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An Inhibitor of Tryptophan-Dependent Biosynthesis of Indole-3-Acetic Acid Alters Seedling Development in *Arabidopsis*

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Abstract Although polar transport and the TIR1-dependent signaling pathway of the plant hormone auxin/indole-3-acetic acid (IAA) are well characterized, understanding of the biosynthetic pathway(s) leading to the production of IAA is still limited. Genetic dissection of IAA biosynthetic pathways has been complicated by the metabolic redundancy caused by the apparent existence of several parallel biosynthetic routes leading to IAA production. Valuable complementary tools for genetic as well as biochemical analysis of auxin biosynthesis would be molecular inhibitors capable of acting in vivo on specific or general components of the pathway(s), which unfortunately have been lacking. Several indole derivatives have been previously identified to inhibit tryptophan-dependent IAA biosynthesis in an in vitro system from maize endosperm. We examined the effect of one of them, 6-fluoroindole, on seedling development of *Arabidopsis thaliana* and tested its ability to inhibit IAA biosynthesis in feeding experiments in vivo. We demonstrated a correlation of severe developmental defects or growth retardation caused by 6-fluoroindole with significant downregulation of de novo synthesized IAA levels, derived from the stable isotope-labeled tryptophan pool, upon treatment. Hence, 6-fluoroindole shows important features of an inhibitor of tryptophan-dependent IAA biosynthesis both in vitro and in vivo and thus may find use as a promising molecular tool for the identification of novel components of the auxin biosynthetic pathway(s).

Keywords Auxin biosynthesis · Inhibitor · *Arabidopsis thaliana* · Indole-3-acetic acid · 6-Fluoroindole

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Introduction

The auxin indole-3-acetic acid (IAA) is involved in regulatory events throughout plant growth and development. We have meanwhile acquired an evolving understanding of how IAA is transported and how its signal is perceived and translated into gene expression (Quint and Gray 2006; Delker and others 2008). However, despite recent findings (Zhao and others 2001; Cheng and others 2006; Tao and others 2008; Pollmann and others 2009), IAA biosynthesis is still unclear. This is most likely due to the complexity of the multiple pathways involved (Pollmann and others 2006) as well as other aspects of its metabolism such as conjugation and oxidative degradation (Cohen and Gray 2006). The amino acid tryptophan (Trp) has long been discussed as the primary precursor for many biosynthetic pathways. Biochemical and genetic studies have elucidated at least three different pathways from Trp to IAA, which might even be interconnected (Delker and others 2008). The indole-3-acetaldoxime (IAOx) pathway is associated with secondary metabolism, for example, indole glucosinolate synthesis in Brassicaceae (Halkier and Gershenzon 2006). It seems to involve conversion of Trp to IAOx by two cytochrome P450 enzymes (Zhao and others 2002). It has also been proposed that the conversion of Trp to IAOx is initiated by the step catalyzed by members of the

YUCCA family (Zhao and others 2001; Cheng and others 2006). Plants have the capacity to form indole-3-acetonitrile (IAN) directly from IAOx, and various nitrilase enzymes might convert it to IAA (Piotrowski 2008). Alternatively, the vast bulk of IAOx produced in *Arabidopsis*, for example, is converted to indole glucosinolate which eventually is metabolized by myrosinase following tissue damage to a number of products, including IAN (Halkier and Gershenzon 2006). Indole-3-pyruvic acid (IPyA) is present in *Arabidopsis* (Tam and Normanly 1998) and the conversion of Trp to IPyA has recently been shown (Stepanova and others 2008; Tao and others 2008). IPyA may be converted further to indole-3-acetaldehyde (IAAld) and finally to IAA by an aldehyde oxidase (Seo and others 1998). Additional evidence for a pathway mostly associated with microorganisms (via indole-3-acetamide) comes from work of Pollmann and others (2003, 2009) showing that an active amidase is present in *Arabidopsis.*

To complicate in vivo analyses further, in addition to the Trp-dependent biosynthesis, it has been discovered that a Trp-independent pathway may be operating in various plant species (Normanly and others 1995). The pathways from two different starting precursors have been associated with different developmental or stress programs such as embryogenesis, fruit ripening, or wounding (Michalczuk and others 1992; Epstein and others 2002; Sztein and others 2002). In maize and *Arabidopsis*, the potential Trp-independent biosynthesis pathway for IAA was associated with Trp synthesis mutants *orange pericarp* and *trp1*, respectively (Wright and others 1991; Normanly and others 1993). Recent studies have linked the tomato *sulfurea* mutant with Trp-independent IAA synthesis (Ehlert and others 2008). However, no genetically deficient mutant has been isolated so far for the core pathway of Trp-independent biosynthesis, although it has been reported that in vitro enzymatic activity from maize converted indole to IAA without a tryptophan intermediate (Östin and others 1999). It is clear from these findings and the apparent complexity of these processes that inhibitors for either pathway would be promising tools to dissect the two pathways biochemically. Likewise, inhibitors could be used as critical genetic tools to screen for the respective mutants in *Arabidopsis* and other plants.

Several potent in vitro inhibitors for Trp-dependent IAA synthesis were previously identified using enzyme extracts from maize (Ilic and others 1999). We have investigated the activity of two of the most promising potential inhibitors in vivo in *Arabidopsis* because its use as the reference species for plant biology makes it ideal for future biochemical and genetic studies of these pathways. Our experiments have identified an inhibitor for Trp-dependent IAA biosynthesis in *Arabidopsis* that also causes developmental defects and thus suggests that it is a candidate for selection of IAA biosynthetic mutants.

Materials and Methods

Plant Material and Growth Assays

All *Arabidopsis thaliana* seedlings employed in this study were in the Col-0 background (INRA AV186). Seeds were sterilized and imbibed/stratified at 4°C for 3 days prior to all reported assays. For the growth inhibition assays, seedlings were grown under sterile conditions on ATS nutrient medium (Lincoln and others 1990) under long-day lighting conditions. Seedlings were grown at 20°C on ATS medium supplemented with the indicated concentrations (Fig. 3) of 2-mercaptobenzimidazole (MBI), 6-fluoroindole (6-FI), 5-hydroxyindole (5-HI), or 2-phenylindole (2-PI) (all from Sigma, St. Louis, MO) (Fig. 1). The compounds were dissolved in ethanol and control growth measurements included the solvent. Growth parameters were analyzed as follows.

For the root growth assays, seeds were germinated on unsupplemented ATS medium for 5 days and then transferred to inhibitor/noninhibitor-containing ATS plates and grown for another 3 days. The root growth (in mm) from day 5 (day of transfer) to day 8 was measured and plotted as percent of root growth inhibition, that is, root growth on ATS + inhibitor compared to root growth on ATS only. For hypocotyl assays, seedlings were incubated at 20°C for the indicated period (Figs. 2 and 3) vertically on supplemented or unsupplemented ATS plates. Hypocotyl length was measured and plotted as percent hypocotyl growth inhibition compared to the unsupplemented control.

For the germination, cotyledon emergence, and true leaf formation assays, sterilized and stratified seeds were planted on supplemented or unsupplemented ATS medium and incubated horizontally at 20°C for the indicated period (Figs. 2 and 3). Seeds were designated as germinated when the seed coat was broken and the radical emerged. Cotyledon emergence and true leaf formation were plotted as percent in comparison to the unsupplemented control.

All reported seedling assays were repeated 4 times with similar results. One representative assay is shown.

Labeling Experiments

Arabidopsis thaliana seeds were germinated and grown in liquid MS medium (Murashige and Skoog 1962) as described by Ludwig-Müller and others (1993). To each flask the appropriate concentration (50–100 μM) of 6-FI dissolved in ethanol (to give a final ethanol concentration not exceeding 1%) was added to 7-day-old seedlings. Incubation time with the inhibitors was 3 or 6 h. After this time the labeled IAA precursor [$^2H_5$]Trp was added at a final concentration of 100 μM. The samples were incubated for an additional 12 h under continuous shaking of

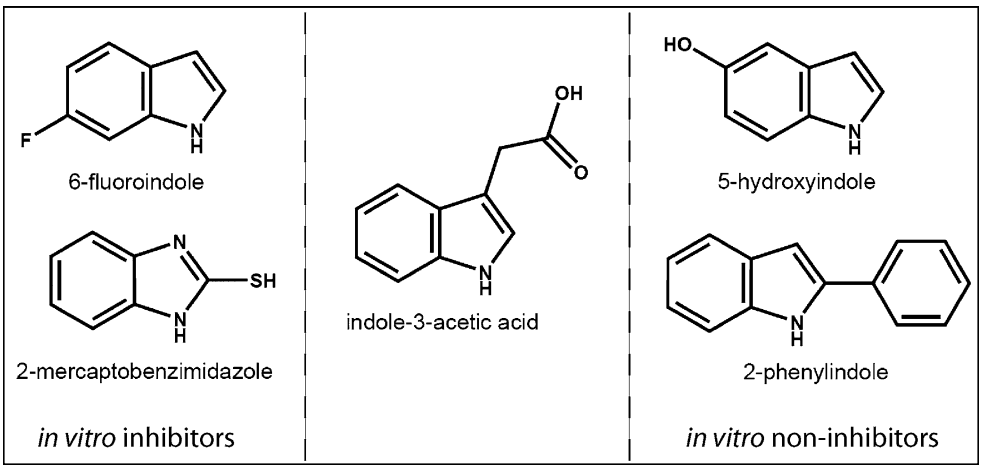

Fig. 1 Indole derivatives tested for in vivo inhibition of IAA biosynthesis. 6-fluoroindole (6-FI) and 2-mercaptobenzimidazole (MBI) have previously been shown to inhibit Trp-dependent IAA biosynthesis in vitro (Ilic and others 1999). 5-hydroxyindole (5-HI) and 2-phenylindole (2-PI) had no inhibitory effect in the same study and function as negative controls for the physiological assays

the flasks. The seedlings were then separated from the liquid culture medium by filtration. The plant material was washed thoroughly with water, the fresh weight was determined, and free IAA was extracted as previously described (Jentschel and others 2007). To correct for losses during the extraction process, 100 ng of [$^{13}C_6$]IAA was added. All isotope-labeled compounds were from Cambridge Isotope Laboratories (Andover, MA, USA). The samples were fractionated on amino (NH_2-) solid-phase extraction (SPE) columns as described by Chen and others (1988). Prior to GC–MS analysis, the samples were methylated with diazomethane (Cohen 1984). GC–MS analysis was carried out on a Varian Saturn 2000 system (Varian Deutschland GmbH, Darmstadt, Germany) according to Jentschel and others (2007). The mass spectrometry settings were as described in Campanella and others (2003). Enrichment of IAA was calculated according to Jentschel and others (2007). Statistical data analysis was carried out using unpaired Student's *t*-test analysis and significance at the $p \leq 0.05$ or at the $p \leq 0.01$ level is indicated.

Glucuronidase Histochemical Staining

For promoter-GUS studies, 6-day-old seedlings were mock, 1-NAA, or 6-FI treated for 5 h and then stained overnight for GUS activity, as previously described (Stomp 1991; Quint and others 2009). For these experiments, transgenic seeds carrying the DR5:GUS construct from Ulmasov and others (1997) were used.

Results and Discussion

As discussed in the Introduction, IAA biosynthesis in *Arabidopsis* most likely is achieved by numerous parallel biosynthetic pathways (Delker and others 2008). This results in functional redundancy of the different routes and may be responsible for significant gaps in our knowledge of this branch of auxin biology. A valuable tool for biochemical and/or genetic approaches to study IAA biosynthesis would be the availability of substances that are readily taken up by the plant and interfere with aspects of IAA biosynthesis. Such pharmacological inhibitors are available for two other levels of auxin biology, transport (Keitt and Baker 1966; Geldner and others 2001) and signaling (Hayashi and others 2008), where they have formed a valuable basis for advances in these areas.

A few indole analogs have been reported to act as antagonists for Trp-dependent IAA biosynthesis, without affecting Trp biosynthesis itself, in an in vitro system using maize liquid endosperm enzyme preparations (Ilic and others 1999). However, these compounds have not been examined in vivo and nothing is known about possible effects on developmental phenotypes. The most potent in vitro inhibitor MBI and the second most potent inhibitor 6-FI (Fig. 1) (Ilic and others 1999) were first examined for possible effects on seedling development.

Effects on Seedling Development

We performed a number of seedling assays on media supplemented with MBI or 6-FI. To assess whether possible phenotypes are caused by potential inhibitory effects on IAA biosynthesis or are rather the result of nonspecific secondary or toxic effects, we included 5-HI and 2-PI (Fig. 1) as negative controls in the same physiological assays. 5-HI and 6-PI are similar indole derivatives that have been shown to have no inhibitory effect on auxin biosynthesis in maize endosperm (Ilic and others 1999). In general, MBI behaved similar to the negative controls. 4-hydroxyindole, another compound that exhibited inhibitory effects similar to 6-FI in the in vitro study (Ilic and others 1999), showed no in vivo effects on plant

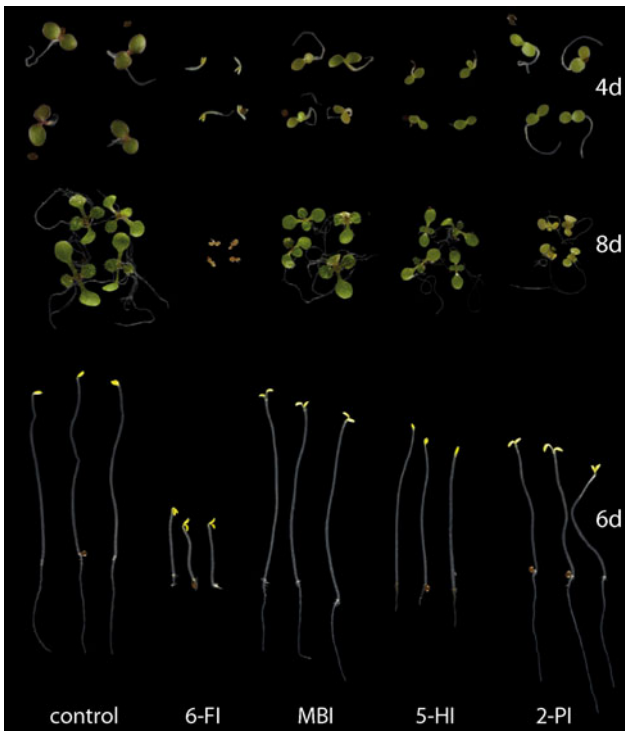

Fig. 2 Developmental defects caused by exogenous 6-FI. Seedlings have been germinated on horizontal plates supplemented with 250 (*upper panel*) or 50 μM (*middle* + *bottom*) of potential inhibitors for the indicated period. *Bottom* Seedlings have been germinated for 6 days on vertical plates in the dark

development (data not shown). However, 6-FI revealed significant effects on seedling development, some of them dramatic (Fig. 2). This is in agreement with data from Widholm (1981), who showed inhibition of carrot and tobacco cell growth in suspension cultures.

Although germination rates and cotyledon emergence were inhibited by 6-FI only at very high concentrations (250–500 μM) (Fig. 3a, b), 6-FI caused more severe defects for other developmental phenotypes. Formation of true leaves after 8 days was inhibited and completely blocked by applying 50 and 100 μM 6-FI, respectively (Fig. 3c). Root elongation was blocked at 50 μM (Fig. 3d), and hypocotyl elongation in the light as well as in the dark was significantly more severely inhibited by 6-FI than by the other three substances (Fig. 3e, f). Because root and hypocotyl elongation are strictly auxin-dependent, these effects provide strong evidence for causal correlations to inhibited IAA biosynthesis. Taken together, although MBI had clear inhibitory effects in previous in vitro experiments on IAA biosynthesis (Ilic and others 1999), we could not identify any developmental defects caused by MBI that were significantly different from the noninhibitor negative controls. MBI-caused defects are therefore most likely not due to possible in vivo inhibition of IAA biosynthesis, but rather to unknown secondary or even toxic effects or alternatively to inefficient uptake into the plant (which was not tested in this study). On the other hand, 6-FI caused dramatic developmental defects in several seedling assays compared to MBI and the negative controls. Among the defects were some known to be dependent on sufficient levels of endogenous IAA. It is generally possible that the fluorine is split from 6-FI *in planta* and the described phenotypes are due to F toxicity. However, fluorine is the most electronegative element in the periodic table. When bound to carbon, as in the case of 6-FI, it forms the strongest bonds in organic chemistry, making it highly unlikely that F is split *in planta* from 6-FI (O'Hagan 2008). Furthermore, we have tested the influence of NaF as a control for the potentially split F on root and hypocotyl elongation and detected no inhibitory effect (data not shown). Based on these observations, 6-FI appears to have potential as a promising tool for genetic approaches using physiological assays in a mutant screen, for example, and thus was subsequently examined for its in vivo inhibitory effects on IAA biosynthesis in labeling experiments.

Several of the observed phenotypes (such as root elongation) are also known to be caused by inhibitory concentrations of auxin itself. It is therefore possible that the indole analog 6-FI possesses auxinic features, which would mean that the described defects are not due to a possible interference with auxin biosynthesis. Instead, they could constitute auxin response phenotypes. To address this question we tested whether 6-FI is able to trigger the expression of the DR5:GUS auxin reporter construct (Ulmasov and others 1997). Although the synthetic auxin NAA induces auxin-responsive GUS expression, various concentrations of 6-FI are unable to do so (Fig. 4). Hence, we can rule out an auxinic function of 6-FI itself.

Effects on Trp-Dependent IAA Biosynthesis

In the maize in vitro system, several indolic substances were identified as potent inhibitors of IAA synthesis (Ilic and others 1999), among them the compounds tested in our study on seedling growth. In maize the most potent inhibitor of Trp-dependent IAA synthesis was MBI, followed by 5-fluoroindole, 5-chloroindole, and 6-FI, which inhibited IAA synthesis by about 60%. However, only 6-FI and MBI did not significantly decrease Trp synthesis itself (Ilic and others 1999).

Having demonstrated that exogenous 6-FI, but not MBI, caused significant defects/retardations in seedling development, we wanted to investigate whether 6-FI also inhibits Trp-dependent IAA biosynthesis in vivo in *Arabidopsis* seedlings. The incorporation of stable isotope-labeled precursors in the free IAA pool was examined in feeding experiments using 7-day-old *Arabidopsis* seedlings. We applied two experimental settings: variation in (1)

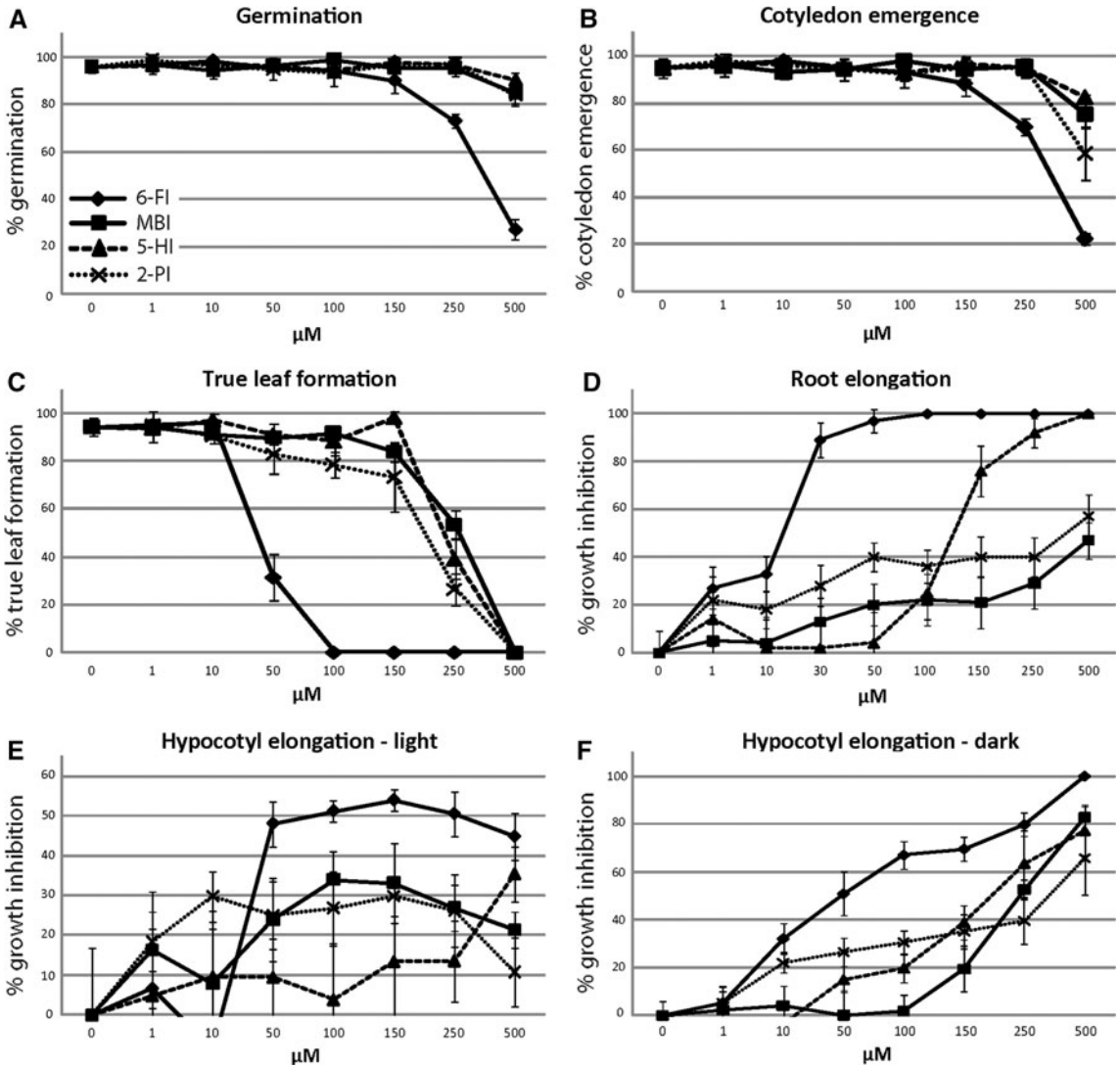

Fig. 3 Physiological effects of potential inhibitors and negative controls on seedling development. **a** Germination rates 96 h after sowing ($n = 100$). **b** Percentage of cotyledon emergence 96 h after sowing ($n = 100$). **c** Percentage of true leaf formation 8 days after sowing ($n = 100$). **d** Inhibition of root elongation; 5-day-old seedlings grown on ATS were transferred to medium containing 6-FI, MBI, 5HI, or 2-PI at the indicated concentrations and grown for 3 additional days ($n = 15$). **e** Inhibition of hypocotyl elongation under white light 10 days after sowing ($n = 15$). **f** Inhibition of hypocotyl elongation in the dark 6 days after sowing. The symbol legend in **a** applies to all graphs. All error bars represent standard deviations from the mean

incubation time and (2) inhibitor concentration. The enrichment of label in IAA from Trp was calculated (Table 1), and we observed that although there was no inhibition of IAA synthesis from Trp at 50 µM 6-FI under our experimental conditions, significant inhibition was shown following application of 100 µM 6-FI for both incubation times tested (3 and 6 h). Therefore, the compound seems quite specific for Trp-dependent IAA synthesis.

Even though the level of IAA biosynthesis inhibition was not as high as in the maize in vitro system, which may be influenced by uptake mechanisms or differences between monocots and dicots, 6-FI has the potential to be used in mutant screens based on the strong developmental phenotype caused after its application. The success of this approach suggests a route where additional inhibitors might be effectively tested in the future to obtain additional compounds useful for selection of mutants in Trp-dependent IAA synthesis.

Because the developmental defects were already significant at 10–50 µM 6-FI, we would have expected similar concentrations to be sufficient for inhibition of IAA biosynthesis in the feeding experiment. However, continuous cultivation on 6-FI for several days, as in the case of the developmental assays, is most likely more efficient than a short incubation period for a few hours, as in the case of the feeding experiments. From this perspective inhibition of

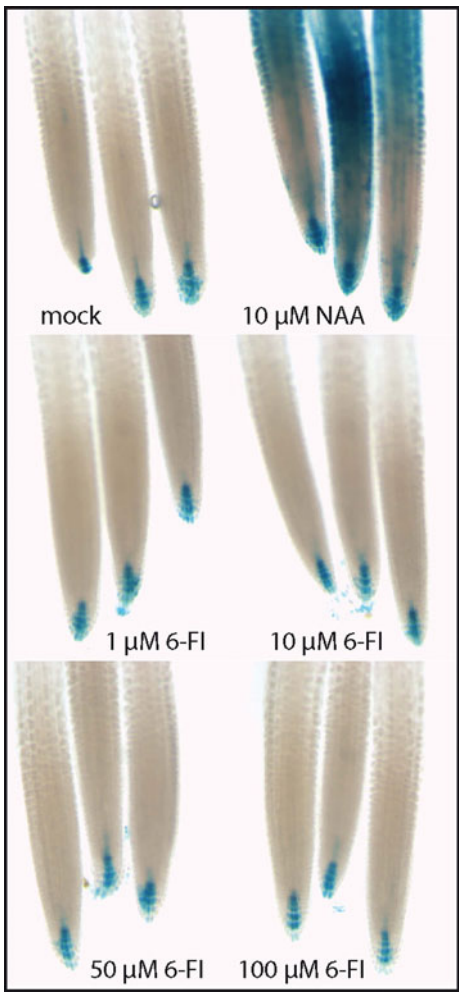

Fig. 4 6-FI does not induce the DR5:GUS reporter in roots. Six-day-old seedlings were treated with NAA (positive control) or various concentrations of 6-FI for 5 h

Table 1 Inhibitory effect of 6-FI on Trp-dependent IAA biosynthesis in *Arabidopsis* seedlings

Condition	% Enrichment	% Inhibition
Control	51.9 ± 10	0
3 h 50 μM 6-FI	60.3 ± 7	–
3 h 100 μM 6-FI	37.5 ± 5*	19
6 h 100 μM 6-FI	36.7 ± 2**	21

The incubation time with the inhibitor was either 3 or 6 h, followed by a 12-h incubation with $[^2H_5]$Trp. For control conditions, five independent experiments consisting of three replicates were conducted. For 6-FI treatments, two experiments with three replicates were performed. Mean values ± SE are given. The degree of significance for the differences between untreated and inhibitor-treated seedlings has been calculated by Student's *t*-test

* Significance at $p \leq 0.05$; ** significance at $p \leq 0.01$

auxin synthesis at 100 μM seems realistic. Furthermore, the level of inhibition for IAA biosynthesis was not as high as expected. We therefore compared our results with recent data on biosynthesis mutants in the Trp-dependent pathway (Tao and others 2008). In mutants, which are defective in the conversion of L-Trp to IPyA, the shade-induced increase in IAA synthesis, indicated by the higher ratio of deuterium-labeled versus unlabeled IAA in wild-type seedlings, did not show up in *shade avoidance3* mutant plants (Tao and others 2008). However, the mutants still showed some incorporation of deuterium. These data are consistent with our experimental results and we can thus conclude that either mutation or chemical inhibition of Trp-dependent biosynthesis leads to a reduction but not a complete block of Trp-dependent IAA synthesis, possibly suggesting in this case partial compensation by at least one route that may be less susceptible to inhibition. Alternatively, 6-FI may affect IAA conjugation which would likewise result in a changed availability of free IAA.

Conclusions

The metabolic redundancy of the several existing parallel auxin biosynthetic pathways has significantly hampered loss-of-function genetics of IAA production in the past. We observed a strong correlation of direct biochemical inhibition of Trp-dependent IAA biosynthesis in our labeling experiments and strong developmental defects caused by 6-FI application. Numerous stages of seedling development, such as root or hypocotyl elongation, are known to be directly regulated by IAA. We observed dramatic effects of 6-FI on these and other phenotypes which suggests that these developmental defects may be the result of the reduced rate of Trp-dependent IAA biosynthesis. However, additional studies are needed to distinguish between the potential impacts of 6-FI on IAA biosynthesis or homeostasis. Another important aspect that needs to be addressed relates to the identification of inhibition targets. A first step toward the dissection of target routes could be the investigation of 6-FI effects on mutants whose role in a specific biosynthetic route is well defined. Taken together, our data suggest that 6-FI may serve as a promising tool for auxin biochemistry and genetic approaches such as mutagenesis selections to dissect the multiple parallel pathways from indole and tryptophan to IAA.

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References

Campanella JJ, Ludwig-Müller J, Bakllamaja V, Sharma V, Cartier A (2003) ILR1 and sILR1 IAA amidohydrolase homologs differ in expression pattern and substrate specificity. Plant Growth Regul 41:215–223

Chen K-H, Miller AN, Patterson GW, Cohen JD (1988) A rapid and simple procedure for purification of indole-3-acetic acid prior to GC-SIM-MS analysis. Plant Physiol 86:822–825

Cheng Y, Dai X, Zhao Y (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes Dev 20:1790–1799

Cohen JD (1984) Convenient apparatus for the generation of small amounts of diazomethane. J Chromatogr 303:193–196

Cohen JD, Gray WM (2006) Auxin metabolism and signaling. In: Hedden P, Thomas S (eds) Plant hormone signaling (Annual Plant Reviews, Vol. 24). Blackwell Publishing, Oxford, pp 37–66

Delker C, Raschke A, Quint M (2008) Auxin dynamics: the dazzling complexity of a small molecule's message. Planta 227:929–941

Ehlert B, Schöttler MA, Tischendorf G, Ludwig-Müller J, Bock R (2008) The paramutated *SULFUREA* locus of tomato is involved in auxin biosynthesis. J Exp Bot 59:3635–3647

Epstein E, Cohen JD, Slovin JP (2002) The biosynthetic pathway for indole-3-acetic acid changes during tomato fruit development. Plant Growth Regul 38:15–20

Geldner N, Friml J, Stierhof YD, Jürgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413:425–428

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333

Hayashi K, Tan X, Zheng N, Hatate T, Kimura Y, Kepinski S, Nozaki H (2008) Small-molecule agonists and antagonists of F-box protein–substrate interactions in auxin perception and signalling. Proc Natl Acad Sci USA 105:5632–5637

Ilic N, Östin A, Cohen JD (1999) Differential inhibition of indole-3-acetic acid and trpytophan biosynthesis by indole analogues. I. Tryptophan dependent IAA biosynthesis. Plant Growth Regul 27:57–62

Jentschel K, Thiel D, Rehn F, Ludwig-Müller J (2007) Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. Physiol Plant 129:320–333

Keitt GW, Baker RA (1966) Auxin activity of substituted benzoic acids and their effect on polar auxin transport. Plant Physiol 41:1561–1569

Lincoln C, Britton JH, Estelle M (1990) Growth and development of the axr1 mutants of Arabidopsis. Plant Cell 2:1071–1080

Ludwig-Müller J, Sass S, Sutter EG, Wodner M, Epstein E (1993) Indole-3-butyric acid in *Arabidopsis thaliana.* I. Identification and quantification. Plant Growth Regul 13:179–187

Michalczuk L, Cooke TJ, Cohen JD (1992) Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry 31:1097–1103

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Normanly J, Cohen JD, Fink GR (1993) *Arabidopsis thaliana* auxotrophs reveal a tryptophan independent biosynthetic pathway for indole-3-acetic acid. Proc Natl Acad Sci USA 90:10355–10374

Normanly J, Slovin JP, Cohen JD (1995) Rethinking auxin biosynthesis and metabolism. Plant Physiol 107:323–329

O'Hagan D (2008) Understanding organofluorine chemistry. An introduction to the C–F bond. Chem Soc Rev 37:308–319

Östin A, Ilic N, Cohen JD (1999) An in vitro system from maize seedlings for tryptophan independent IAA biosynthesis. Plant Physiol 119:173–178

Piotrowski M (2008) Primary or secondary? Versatile nitrilases in plant metabolism. Phytochemistry 69:2655–2667

Pollmann S, Neu D, Weiler EW (2003) Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid. Phytochemistry 62:293–300

Pollmann S, Müller A, Weiler EW (2006) Many roads lead to "auxin": of nitrilases, synthases, and amidases. Plant Biol 8:326–333

Pollmann S, Düchting P, Weiler EW (2009) Tryptophan-dependent indole-3-acetic acid biosynthesis by 'IAA-synthase' proceeds via indole-3-acetamide. Phytochemistry 70:523–531

Quint M, Gray WM (2006) Auxin signaling. Curr Opin Plant Biol 9:448–453

Quint M, Barkawi LS, Fan K-T, Cohen JD, Gray WM (2009) Arabidopsis IAR4 modulates auxin response by regulating auxin homeostasis. Plant Physiol 150:748–758

Seo M, Akaba S, Oritani T, Delarue M, Bellini C, Caboche M, Koshiba T (1998) Higher activity of an aldehyde oxidase in the auxin-overproducing *superroot1* mutant of *Arabidopsis thaliana*. Plant Physiol 116:687–693

Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie D-Y, Dolezal K, Schlereth A, Jürgens G, Alonso JM (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 133:177–191

Stomp A-M (1991) Histochemical localization of b-glucuronidase. In: Gallagher SR (ed) GUS protocols. Academic Press, London, pp 103–113

Sztein AE, Ilić N, Cohen JD, Cooke TJ (2002) Indole-3-acetic acid biosynthesis in isolated axes from germinating bean seeds: the effect of wounding on the biosynthetic pathway. Plant Growth Regul 36:201–207

Tam YY, Normanly J (1998) Determination of indole-3-pyruvic acid levels in *Arabidopsis thaliana* by gas chromatography–selected ion monitoring-mass spectrometry. J Chromatogr 800:101–108

Tao Y, Ferrer J-L, Ljung K, Pojer F, Hong F, Long JA, Li L, Moreno JE, Bowman ME, Ivans LJ, Cheng Y, Lim J, Zhao Y, Ballaré CL, Sandberg G, Noel JP, Chory J (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell 133:164–176

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

Widholm JM (1981) Utilization of indole analogs by carrot and tobacco cell tryptophan synthase in vivo and in vitro. Plant Physiol 67:1101–1104

Wright AD, Sampson MB, Neuffer MG, Michalczuk L, Slovin JP, Cohen JD (1991) Indole-3-acetic acid biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph. Science 254:998–1000

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory C (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 291:306–309

Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, Ecker JE, Normanly J, Chory J, Celenza JC (2002) Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev 16:3100–3112