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SHORT COMMUNICATION

Mechanism by Which *Bacillus*-Derived 2-Aminobenzoic Acid Inhibits the Growth of *Arabidopsis thaliana* Roots

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To analyze the growth inhibitory mechanism of a 2-aminobenzoic acid (2-AA) derived from *Bacillus cereus* EJ-121, we treated *Arabidopsis thaliana* plants with 2-AA, 2-AA analogs, auxin (NAA), a known auxin transport inhibitor [2,3,5-triiodobenzoic acid (TIBA)], and an ethylene action inhibitor [silver thiosulfate (Ag)]. Root development was significantly inhibited by 50 μM 2-AA, whereas the growth of bacteria and yeast was undeterred. The application of two 2-AA analogs -- 3-aminobenzoic acid (3-AA) and 4-aminobenzoic acid (4-AA) -- did not impair *Arabidopsis* root growth at concentrations below 100 μM. These results suggest that the effect of 2-AA is not due to its chemical structure, but because of its conversion to another metabolite, IAA. To confirm this, we supplemented TIBA in the growth medium, and found that the degree of inhibition was significantly reduced. Similarly, when plants were co-treated with 100 μM Ag, the negative effect of 50 μM 2-AA was greatly diminished. All of these observations support the proposal that this inhibition results from the conversion of 2-AA to IAA. Furthermore, the increased auxin level leads to a rise in ethylene synthesis, which then blocks root growth and, ultimately, retards overall plant development.

Keywords: 2-aminobenzoic acid, auxin, ethylene, plant growth inhibitor

The effect of rhizobacteria on plant growth can be positive, negative, or neutral (Nehl et al., 1996). For example, allelopathic rhizobacteria can inhibit development by secreting allelochemicals, either directly or indirectly, into the rhizosphere (Barazani and Friedman, 1999). Because of this property, some organic compounds produced by rhizobacteria can serve as weed-killers (Bernart and Gerwick, 1990).

Many reports have described how microbe-originated compounds inhibit plant growth. For example, hydroxysuochrin and sulochrin isolated from a fungus negatively affect the development of tea pollen tubes (Shimada et al., 2001), while penicillic acid, also from a fungus, prohibits root elongation in rice (Sassa et al., 1971). In fact, phosphinothricin and bialaphos have been used as commercial herbicides in agriculture (Barazani and Friedman 1999).

We previously isolated a 2-aminobenzoic acid (2-AA) from *Bacillus cereus* EJ-121, and confirmed its inhibitory effect on the growth of lettuce seedlings (Hoang et al., 2005). Here, we have focused on analyzing the mechanism of 2-AA as a growth inhibitor in *Arabidopsis thaliana*.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

We germinated 20 seeds of wild-type *A. thaliana* (L.) Heynh. (ecotype Columbia) and grew them in 100 × 100 × 15 mm square plates containing a full-strength Murashige and Skoog (MS; Murashige and Skoog, 1962) agar medium (pH 5.8) supplemented with 2% (w/v) sucrose. These plates were maintained in a growth chamber at 23°C, under a 12-h photoperiod provided by cool-white fluorescent tubes (photon flux density approx. 80 μE m^{-2} s^{-1}) (Kim and Lee, 2007).

Growth of Bacteria and Yeast

Escherichia coli and *Saccharomyces cerevisiae* were grown overnight in liquid LB and liquid YPD (yeast extract, peptone, and dextrose), respectively. They were then inoculated to fresh media containing various concentrations of 2-AA and incubated for 24 h further before their ODs were measured spectrophotometrically at 600 nm.

RESULTS AND DISCUSSION

We have now confirmed the inhibitory influence of 2-AA in *Arabidopsis* (Fig. 1). When 50 μM 2-AA was applied to the media from the beginning of seed germination, root elongation was significantly blocked (Fig. 1A). This effect was also observed during later growing stages (Fig. 1B). A concentration of 45 μM 2-AA was required to inhibit 50% (IC_{50}) of this *Arabidopsis* development. However, the growth of both *E. coli* and *S. cerevisiae* was not inhibited by treatment with 50 μM of 2-AA (Fig. 2). These microorganisms showed tolerance up to a concentration of 5 mM. Therefore, this deleterious effect seems to be specific to plants.

To determine whether this phenomenon was due to either the chemical structure of 2-AA itself, or its conversion to another metabolite, we tested two analogs of 2-AA -- 3-aminobenzoic acid (3-AA) and 4-aminobenzoic acid (4-AA). Applications of either did not inhibit *Arabidopsis* root growth at concentrations lower than 100 μM (Fig. 3). Therefore, this suggests that 2-AA is effective because of its conversion.

Normanly et al. (1993) have shown that applying 2-AA

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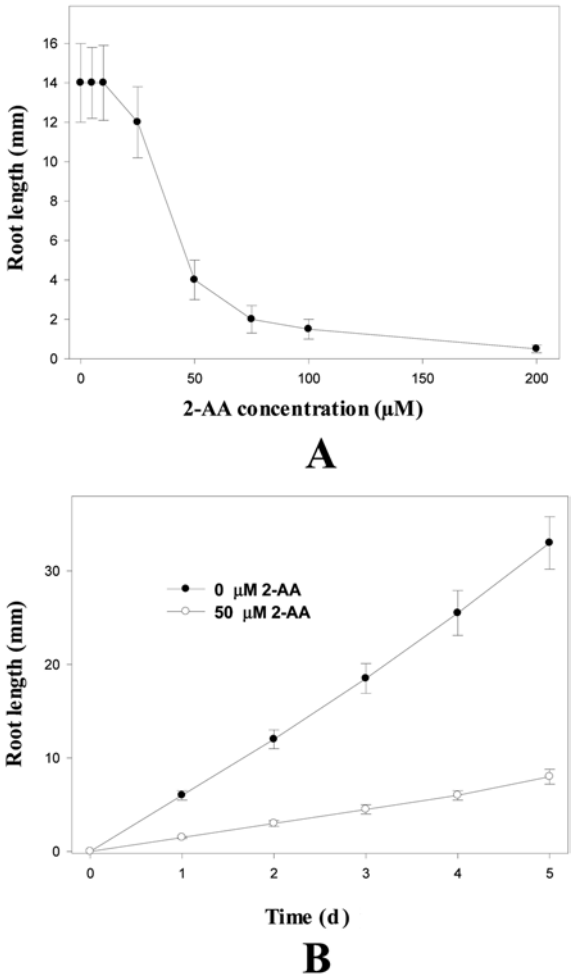

Figure 1. Inhibitory effect of 2-AA on root growth. (**A**) *Arabidopsis* seeds were germinated and grown for 6 d on MS media containing various 2-AA concentrations. Root lengths were measured from 6-d-old seedlings. (**B**) Six-d-old seedlings were transferred to fresh MS medium containing either 0 or 50 µM 2-AA, then incubated 5 d further. Root elongation was monitored by periodically measuring lengths. Values are means ± SE of 5 replicates.

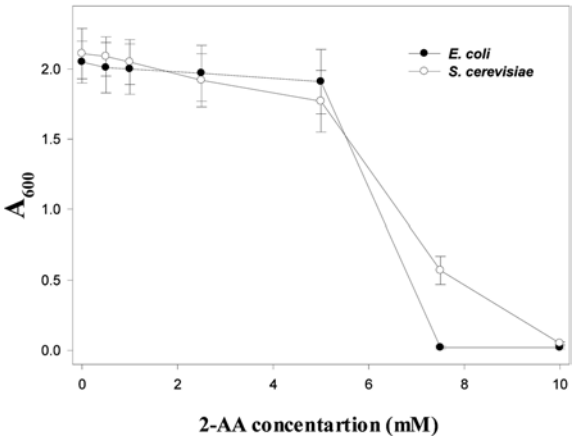

Figure 2. Effect of 2-AA on microbial growth. Bacteria and yeast were inoculated in liquid LB and YPD, respectively, and grown overnight until A_{600} values were near 2.0. Microorganisms were then transferred to fresh media and incubated for 24 h in various 2-AA concentrations. A_{600} was measured spectrophotometrically; values are means ± SE of 3 replicates.

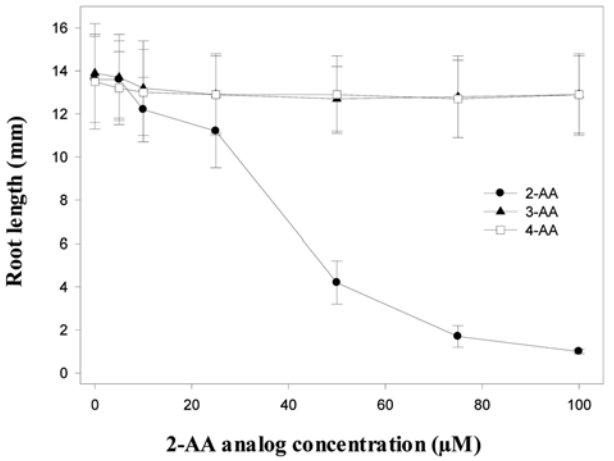

Figure 3. Effect of 2-AA analogs on root growth. *Arabidopsis* seeds were germinated and grown for 6 d on MS media containing various concentrations of 2-AA analogs. Roots were measured from 6-d-old seedlings; values are means ± SE of 5 replicates.

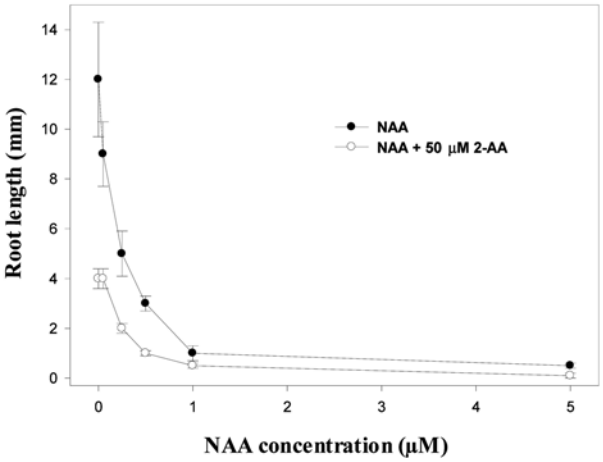

Figure 4. Inhibitory effect of NAA on root growth. *Arabidopsis* seeds were germinated and grown for 6 d on MS media containing various NAA concentrations. Roots were measured from 6-d-old seedlings; values are means ± SE of 5 replicates.

to *Arabidopsis* plants causes an increase in their level of indole-3-acetic acid (IAA) via the tryptophan-independent biosynthetic pathway. Thus the effect of 2-AA on plant growth again seems to result from its conversion to IAA. Here, we also treated seedlings with alpha-naphthaleneacetic acid (NAA) to confirm that auxin inhibits root growth under our experimental conditions (Fig. 4). The negative influence of NAA was slightly increased when it was applied in conjunction with 50 µM 2-AA. This suggests that the 2-AA is converted to IAA, such that the auxin effect is further enhanced.

It is thought that plants take up exogenous 2-AA from the roots and transport it to the leaves, where it is converted into IAA. This newly synthesized IAA is then moved to the roots, where elongation is inhibited. In a separate experiment, we added an auxin transport inhibitor, 2,3,5-triiodobenzoic acid (TIBA), to the growth medium at the beginning of seed germination, and found that the inhibitory effect of 2-AA was significantly reduced (Fig. 5A). This also occurred

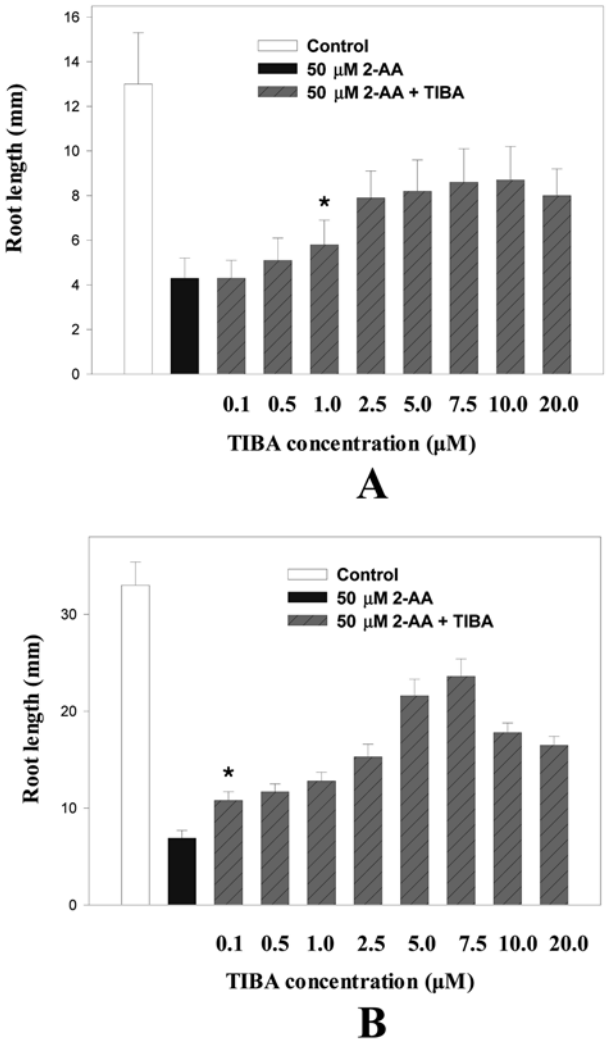

Figure 5. Auxin transport inhibitor TIBA induces recovery of root growth that was inhibited by 2-AA. (**A**) *Arabidopsis* seeds were germinated and grown for 6 d on MS media containing various concentrations of either 2-AA or 2-AA plus TIBA. Roots were measured from 6-d-old seedlings. (**B**) Six-d-old seedlings were transferred to fresh medium containing either 2-AA or 2-AA plus TIBA, then incubated 5 d further. Roots were measured at end of incubation; values are means ± SE of 5 replicates. Asterisk indicates significant differences (Student's *t* test; $P < 0.05$) for comparisons with 50 μM 2-AA treatment.

if we instead added the TIBA at the onset of seedling growth (Fig. 5B).

The inhibitory effect of auxin on plant root growth seems to be associated with an increase in ethylene biosynthesis (Yang and Hoffman, 1984; Abidur et al., 2001). Therefore, we also supplemented the growth medium with an ethylene action inhibitor, silver thiosulfate (Ag). With the addition of 100 μM Ag, we noted that the negative impact from 50 μM of 2-AA was strongly reduced (Fig. 6).

All of these results support the theory that the inhibitory effect of 2-AA on plant root growth follows from its conversion to IAA, and that this increased level of auxin leads to greater ethylene synthesis. This ethylene impairs root elongation and, in turn, retards overall plant development.

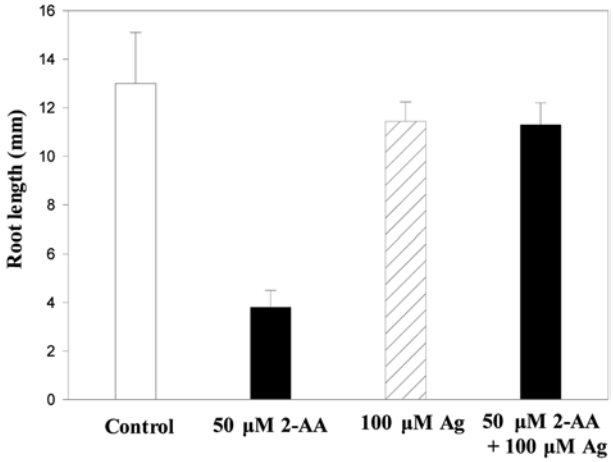

Figure 6. Ethylene action inhibitor Ag induces recovery of root growth that was impaired by 2-AA. (A) *Arabidopsis* seeds were germinated and grown for 6 d on MS media containing either 50 μM 2-AA or 50 μM 2-AA plus 100 μM Ag. Roots were measured from 6-d-old seedlings; values are means ± SE of 5 replicates.

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