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Suppression of Elongation and Growth of Tomato Seedlings by Auxin Biosynthesis Inhibitors and Modeling of the Growth and Environmental Response

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To develop a growth inhibitor, the effects of auxin inhibitors were investigated. Application of 30 μM L-α-aminooxy-β-phenylpropionic acid (AOPP) or (S)-methyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate (KOK1101), decreased the endogenous IAA levels in tomato seedlings at 8 days after sowing. Then, 10–1200 μM AOPP or KOK1101 were sprayed on the leaves and stem of 2–3 leaf stage tomato plants grown under a range of environmental conditions. We predicted plant growth and environmental response using a model based on the observed suppression of leaf enlargement. Spraying AOPP or KOK1101 decreased stem length and leaf area. Concentration-dependent inhibitions and dose response curves were observed. Although the effects of the inhibitors on dry weight varied according to the environmental conditions, the net assimilation rate was not influenced by the inhibitors. Accordingly, the observed decrease in dry weight caused by the inhibitors may result from decreased leaf area. Validation of the model based on observed data independent of the dataset showed good correlations between the observed and predicted values of dry weight and leaf area index.

S oeno et al.[1] reported that L–α–aminooxy–β–phenylpropionic acid (AOPP; $C_6H_5CH_2CH(ONH_2)COOH$) inhibited root development of Arabidopsis (*Arabidopsis thaliana*) through its effects on elongation of the main root, root gravitropism, and root hair formation, although this inhibition could be eliminated by exogenous application of indoleacetic acid (IAA). They also found that AOPP decreased the endogenous IAA levels in tomato and rice seedlings and acts as an inhibitor that directly blocks auxin biosynthesis. Since auxins regulate many processes during plant growth and development, auxin biosynthesis inhibitors are likely to have more effects than the inhibition of root development, and accordingly, are potentially useful new agrichemicals or plant growth regulators such as growth retardants. To develop a practical inhibitor for horticultural use, it is necessary to confirm the influence of these inhibitors on many plants. We also need to investigate suitable application techniques for when the inhibitor is applied as an agrichemical or plant growth regulator. It is not easy to apply inhibitors to the root zone, since commercial plants are grown in large volumes of soil or substrate or in nutrient solution. To develop a more practical application method, we applied the inhibitor by spraying the leaves and stem. Although AOPP inhibits auxin biosynthesis, AOPP is known as an inhibitor of phenylalanine ammonia-lyase (PAL)[2,3,4]. We have targeted to develop a new inhibitor that inhibits only auxin biosynthesis. We investigated a new compound that had a chemically-improved structure introducing a phthaloyl substituent on the chemically reactive aminooxy group of AOPP. Since plant growth may be influenced by many environmental factors, we investigated the combined influences of the inhibitors and of three environmental factors (light, temperature, and CO_2 level) on tomato growth.

It is well known that auxins affect plant elongation, since auxins promote the release of hydrogen ions from the plant cell and relax the stress on the cell wall[5,6,7]. Thus, an auxin biosynthesis inhibitor may also inhibit the cell's relaxation response, thereby inhibiting stem and leaf elongation. Since plant growth depends on dry matter production by the leaves, thereby increasing the photosynthate production capacity and availability, the inhibition of leaf enlargement could affect total dry matter production and thus, decrease plant growth. To develop a

Table 1 | Effects of the inhibitors spraying on the growth characteristics of tomato seedlings grown under different environmental conditions; solar radiation (233 μmol·m⁻²·s⁻¹ averaged PPFD), ambient CO_2 (370 μmol·mol⁻¹), and low temperature (18–11°C, day–night) at 21 days after sowing (LT-AC); fluorescent lamps (400 μmol·m⁻²·s⁻¹), ambient CO_2, and high temperature (30–25°C) at 16 days after sowing (HT-AC); fluorescent lamps, a high CO_2 concentration (900 μmol·mol⁻¹), and moderate temperature (23–17°C) at 20 days after sowing (MT-HC). SLA, specific leaf area; RGR, relative growth rate; NAR, net assimilation rate

Condition	Inhibitor (μM)		Aboveground dry weight (g per plant)		Leaf number (leaves per plant)		Stem length (cm per plant)		Leaf area (cm² per plant)		Dry matter content (g·g⁻¹)		SLA (m²·g⁻¹)		RGR (g·g⁻¹·d⁻¹)		NAR (g·m⁻²·d⁻¹)	
LT-AC	AOPP	100	0.048	**¹	3.0	NS	6.2	*	12.6	*	0.112	NS	0.026	NS	0.068	*	2.63	NS
	–	0	0.055		3.1		6.5		14.5		0.112		0.027		0.081		2.93	
HT-AC	AOPP	600	0.046	b²	2.8	b	6.5	c	9.0	b	0.107	a	0.021	b	0.192	b	7.88	a
	AOPP	100	0.061	a	3.3	a	6.0	b	16.0	a	0.101	b	0.026	a	0.259	a	10.43	a
	–	0	0.061	a	3.0	ab	7.7	a	14.3	a	0.097	b	0.024	ab	0.257	a	10.75	a
MT-HC	AOPP	100	0.059	a	3.9	a	4.6	c	9.7	c	0.144	a	0.017	b	0.17	a	8.72	a
	AOPP	10	0.069	a	4.0	a	4.7	bc	14.9	ab	0.109	c	0.022	a	0.19	a	8.80	a
	KOK1101	100	0.073	a	3.8	a	4.6	c	12.6	b	0.132	ab	0.018	b	0.20	a	10.27	a
	KOK1101	10	0.067	a	4.1	a	5.0	ab	15.6	a	0.103	c	0.025	a	0.19	a	8.23	a
	–	0	0.073	a	4.1	a	4.8	bc	15.1	a	0.108	c	0.022	a	0.20	a	8.80	a

¹NS: non-significant; * and ** indicate significant differences at the 0.05 and 0.01 levels, respectively, by t-test; n = 25 except for leaf area (n = 10).
²Values within a column followed by different letters differ significantly within the same condition (P < 0.05; ANOVA followed by Tukey's multiple-comparison test; n = 15 (HT-AC), or 20 (MT-HC)).

growth inhibitor suitable for practical horticultural use, and to investigate the direct and indirect effects of auxin biosynthesis inhibitors, we focused on the ability of these substances to decrease plant growth, and developed a model to predict growth with and without the inhibitors. Using the model, we tried to predict the growth of plants to which the inhibitors had been applied under a range of environmental conditions. We then validated the model by comparing its predictions with data observed independently of the data used to develop the model.

Results

Effects of auxin biosynthesis inhibitors on the endogenous IAA level. The endogenous IAA levels in the root of tomato seedlings applied with AOPP or KOK1101 were significantly lower than that of not-treated (Fig. 3). There was no significant difference in the levels in the root between AOPP and KOK1101 treatments. Although there was no significant difference in the IAA levels in the shoot of tomato seedlings, the levels applied with AOPP or KOK1101 were slightly lower than that of not-treated. The IAA levels of Arabidopsis seedlings applied with AOPP or KOK1101 were also significantly lower than that of not-treated. There was also no significant difference in the IAA levels of the Arabidopsis seedlings between AOPP and KOK 1101 treatments.

Effects of the inhibitors under different environmental conditions. Table 1 shows the growth characteristics of tomato seedlings sprayed with the inhibitors. Under LT-AC, aboveground dry weight, stem length, and leaf area were significantly lower in the plants sprayed with 100 μM AOPP than in 0 μM. There was no significant difference in the number of leaves, the dry matter content, or SLA. Although RGR was significantly lower in the sprayed plants, there was no significant difference in NAR. Accordingly, spraying 100 μM AOPP may not affect the assimilation efficiency but may instead decrease the growth in plant mass.

Under HT-AC, stem length was also significantly lower in plants sprayed with 600- or 100 μM AOPP than in plants sprayed with 0 μM (Table 1). Except for the stem length, there was no significant difference between the 100 μM and 0 μM AOPP sprays. The aboveground dry weight, stem length, leaf area, and RGR were significantly lower and dry matter content was significantly higher in the plants sprayed with 600 μM AOPP than in the other treatments. Since there was no significant difference in NAR among the treatments, spraying AOPP does not appear to affect the assimilation efficiency. The

difference in RGR therefore appears to result from decreased LAI rather than decreased NAR. These results also suggest that AOPP decreased the growth of plant mass without directly influencing the assimilation efficiency.

Under MT-HC, there was no significant difference in the aboveground dry weight, number of leaves, RGR, and NAR (Table 1). Stem length and SLA were significantly lower in plants sprayed with 100 μM KOK1101 than in plants sprayed with 10 μM of the inhibitor. Leaf area was significantly lower in plants sprayed with 100 μM AOPP than in plants sprayed with 100 μM KOK1101, but only the leaf areas and SLA in the plants sprayed with 100 μM AOPP or KOK1101were significantly lower than that in plants sprayed with 0 μM. The dry matter content in plants sprayed with 100 μM AOPP or KOK1101 was significantly higher than in plants sprayed with 0 μM. These results suggest that KOK1101 also decreases the growth of plant mass to almost the same extent as AOPP.

Modeling of growth and environmental responses of plants sprayed with the inhibitor. Figure 4 shows the averaged aboveground dry weight and leaf area against common logarithms of AOPP concentration. Both aboveground dry weight and leaf area decreased as increased AOPP concentration. Concentration-dependent inhibitions of the dry weight and leaf area were observed at range of 10–1200 μM AOPP. We obtained the regression lines of dry weight and leaf area that assumed the dose response curve ($r^2 = 0.990$ and 0.998, respectively). Since EC_{a50} (321) was lower than EC_{w50} (589), the leaf enlargement was inhibited at lower AOPP concentration. This result implied that the leaf enlargement was inhibited prior to decrease in dry matter production, and that the leaf enlargement inhibition could cause the inhibition of dry matter production.

Using our model, we predicted the plant growth with or without AOPP under different environmental conditions (i.e., the conditions in HT-AC and MT-HC). Figure 5 shows that dry weight and LAI decreased after spraying with AOPP, and that the magnitude of the decrease varied with the environmental conditions. The predicted dry weight was strongly and significantly correlated with the observed values ($r = 0.97$, $P < 0.01$). The predicted LAI was also strongly and significantly correlated with the observed data ($r = 0.89$, $P < 0.05$).

Table 2 shows prediction of aboveground dry weight and LAI with or without AOPP under low and high PPFD. Predicted aboveground dry weight and LAI with AOPP were lower than those without

Table 2 | Prediction of aboveground dry weight and leaf area index (LAI) with or without 100 μM AOPP spraying under low and high PPFD

PPFD[1] (mol·m⁻²·d⁻¹)	Days	AOPP 100 μM	Aboveground dry weight^y (g·m⁻² (%))		LAI[2] (m²·m⁻² (%))	
11.4	14	Application	91.2	(92)	1.9	(73)
		None	99.1	(100)	2.6	(100)
29.7	6	Application	91.8	(94)	1.7	(79)
		None	97.7	(100)	2.2	(100)

[1]50% or 130% of daily PPFD in HT-AC (400 μmol·m⁻²·s⁻¹ PPFD, 16-h day length, 370 μmol·mol⁻¹ CO_2, 30°C day, 25°C night).
[2]Dry weight and LAI at the start of spraying were used as the initial values in HT-AC.

AOPP. Percentages of them with AOPP were slightly lower under low PPFD than high PPFD.

Discussion

The AOPP or KOK1101 application to the seedlings decreased the endogenous IAA levels significantly in the tomato roots and Arabidopsis, and slightly in the tomato shoots (Fig.3). These indicate that KOK1101 also blocks auxin biosynthesis as well as Soeno et al.'s report[1] on AOPP treatment. The role of auxins on the promotion of plant cell elongation is well known[5,6,7]. However, Keller et al.[14] reported that the applications of auxins and of auxin transport inhibitor elevated the auxin level in leaves and then inhibited leaf expansions in bean and Arabidopsis. Controlling cell elongation and leaf expansion by auxins are complicated, and their mechanisms are still unclear. Although the mechanism of the inhibition by our auxin biosynthesis inhibitors and the active site of inhibitors also remain unclear, it appears that the inhibitors inhibit the leaf enlargement (Table 1, and Fig. 4). The increase in plant mass was limited both by AOPP and by KOK1101 in the present study (Table 1). These results suggest that KOK1101 functions similarly to AOPP. Although the endogenous IAA level of the seedlings decreased by AOPP or KOK1101 (Fig. 3), to determine the mechanism of the inhibition by the auxin biosynthesis inhibitors and their active site, it will be necessary to investigate by a biochemical approach.

Interactions between plant hormones such as auxin and ethylene influenced the stomatal conductance of leaves[15]. If the interaction and stomatal closure might occur by the auxin biosynthesis inhibitors in our experiment, the leaf photosynthetic rate, and thereby NAR might also decreased. However, since there was no significant difference in NAR in any of the three conditions (Table 1), it appears that these auxin biosynthesis inhibitors do not directly affect the assimilation efficiency. Although the effects of the inhibitors on growth characteristics such as dry matter content and RGR differed among the experimental conditions, spraying the inhibitors on the leaves and stem of tomato seedlings appears to decrease parameters such as stem growth and leaf area that lead to increased plant mass. Those results were also supported that EC_{a50} was lower than EC_{w50} in Figure 4 (i.e., the leaf enlargement decreased prior to decrease in dry matter production).

Soeno et al.[1] reported that adding 50 μM AOPP to Arabidopsis seedlings inhibited elongation of the main root, root gravitropism, and root skewing. Although elongation of the stem and leaves of tomato seedlings were inhibited by spraying the inhibitors on the leaves and stem in our experiment, we did not observe any inhibition of the effects of stem and leaf gravitropism. Additional research is necessary to determine whether this difference between Arabidopsis and tomato resulted from different responses of different plant parts (e.g., roots versus aboveground parts), interspecies differences in auxin metabolism, or differences in absorption of the biosynthesis inhibitors by different treatments. Although plants absorbed the inhibitor from the surface of whole plants including root zone in Soeno et al.'s study[1], the inhibitor only contacted the surface of the leaves and stem of the plants in our spraying experiments.

Root gravitropism results from differences in water permeability at the upper and lower sides of the root cells[16]. Auxins may regulate this process, and auxin biosynthesis inhibitors may therefore inhibit root gravitropism[1]. Recently, Takahashi et al.[17] reported that hypocotyl elongation was regulated by auxins through phosphorylation of the penultimate threonine. Although the molecular mechanisms responsible for elongation in response to auxins have been ascertained, this knowledge may be insufficient to support their practical use in crop production. This is because crops are produced under a wide range of environmental conditions, and as the present results show, different conditions may produce different results. Thus, even if plant elongation could be regulated by a biosynthesis inhibitor, the effect on plant growth and development would be strongly affected by differences in factors such as light, temperature, and CO_2. Our results confirm the importance of environmental factors, since the effects of the biosynthesis inhibitors on dry weight and RGR differed in the three experiments under different environmental conditions, although decreased aboveground biomass was observed under all three experimental conditions (Table 1). To apply the inhibitors in crop production, it will be necessary to investigate the plant responses to the inhibitors under a wider range of environmental conditions than those in the present study.

We modeled the suppression of elongation and of plant mass and dry matter production by auxin biosynthesis inhibitors. Our model of the suppression of leaf enlargement was able to predict the decrease in dry weight and LAI of plants sprayed with AOPP under the different environmental conditions (Fig. 5). However, the predicted values were slightly lower than the observed values for all combinations of AOPP application, temperature, and CO_2 level; the slopes of the regression lines for dry weight and LAI were 0.93 and 0.87, respectively. Since SLA was lower under MT–HC than under HT–AC (Table 1), the change in SLA, which we defined as v_1 in the model, may also have affected the results. The results might be because our model did not account temperature and CO_2 level, though improvement on NAR under MT–HC was not observed (Table 1).

The model successfully predicted that the growth suppression by AOPP would be more prominent under low light than under high light (Table 2). Our results suggest that elongation in response to auxins is more advantageous for plant growth under conditions that lead to low production of dry matter, such as low light intensity, than under conditions that lead to high production of dry matter, such as high light intensity, since dry matter production would reach its upper limit under high light intensity, all other conditions being equal. Our models would therefore be useful to support practical
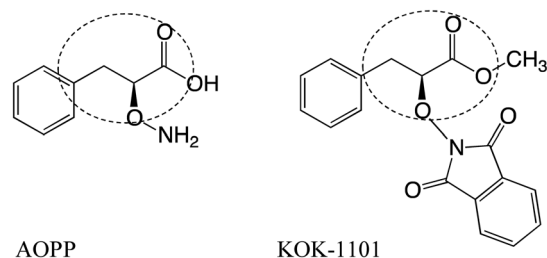

Figure 1 | The auxin biosynthesis inhibitors used in the present study: L–α–aminooxy–β–phenylpropionic acid (AOPP), and the new compound with partially similar backbone (dashed circle): (S)-methyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate (KOK1101).
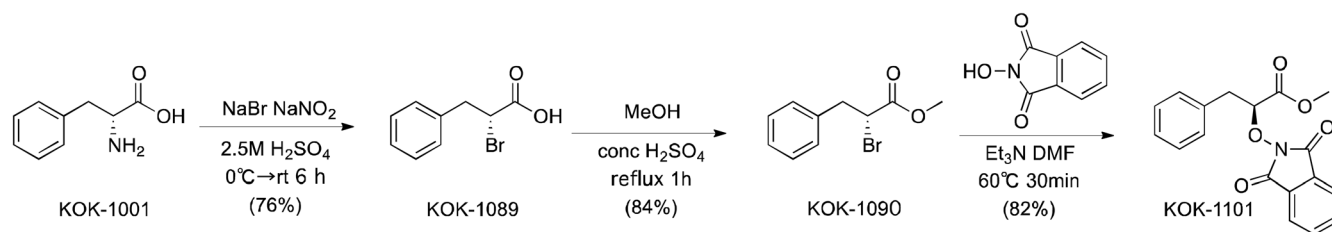
Figure 2 | Synthesis of KOK1101; (S)-methyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate.

application of the inhibitors and to investigate the function of auxins in plant growth and environmental responses.

Conclusions

Based on the present results, we conclude that the auxin biosynthesis inhibitors AOPP and KOK1101 decreased the endogenous IAA levels in tomato seedlings, and that spraying of them on the leaves and stem of tomato plants at 2–3 leaf stage can decrease the growth of plant mass by decreasing parameters such as stem length and leaf area; The concentration-dependent inhibition by AOPP is observed. However, the efficiency of dry matter production (here, measured as NAR) was not affected by the inhibitors. Accordingly, AOPP or KOK1101 inhibited the leaf enlargement prior to decrease in dry matter production. Then, total aboveground dry weight and RGR decreased by decrease in the leaf area. The dry weight and LAI predicted by our model based on the suppression of leaf and stem enlargement were significantly correlated with the observed values using a dataset independent of the one used to develop the model. Thus, the model successfully predicted plant growth and under the suppression effect of the inhibitors under a range of environmental conditions.

Methods

Auxin biosynthesis inhibitors. *Effects of auxin biosynthesis inhibitors on the endogenous IAA level.* As auxin inhibitors, we tested AOPP (MW 181.19; Wako, Osaka, Japan) and a new compound ((S)-methyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate; KOK1101) (Fig. 1)[8]. The compound KOK1101 was synthesized as described later. To confirm that the compounds act as an auxin biosynthesis inhibitor, we applied the inhibitors to seedlings and measured endogenous IAA levels of the seedlings. Tomato seeds (*Solanum lycopersicum* 'Momotaro York', Takii, Kyoto, Japan) were germinated in the dark for 24 h at 30°C. The seedlings were grown on 0.8% agar for 7 days, at a 16-h day length, and temperatures of 24 and 20°C (day and night). Then, the seedlings were transferred to a culture tube containing water on rotary shaker (60 rpm) at the same condition for 1 day, and were treated with the inhibitors (AOPP, or KOK1101) at 30 μM for 3 h. The seedlings were divided into aerial part and root, and IAA extraction and quantitative analysis was performed by LC-MS/MS using $[^2H_5]$-IAA as an internal standard as described by Soeno et al.[1] with minor modifications. We also applied 30 μM AOPP or KOK1101 to Arabidopsis seedlings grown in a half-strength MS liquid medium, and measured the endogenous IAA levels of the seedlings with the same method.

Synthesis of the new compound; (S)-methyl 2-((1,3-dioxoisoindolin-2-yl)oxy)-3-phenylpropanoate (KOK1101). The compound; KOK1101, was synthesized from D-(+)-phenylalanine (KOK1001) via (S)-2-bromo-3-phenylpropanoic acid (KOK1089) and (S)-methyl 2-bromo-3-phenylpropanoate (KOK1090) (Fig. 2). KOK1001 (5.00 g, 30.27 mmol) and sodium bromide (12.67 g, 105.94 mmol) were dissolved in 2.5 M sulfuric acid (39 mL) and stirred. Sodium nitrate (2.61 g, 37.84 mmol) aqueous solution (3 mL) was added dropwise to the mixture and stirred for 1 h at 0°C following 6 h at rt. The reaction mixture was extracted with ethyl acetate three times, washed with saturated sodium chloride, and the organic phase was dried over anhydrous sodium sulfate. The crude product was filtered and concentrated under reduced pressure, and the residue was purified with a silica gel column chromatography (hexane:ethyl acetate:acetic acid = 50:50:1) to give KOK1089 (5.26 g, 76%, colorless oil). To the solution KOK1089 (4.39 g, 19.17 mmol) in methanol (38 mL), 0.6 mL sulfuric acid was added to the solution and refluxed for 1 h. The corresponding methyl ester in methanol was concentrated and the residue was purified by a silica gel column chromatography (hexane:ethyl acetate = 10:1) to give KOK1090 (3.90 g, 84%, colorless oil). KOK1101 was synthesized according to methods described by Moumne et al.[9] KOK1090 (2.66 g, 10.94 mmol), N-hydroxyphtalimide (2.0 g, 10.94 mmol) and trietylamine (1.22 g, 12.04 mmol) was dissolved in N,N-dimethylformamide (DMF; 10 mL) and stirred at 60°C for 30 min. Water was added to the solution and extracted with ethyl acetate for 3 times, washed with water for 3 times, washed with saturated sodium chloride, and the organic phase was dried over anhydrous sodium sulfate. The resulting suspension was filtered and concentrated under reduced pressure, and then the residue was purified by a silica gel column chromatography (gradient, hexane:ethyl acetate = 3:1, 1:1, 0:1) to yield KOK1101 (2.92 g, 82%, white solid); ESI-MS *m/z* calcd for $C_{18}H_{16}NO_5$ ($[M^+ H]^+$) 326.1, found 326.1.

Effects of spraying auxin biosynthesis inhibitors on tomato seedlings grown under different environmental conditions. *Preparation of tomato seedlings.* Tomato seeds were sown on wet filter paper at 30°C, and maintained for 2 days in the dark. They were then transplanted at a density of 1600 plants·m⁻² into seedling trays (288 holes per tray, 450 × 900 mm) that contained granulated rockwool (Rock-fiber 66R, Nittobo, Tokyo, Japan). The trays were placed in a seedling growth chamber (Seedling Terrace, MKV Dream, Tokyo, Japan). The plants were fertilized from below the trays using High-Tempo nutrient solution (Sumitomo Chemicals, Tokyo, Japan); it consisted of 10.7 mM NO_3^-, 6.3 mM K^+, 5.4 mM Ca^{2+}, 1.9 mM Mg^{2+}, 2.4 mM $H_2PO_4^-$, 3.8 mg L⁻¹ Fe, 0.38 mg L⁻¹ Mn, 0.26 mg L⁻¹ B, 0.15 mg L⁻¹ Zn, 0.05 mg L⁻¹ Cu, and 0.07 mg L⁻¹ Mo) adjusted to 1.8 dS m⁻¹ electric conductivity every 2 days. The experiments used a complete randomized block design (CRBD) in two or three blocks.

Natural light, low temperature, and ambient CO₂ level (LT-AC). The seedlings were sown on 21 January 2010, and illuminated with fluorescent lamps, using a 16-h day length and a photosynthetic photon flux density (PPFD) of 397 ± 39 μmol·m⁻²·s⁻¹ (mean ± SD), 900 μmol·mol⁻¹ CO₂, and air temperatures of 30 and 23°C (day and night). Six days after sowing, the trays were moved into a glasshouse (18 m in length, 8 m in width, and 4 m in height) at the National Agriculture and Food Research Organization's Institute of Vegetables and Tea Science (Taketoyo, Aichi, Japan). The air temperature in the greenhouse at which
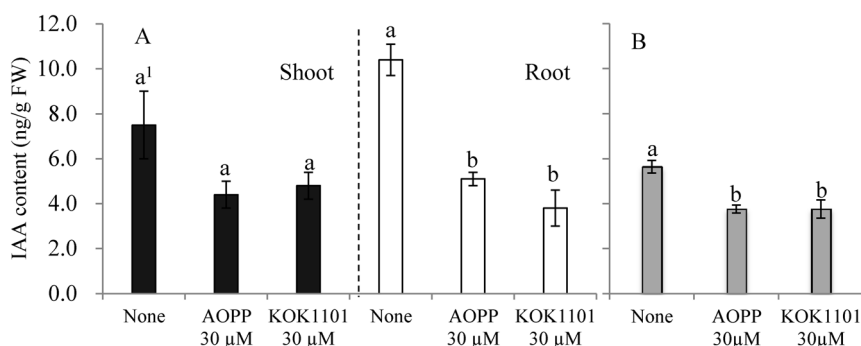

Figure 3 | Effects of AOPP or KOK1101 on endogenous IAA levels in shoot and root of tomato seedlings (A) and in Arabidopsis seedlings (B), at 8 days after sowing. [1]Different letters indicate significant differences within the same plant part at $P < 0.05$ by ANOVA followed Tukey's multiple comparison test ($n = 3$ (A), 4–10 (B)). The error bars show SEs.
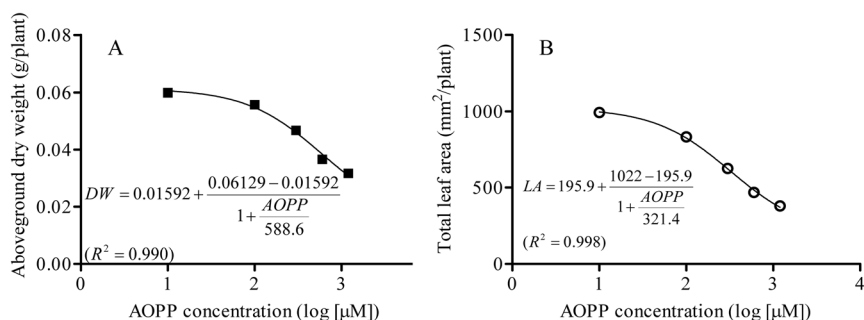
Figure 4 | Effects of AOPP concentration on (A) the aboveground dry weight and (B) leaf area of tomato seedlings grown under fluorescent lamps (368 μmol·m^{-2}·s^{-1}), ambient CO$_2$ (370 μmol·mol^{-1}), and moderate temperature (23°C day, 17°C night) at 16 days after sowing. The regression line assumed the dose response curve with a standard slope (Equations 8 and 9); EC_{w50} and EC_{a50} are 589 and 321, respectively; $n = 20$.

heating began was set at 13°C. The root zone of the seedlings was also heated directly using electrical heating wires to maintain a temperature greater than 10°C. Otsuka-A nutrient solution (Otsuka AgriTechno, Tokyo, Japan; it consisted of 9.3 mM NO^{3-}, 4.3 mM K$^+$, 4.1 mM Ca^{2+}, 1.5 mM Mg^{2+}, 0.9 mM H$_2$PO$_4^-$, 2.7 mg L^{-1} Fe,1.2 mg L^{-1} Mn, 0.51 mg L^{-1} B, 0.09 mg L^{-1} Zn, 0.03 mg L^{-1} Cu, and 0.03 mg L^{-1} Mo) adjusted to 1.0 dS·m^{-1} electrical conductivity was provided to the plants daily from below the trays. Air and root temperatures, solar radiation, and PPFD were measured with thermocouples, a pyranometer (LI-200SB, LI-COR, Lincoln, NE, USA), and a quantum sensor (LI-190SB, LI-COR), respectively. These data were recorded at 2-min intervals by a datalogger (GL-200, Graphtech, Yokohama, Japan). The mean day and night temperatures, solar radiation, and PPFD during the experimental period were 18.1 and 11.3°C, 4.0 MJ·m^{-2}·d^{-1}, and 8.0 mol·m^{-2}·d^{-1} (ca. 9.5 h day length, 233 μmol·m^{-2}·s^{-1}), respectively.

Between 10:00 and 11:00 each day at 11 to 13 and 18 to 20 days after sowing, we sprayed the leaves and stem of each plant with ca. 17 mL 100 μM (12.1 μg actual mass of active ingredient (a.i.) per plant) or 0 μM AOPP, in three blocks with 84 plants per block and 25 plants per treatment (control versus auxin biosynthesis inhibitors) in each block. AOPP was dissolved in dimethyl sulfoxide (DMSO, [CH$_3$]$_2$SO; Wako) and diluted to 100 μM in water. These solutions were prepared just before each spraying to prevent changes in their properties.

At 10 and 21 days after sowing, 25 plants in each treatment (one block) were sampled. We measured the number of leaves (>5 mm length), stem length, fresh and dry aboveground weight (total per plant), and the dry matter content (g dry weight/g fresh weight for the aboveground plant parts). We also measured the leaf area of 10 plants per treatment by scanning with a GT–9300UF flatbed scanner (Epson, Tokyo, Japan) and image analysis (LIA32 ver.0376 β1, Yamamoto, Nagoya Univ.). We calculated the relative growth rate (RGR) and net assimilation rate (NAR) using the following equations:

$$RGR = \{\ln(W_2) - \ln(W_1)\}/(t_2 - t_1) \tag{1}$$

$$NAR = (W_2 - W_1)/(t_2 - t_1)\cdot\{(\ln(A_2) - \ln(A_1))/(t_2 - t_1)\} \tag{2}$$

where W_1 and W_2 represent the aboveground dry weight (g) at times t_1 and t_2, respectively, and A_1 and A_2 represent the leaf area (m^2) at t_1 and t_2, respectively.

Fluorescent lamps, high temperature, and ambient CO$_2$ (HT-AC). Tomato seeds were sown in the three seedling trays on 9 February 2010 and placed in the growth chamber under fluorescent lamps, with a 16-h day length, ca. 400 μmol·m^{-2}·s^{-1} PPFD, 370 μmol·mol^{-1} CO$_2$, and air temperatures of 30 and 25°C (day and night). We used four treatment solutions (AOPP [μM]–DMSO [mM]): 600–469, 100–78, and 0–469, with three blocks and 25 plants per treatment in each block. The solutions were prepared just before each spraying, and we sprayed ca. 17 mL on the leaves and stem of the plants (600–469, 72.5 (μg a.i. per plant); 100–78, 12.1) in each treatment daily between 10:00 and 11:00 for 6 days, starting 10 days after sowing. At 10 and 16 days after sowing, we measured 15 plants in each treatment using the same approach described in LT-AC.

Fluorescent lamps, moderate temperature, and high CO$_2$ (MT-HC). Tomato seeds were sown in the three seedling trays on 28 July 2010 and placed in the growth chamber with fluorescent lamps, a 16-h day length, ca. 400 μmol·m^{-2}·s^{-1} PPFD, 900 μmol·mol^{-1} CO$_2$, and air temperatures of 23 and 17°C (day and night). We prepared five treatment solutions just before each spraying: 100 μM AOPP (78 [mM DMSO]), 10 μM AOPP (8), 100 μM KOK1101 (78), 10 μM KOK1101 (8), and 0 μM inhibitor (156), with two blocks and 25 plants per treatment in each block. From 10:00 to 11:00 each day for 6 days, starting 14 days after sowing, we sprayed ca. 25 mL of each treatment solution on the leaves and stem of the plants (100 μM AOPP, 18.1 (μg a.i. per plant); 10 μM AOPP, 1.8; 100 μM KOK1101, 32.5; 10 μM KOK1101, 3.3), and 0 μM inhibitor in each treatment. At 13 and 20 days after sowing, we measured 20 plants in each treatment using the same approach described in LT-AC.

Modeling of growth and environmental responses of plants sprayed the inhibitor. *Modeling of the plant growth and growth suppression.* The increase in leaf area index (LAI, m^2·m^{-2}) can be described using the following equation:

$$dA/dt = v_l\cdot dM/dt \tag{3}$$

where A represents LAI (m^2·m^{-2}), M represents dry matter weight per area (g·m^{-2}), and v_l represents the rate of increase in LAI per unit dry matter (m^2·g^{-1}).

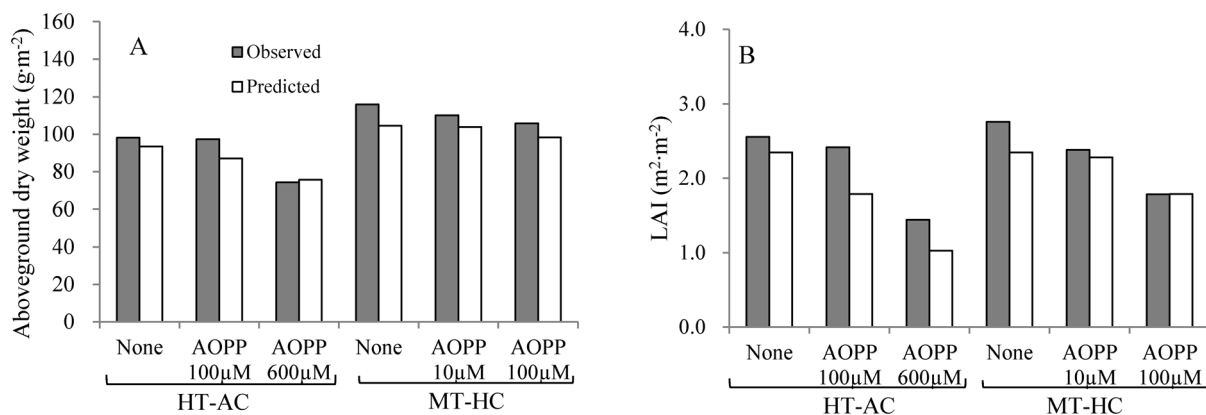We described the suppression of leaf enlargement (A_i) using the following equation:

Figure 5 | Predicted and observed (A) total aboveground dry weight and (B) leaf area index (LAI) in tomato plants sprayed with AOPP and non-sprayed plants. HT-AC, high temperature, and ambient CO$_2$ (370 μmol·mol^{-1} CO$_2$, 30°C day, 25°C night); MT-HC, moderate temperature, and high CO$_2$ (900 μmol·mol^{-1} CO$_2$, 23°C day, 17°C night).

$$dA_i/dt = i \cdot v_l \cdot dM/dt \tag{4}$$

where i represents the suppression coefficient.

Since light interception by plants is determined by LAI and the light extinction coefficient within the canopy, dry matter production by plants can be described using the following equation[10]:

$$dM_p/dt = LUE \cdot (1 - e^{-kLAI}) \cdot Sr \tag{5}$$

where M_p represents potential dry matter weight (i.e., the level with no down-regulation of photosynthesis), LUE represents the light-use efficiency (g·mol^{-1} PPFD), k represents the light-extinction coefficient, and Sr represents PPFD (μmol·m^{-2}·s^{-1}).

Plants grown under elevated CO_2 or high light levels show a down-regulation of photosynthesis[11]. In this phenomenon, the photosynthetic rate may decrease due to an excessive accumulation of photoassimilate in the leaves[12], leading to decreased dry matter production. The potential dry matter production represents the dry matter production under the assumption of no restriction by this down-regulation of photosynthesis. We assumed that the dry matter production was decreased by photoassimilate accumulation in this experiment, and that the assimilate reservoir and its utilization rate were determined by plant size. Accordingly, the upper limit of the growth rate in our model may increase with increasing plant weight. Thus, the limit would be higher in large plants than in small plants. The limit of dry matter production can be described using the following equation:

$$l = m \cdot M \tag{6}$$

where l represents the upper limit of dry matter production (g·m^{-2}·d^{-1}), m represents a coefficient for the upper limit of dry matter production that is related to the reservoir size and the utilization rate of assimilate (g·g^{-1}·d^{-1}), and M represents dry weight (g·m^{-2}). We assumed that actual dry matter production (M; g·m^{-2}) can be described using the following equations:

$$\text{If } l \cdot \left(1 - e^{-dM_p/dt}\right) \geq dM_p/dt, \text{ then } dM/dt = dM_p/dt$$

$$\text{If } l \cdot \left(1 - e^{-dM_p/dt}\right) < dM_p/dt, \text{ then } dM/dt = l \cdot \left(1 - e^{-dM_p/dt}\right) \cdot dM_p/dt \tag{7}$$

Dose response relationship between the AOPP concentration and plant enlargement. To obtain the suppression coefficient; i, in the equation [4], we investigated relationship between the AOPP concentrations and plant enlargement. Tomato seedlings were grown in the growth chamber under fluorescent lamps, with a 16-h day length, ca. 368 μmol·m^{-2}·s^{-1} PPFD, 370 μmol·mol^{-1} CO_2, and air temperatures of 23 and 17°C (day and night). The experiment was conducted using a CRBD with two blocks and 25 plants per treatment in each block. We used six treatment solutions; 0, 10, 100, 300, 600, and 1200 μM AOPP with 391 mM DMSO. The solutions were prepared just before each spraying, and were sprayed ca. 25 mL on the leaves and stem of the plants (0, 1.8, 18.1, 54.4, 108.7, and 217.4 μg a.i. per plant) in each treatment daily between 10:00 and 11:00 for 6 days, starting 10 days after sowing. At 10 and 16 days after sowing, we measured 10 plants in each treatment using the same approach described in LT-AC. We obtained following regression lines that assumed a dose response curve with standard slope based on the averaged aboveground dry weight and leaf area.

$$W_p = B_w + (T_w - B_w)/(1 + C/EC_{w50}) \tag{8}$$

$$A_p = B_a + (T_a - B_a)/(1 + C/EC_{a50}) \tag{9}$$

where W_p and A_p represent the aboveground dry weight (g) and total leaf area (mm2) per plant, respectively, and B_w and B_a represent the maximally inhibited response of the dry weight and leaf area, respectively, and T_w and T_a represent the maximal response of the dry weight and leaf area, respectively. C represents AOPP concentration (mM); EC_{w50} and EC_{a50} represent half maximal effective concentration on the dry weight and leaf area, respectively.

Validation of the model and growth prediction under low and high light level. We post-predicted the effects of AOPP spraying on plant growth under similar light conditions in HT-AC and MT-HC. We obtained the parameters of this model from data under LT-AC, as follows. Since the rate of leaf area increase at 100 μM AOPP was 0.74 times the value at 0 μM AOPP in LT-AC, we defined $i = 0.74$ as the suppression coefficient for 100 μM AOPP, $i_{0.1} = 0.97$, and $i_6 = 0.359$ as the coefficients for 10 and 600 μM AOPP, respectively, based on the dose response curve; the equation [9]. We calculated $LUE = 0.795$ g·mol^{-1} as the slope of a linear regression for the total cumulative dry matter production as a function of the cumulative intercepted photosynthetic photon flux at the two sampling dates in LT-AC. Based on data from Higashide and Heuvelink[13], we defined the light-extinction coefficient as $k = 0.8$. We defined $m = 0.260$ (g·g^{-1}·d^{-1}) as the coefficient for the upper limit of dry matter production by reference to the maximum RGR of the tomato plants in our study

(Higashide, unpublished data). We also defined the specific leaf area (SLA, the leaf area per leaf biomass) at the start of spraying the inhibitor as v_l for each experimental condition. Dry weight and LAI at the start of spraying were used as the initial values in each condition.

Based on these parameters and the cumulative PPFD on each day in HT-AC and MT-HC, we predicted the total aboveground dry weight per area and LAI on each day. Influences of temperature and CO_2 level are not reflected directly in this model. To validate the model, we calculated Pearson's correlations between the predicted and observed dry weights and LAI values using a dataset that was independent from the one used to develop the model.

After the validation, we predicted aboveground dry weight and LAI with or without AOPP 100 μM under low and high PPFD. We assumed two light levels, 11.4 and 29.7 mol·m^{-2}·d^{-1}; those were equal to 50% and 130% of daily PPFD in HT-AC, respectively. The dry weight and LAI at the start of spraying were used as the initial values in HT-AC. The prediction was conducted until the dry weight reached ca. 90–100 g·m^{-2} in each light condition.

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Acknowledgments

Author contributions

T.H. wrote the main manuscript text and prepared tables 1–2, and figures 4–5. M.N. and Y.S. prepared figures 1–2. K.S. prepared figure 3. All authors reviewed the manuscript.

Additional information