

Bacterial isolates showing a range of IAA production were verified for IAA production using a method based on the quantitative colorimetric assay of Gordon and Weber (1951). For the colorimetric IAA assay of each isolate, a 24- to 72-h half-strength tryptic soy broth culture was diluted in sterile water to obtain an O.D. of 0.5 at 500 nm. The diluted broth suspension (1 mL)

was added to 14 ml of growth medium in a 30-mL tube. The growth medium contained (in g $\cdot L^{-1}$): glucose, 5.0; yeast extract, 0.025. L-tryptophan at 0.204 g $\cdot L^{-1}$ was added in the growth medium as a substrate for IAA production. Controls were prepared by substituting sterile water for bacterial suspension. Tubes were capped, vortexed, and statically incubated in the dark at 27 ℃ for no more than 72 h. For the analysis of IAA production, turbidity of each culture was adjusted to 10^8 cells ml^{-1} at 500 nm absorbance with sterile water based on previously determined growth curve before filtration through 0.2-μm filter membranes. Each culture filtrate was dispensed in three replicate wells of 96-well microplates (Corning) for the assays using the microplate method, a modification of the standard method (Gordon and Weber, 1951) developed by Sarwar and Kremer (1995). IAA in the microplate assays was determined spectrophotometrically at 530 nm using a microplate reader (Dynatech MR 5000).

3. Soil preparation

Square plastic pots (260.47 cm^3) were prepared with a bottom layer of perlite approximately 1 cm deep over which was dispensed approximately 223.26 cm^3 of a soil-perlite mixture. Soil was classified as a Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualf) with pH 6.8 and 3.3% organic matter and mixed with perlite [30% (v/v)]. The soil-perlite mixtures were exposed to microwave radiation (2 min $\cdot kg^{-1}$ soil; 625 W heating power; 2,450 MHz). The microwave treatment is known to remarkably reduce soil fungal species such as *Pythium* and *Fusarium* with little effect on bacterial species (Ferriss, 1984; Larkin et al., 1995).

4. Response of morningglory seedlings to *Bradyrhizobium japonicum* isolate GD3

B. japonicum cultured on TSA provided inocula for the dose response assay with morningglory seedlings. For inocula preparation, bacterial cultures were suspended in peptone broth (0.1%), spectrophotometrically adjusted to 10^8 cells $\cdot mL^{-1}$ at 500 nm, and serial-diluted in sterilized distilled water to appropriate inoculum densities. Five pre-germinated morningglory seeds were aseptically sown about 1 cm below the soil surface in a pot and inoculated with 2 mL of bacterial inoculum two times at an interval of 2 days. For the control, peptone broth (0.1%) was dispensed onto the seeds. The pouches were placed at ambient temperature (19~24℃) under 12-h light and 12-h dark period supplemented with fluorescent lamps. Six replicate pots were prepared per treatment.

5. Variables Measured and Statistical Analysis

To determine dose response of morninggloy to IAA solutions, seedling radicles were measured 48 h after IAA solutions were applied. For the concentration response of morningglory to isolate GD3, roots were weighed and rated 12 days after pre-germinated seeds were inoculated. The rating ranged from 0 to 5 based on development of root necrosis (discoloration), with 0 = healthy without discoloration and 5 = primary roots entirely necrotic and most of the secondary roots showing discoloration. Each root system was spread out by suspending in distilled water for rating. GR_{50} is defined as 50% growth reduction (Lavy and Santelmann, 1986) and was estimated based on regression equations obtained from the response of morningglory seedlings to cell concentration of isolate GD3 and IAA dose. The data were subjected to analysis of variance by PROC ANOVA (SAS Institute, 2001) and, where the F-tests were significant, treatment means were separated using Fisher's protected LSD (α=0.05).

Ⅲ. Results and Discussion

1. Response of morningglory seedlings to IAA solution

Suppressive effects of IAA solutions on pre-germinated seedlings varied depending on IAA concentration (Fig. 1). Seedling radicle length was not affected at concentrations less than 1.0 μM of IAA; however, length was significantly inhibited by 1.0 μM of IAA solution, which reduced growth by ca. 29%, compared to the control, 48 h after application (Fig. 1). IAA concentration for 50% inhibition of radicle length (GR_{50}) was $10^{2.40}$ μM (Fig. 1). IAA concentration at $10^{2.40}$ μM for GR_{50} of seedling radicle length was calculated as 251.19 μM. Suppressive effects of IAA standard solution and isolate GD3 cell cultures on pre-germinated morningglory seedlings were detected as reductions in both radicle length and biomass. IAA production by isolate GD3 at $10^{4.82}$ cfu, the GR_{50} for radicle biomass reduction, was 0.24 μM, which is much lower than the IAA concentration (117.48 μM) ml^{-1} needed for GR_{50} of radicle length. Therefore, excess IAA production of isolate GD3 may be more detrimental to radicle biomass of morningglory than radicle growth inhibited by IAA solution, which also somewhat confirmed the suppressive effect of isolate GD3 on morningglory seedlings grown in a hydroponic system (Kim and Kremer, 2005). Amending soil with rhizobacteria able to producing high levels of IAA may suppress morningglory seedling growth and act synergistically with glyphosate in managing this

herbicide-tolerant weed.

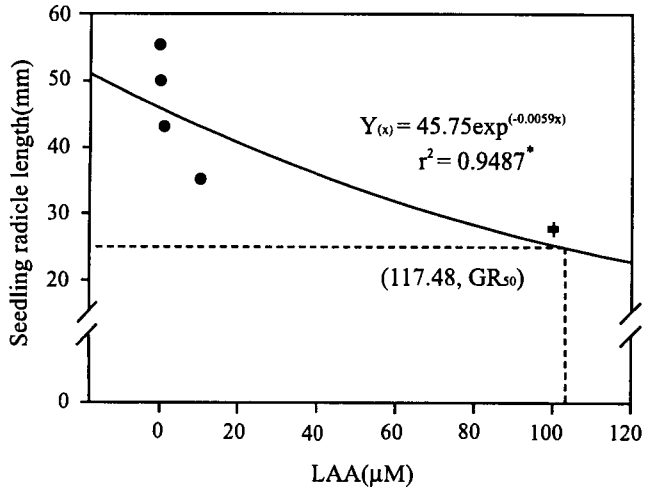

Fig. 1. Effect of IAA solution on radicle growth of seedlings two days after dispensed. Curve is predicted non-linear regression models using single and 2- parameter equation [$Y_{(x)} = Y_0\exp^{kx}$], where Y_0 and Y_x are the initial and suppressive radicle length at IAA concentration per a plant of morningglory seedling (x) when k = -0.0059. * represents significance at $P < 0.05$. † represents IAA concentration at GR_{50}. ‡ represents 50% of growth reduction.

2. Response of morningglory seedlings to *Bradyrhizobium japonicum* isolate GD3

Bradyrhizobium japonicum isolate GD3 was selected based on its high levels of IAA production (365.38 μM; Table 1) and its ability to suppress growth of ivyleaf morningglory seedlings grown in a hydroponic system (Kim and Kremer, 2005). The isolate was inoculated onto the pre-germinated seedlings in soil with appropriate cell concentrations. Suppressive effects of isolate GD3 on seedlings varied when measured as radicle biomass and injury rating (Figs. 2 and 3). The cell concentration for 50% inhibition of radicle biomass at GR_{50} was $10^{9.04}$ cfu (Fig. 2). IAA production at $10^{9.04}$ cfu for GR_{50} of seedling radicle biomass was calculated as 4006.31 μM based on the 365.85 μM IAA concentration produced by isolate GD3 at 10^8 cfu. The line describing the isolate GD3 cell concentration and root rating of morningglory seedlings relationship also illustrated the suppressive effect of isolate GD3 cell concentration on seedling growth (Fig. 3). Injury rating agreed closely with reductions in radicle biomass demonstrating

accuracy at the visual rating system (Fig. 4). Results may have implications for other cropping systems using herbicide-resistant crops or those systems using biological agents for weed management. Finally, the intense growth suppressive effect of isolate GD3 may be related with other phytotoxins, acting synergistically with IAA produced by isolate GD3 to reduce radicle growth of morningglory seedlings. Therefore, future research is required to verify involvement of other growth-inhibiting mechanisms by rhizobacteria on morningglory growth.

Table 1. IAA production of bacterial isolates by colorimetric IAA assay.

	Isolate	IAA production (μM)
A	*Pseudomonas syringae* 96-1-1C	0.00
B	*P. syringae* 116-1-2C	10.46
C	*P. syringae* 98-1-1C	18.56
D	*Bradyrhizobium japonicum* GD3	365.38
E	*Phyllobacterium rubiacearum* 93-2-11C	686.38

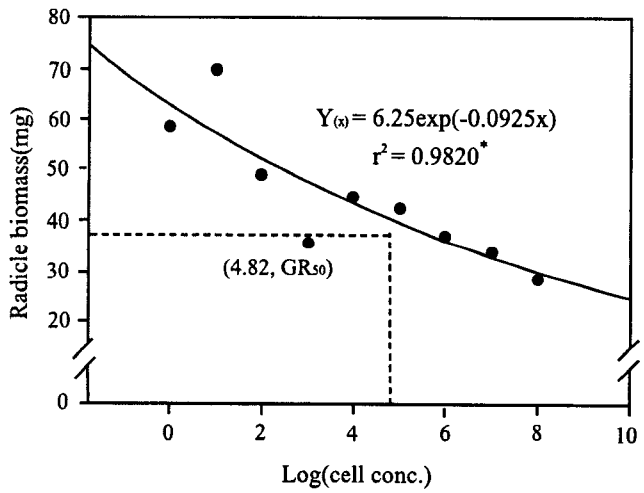

Fig. 2. Effect of isolate GD3 cell concentration on radicle biomass of morningglory seedlings. Curve is predicted non-linear regression models using single and 2- parameter equation [$Y_{(x)} = Y_0 \exp^{kx}$], where Y_0 and Y_x are the initial and suppressive biomass at Log total isolate GD3 cell concentration inoculated per a plant of morningglory (x) when k=-0.0925. *represents significance at $P < 0.05$. †represents Log cell conc. at GR50. ‡represents 50% of growth reduction.

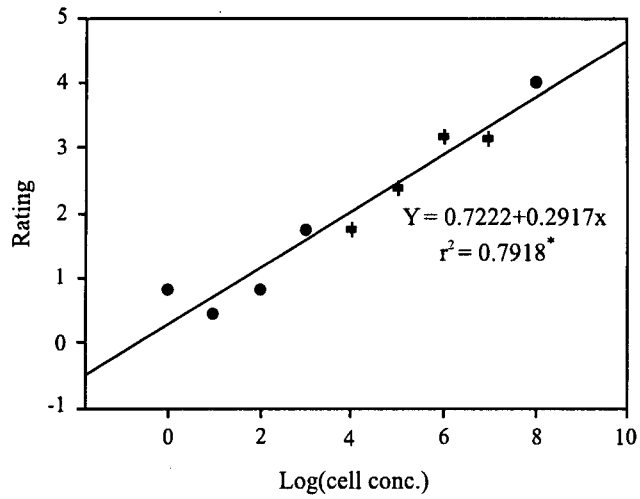

Fig. 3. Effect of isolate GD3 cell concentration on growth rating of morningglory seedlings. Line is composed of predicted values based on linear regression models of equation [$Y_{(x)} = Y_0 + kx$], where Y_0 and Y_x are the initial and suppressive rating of seedlings at Log total isolate GD3 cell concentration inoculated per a plant of morningglory (x) when k=0.2917. *represents significance at $P < 0.05$.

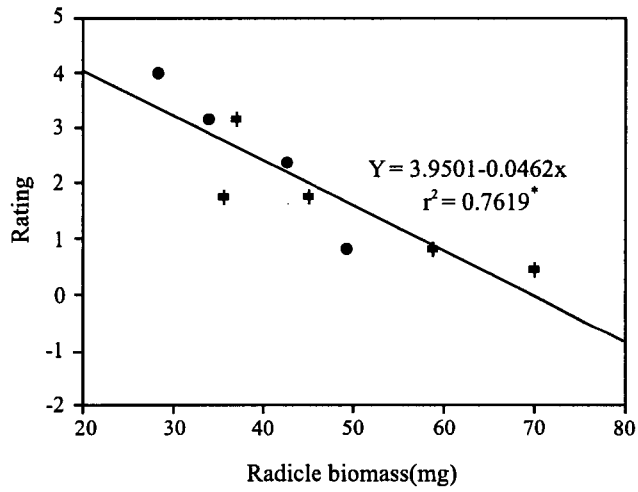

Fig. 4. Relationship of growth suppressive rating with radicle biomass of morningglory seedlings. Line is composed of predicted values based on linear regression models of equation [$Y_{(x)} = Y_0 + kx$], where Y_0 and Y_x are the initial and suppressive rating of seedlings at radicle biomass(x) when k=- 0.0462. *represents significance at $P < 0.05$.

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