Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF *ARABIDOPSIS* and YUCCAs in *Arabidopsis*

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Auxin is an essential hormone, but its biosynthetic routes in plants have not been fully defined. In this paper, we show that the TRYPTOPHAN AMINOTRANSFERASE OF *ARABIDOPSIS* (TAA) family of amino transferases converts tryptophan to indole-3-pyruvate (IPA) and that the YUCCA (YUC) family of flavin monooxygenases participates in converting IPA to indole-3-acetic acid, the main auxin in plants. Both the YUCs and the TAAs have been shown to play essential roles in auxin biosynthesis, but it has been suggested that they participate in two independent pathways. Here, we show that all of the *taa* mutant phenotypes, including defects in shade avoidance, root resistance to ethylene and *N*-1-naphthylphthalamic acid (NPA), are phenocopied by inactivating *YUC* genes. On the other hand, we show that the *taa* mutants in several known auxin mutant backgrounds, including *pid* and *npy1*, mimic all of the well-characterized developmental defects caused by combining *yuc* mutants with the auxin mutants. Furthermore, we show that overexpression of *YUC1* partially suppresses the shade avoidance defects of *taa1* and the sterile phenotypes of the weak but not the strong *taa* mutants. In addition, we discovered that the auxin overproduction phenotypes of *YUC* overexpression lines are dependent on active *TAA* genes. Our genetic data show that *YUC* and *TAA* work in the same pathway and that *YUC* is downstream of *TAA*. The *yuc* mutants accumulate IPA, and the *taa* mutants are partially IPA-deficient, indicating that *TAA*s are responsible for converting tryptophan to IPA, whereas *YUC*s play an important role in converting IPA to indole-3-acetic acid.

Auxin is an essential regulator for various plant developmental processes. Indole-3-acetic acid (IAA), the main auxin in plants, can be synthesized from tryptophan (Trp) -dependent and -independent pathways (1). Several auxin biosynthesis routes have been proposed (Fig. 1*A*), but none of the proposed pathways in plants have been fully determined (1). In some plant pathogenic bacteria, IAA is synthesized from Trp by the Trp monooxygenase iaaM and the hydrolase iaaH (Fig. 1*A*). The iaaM converts Trp to indole-3-acetamide (IAM) that is subsequently hydrolyzed to IAA by iaaH (2). Plants also make IAM, but the biosynthesis routes of IAM in plants are not defined (3). IAM can be converted to IAA by *Arabidopsis* AMIDASE1 (4). Trp can also be converted into indole-3-acetaldoxime (IAOx) by the P450 enzymes CYP79B2 and -B3 (Fig. 1*A*) (5). The routes from IAOx to IAA are not understood, although both IAM and indole-3-acetonitrile have been suggested as intermediates (Fig. 1*A*) (3). IAOx is probably not a main auxin biosynthesis intermediate, because (*i*) a complete elimination of IAOx production in *Arabidopsis* only leads to subtle growth defects (5), (*ii*) the enzymes CYP79B2 and -B3 seemed only to exist in a small group of plants, and (*iii*) there is no detectable IAOx in rice and maize (3).

The most important plant auxin biosynthetic enzymes are the YUCCA (YUC) family of flavin-containing monooxygenases (6, 7) and the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS* (*TAA*) (8, 9) family of aminotransferases, because inactivating members of either family causes dramatic developmental defects (Fig. 1*A*). Both families are widely distributed among all sequenced plant genomes. The *YUC* genes were first identified as auxin biosynthesis enzymes, because overexpression of *YUC*s leads to auxin overproduction in *Arabidopsis* (7). *YUC* genes are essential for embryogenesis, seedling development, vascular patterning, and flower development (6, 10). Furthermore, the *yuc* phenotypes can be rescued by expressing the iaaM gene under the control of a *YUC* promoter (6). The *TAA* genes were independently isolated from three genetic screens. Inactivation of *TAA1* leads to altered responses to shade (9), ethylene (8), and the auxin transport inhibitor NPA (11). Furthermore, simultaneously inactivating *TAA1* and its close homologs (*TAR* genes) lead to defects in embryogenesis, vascular patterning, and flower development (8). YUCs were proposed to catalyze the conversion of tryptamine to *N*-hydroxyl tryptamine, which may be converted to IAOx (7, 12). However, our recent biochemical analysis indicates that YUCs do not play a major role in IAOx production (3). Recent work has also questioned whether YUCs are involved in the tryptamine pathway (13). Flavin monooxygeanses are known to use a broad range of substrates in vitro, because they use the stable C4a-hydroperoxyl flavin, an activated intermediate, for catalysis (14). Therefore, additional genetic and biochemical analyses are needed to define the in vivo roles of YUCs in auxin biosynthesis. The *TAA* family proteins catalyze the conversion of Trp to indole-3-pyruvate (IPA), but the in vitro data suggest that the reaction from IPA to Trp is actually more favorable (9). Furthermore, the mechanism by which IPA is converted to IAA is still not understood. It has been widely speculated that IPA is converted to indole-3-acetaldehyde (IAAld) by IPA decarboxylase. IAAld then is believed to be converted to IAA by aldehyde oxidases or dehydrogenases (Fig. 1*A*) (1).

Interestingly, some of the *yuc* mutant phenotypes are very similar to those phenotypes of *taa* mutants. For example, *yuc1 yuc4 yuc10 yuc11* quadruple mutants fail to make the basal parts of *Arabidopsis* embryos (10), a phenotype that is also observed in *wei8 tar1 tar2-1* (8). Mutant alleles of *taa1* are also called *wei8* (8), *sav3* (9), and *tir2* (11), which reflect the three genetic screens that identified the mutant alleles. We keep using the *wei8*, *sav3*, and *tir2* allele names in this paper so that readers can easily track

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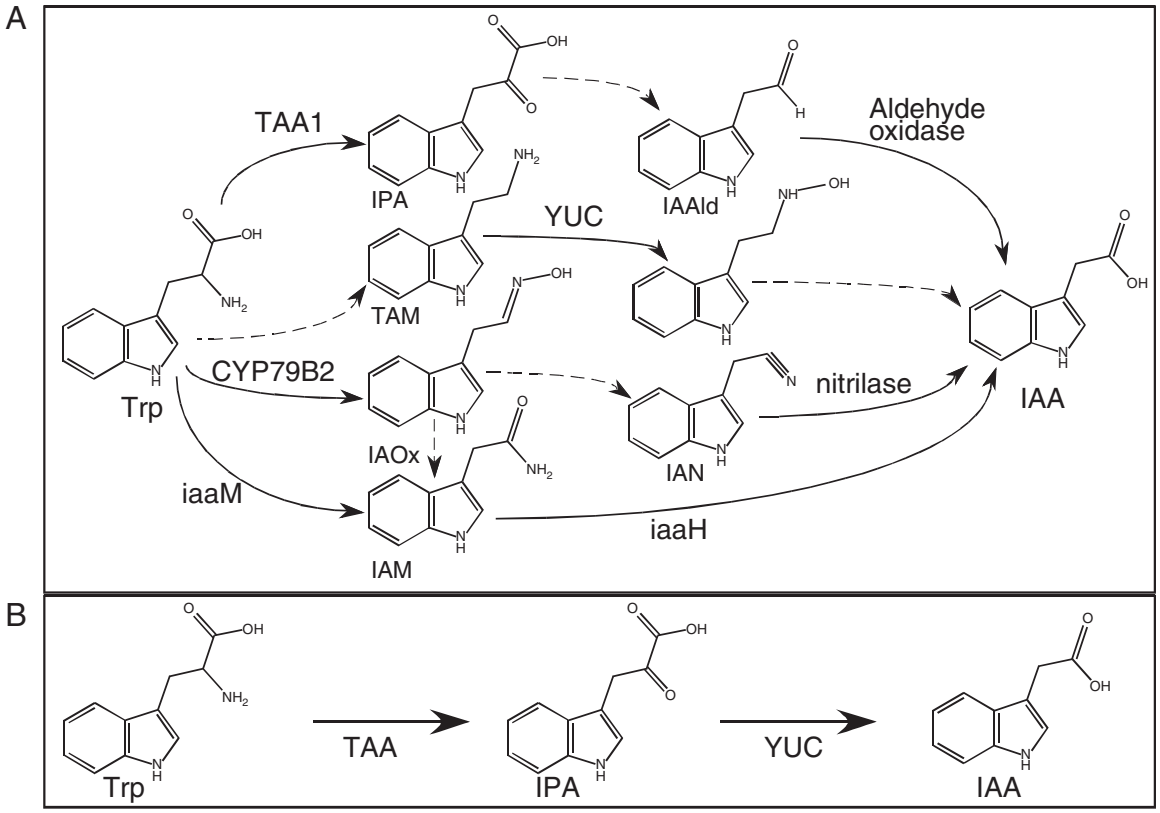

Fig. 1. Trp-dependent auxin biosynthesis routes. (*A*) The proposed pathways for converting Trp to IAA. (*B*) A complete two-step auxin biosynthesis pathway. IPA, indole-3-pyruvate; TAM, tryptamine; IAOx, indole-3-acetaldoxime; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAAld, indole-3-acetaldehyde; IAA, indole-3-acteic acid. A solid line indicates that a gene has been suggested for the step, whereas no genes have been suggested for the step indicated by a dotted line.

the alleles of *taa1* that are used. A list of mutants used in this work is shown in Table S1. Vascular defects in *yuc1 yuc2 yuc4 yuc6* are also very similar to those defects in *wei8 tar2-1* (6, 8). Both *yuc* and *wei8 tar2* mutants also have defects in flower development (6, 8). The observed phenotypic similarities between *yuc* and *taa* mutants have prompted speculations that YUC and TAA may participate in the same pathway to produce auxin (1, 15). The observation that inactivation of the *SPI1*/*YUC* gene in the *vt2*/*taa1* mutant background in maize did not further enhance the phenotypes of *vt2* also suggests that the *YUC* and *TAA* genes might belong to the same pathway (16).

Although there are many similarities between *yuc* and *taa* mutants, there are several key differences. First, the *yuc* mutants analyzed so far have not been shown to affect shade avoidance responses, whereas *taa1*/*sav3* alone showed dramatic defects in shade avoidance (9). Second, the *yuc* mutants have not been reported to affect ethylene responses in roots, but *taa* mutants are insensitive to the inhibitory effects of ethylene in roots (8). Third, the *yuc* mutants have not been shown to alter responses to NPA in roots, whereas *taa* is resistant to NPA (11). Fourth, the *wei8*/*sav3* alone dramatically reduced IAA levels in seedlings, whereas quadruple *yuc* mutants did not show a significant decrease in auxin levels (9), although the *yuc* mutants have phenotypes much more severe than those phenotypes of *wei8* or *sav3*.

Some of the phenotypic defects observed in *yuc* mutants have not been observed in *taa* mutants or have not been analyzed. For example, the floral defects of *yuc1 yuc4* seem to be different from those defects in *wei8 tar2* mutants (6). The *yuc1 yuc4* double mutants have fewer floral organs, whereas *wei8 tar2* flowers are defective; however, no floral organs are missing. We have shown that *yuc* mutants have synergistic interactions with known auxin mutants, including *pid*, *pin1*, and *npy1* (10, 17, 18). It will be informative to analyze the genetic interactions between the known auxin and *taa* mutants.

In this paper, we show that all of the phenotypes observed in *taa* mutants, including root resistance to ethylene treatment, altered shade avoidance responses, and root resistance to NPA treatments, are phenocopied by inactivating certain combinations of *YUC* genes. We also show that all of the synergistic effects between *yuc* and known auxin mutants are observed when *TAA* genes are inactivated in the known auxin mutant backgrounds. Furthermore, we show that overexpression of *YUC1* in *taa1*/*sav3* can partially rescue the shade avoidance defects. Overexpression of *YUC1* can also rescue the sterile phenotypes of *wei8 tar2-2*, a weak *taa* double mutant. However, the characteristic long hypocotyl phenotypes associated with *YUC* overexpression are not observed in the strong *wei8 tar2-1* mutant background. Our genetic analysis put the *YUC* and *TAA* genes in the same auxin biosynthesis pathway, with *YUC*s downstream of *TAA*s. We discovered that IPA levels in *wei tar2* are decreased, whereas in *yuc1 yuc2 yuc6*, they are increased, further supporting our hypothesis that *YUC*s function downstream of *TAA*s. We propose that auxin is synthesized by a two-step pathway in which TAAs convert Trp into IPA and YUCs are responsible for converting IPA into IAA (Fig. 1*B*).

Results

***yuc* Mutants Phenocopied the *taa* Mutants.** Alleles of *taa1* were isolated from genetic screens for mutants with altered responses to ethylene (*wei8* mutants) (8), shade (*sav3* mutants) (9), and NPA (*tir2* mutants) (11). Interestingly, no *yuc* mutants were

identified from the three genetic screens. It is likely that genetic redundancy among the *YUC* genes might have prevented the identification of *yuc* mutants in the screens.

Our previous work on *yuc* mutants mainly focused on the development of aerial parts of *Arabidopsis* (6, 10). We hypothesized that a different set of *YUC* genes might be responsible for root development. We investigated whether the *yuc3*, *yuc5*, *yuc7*, *yuc8*, and *yuc9* quintuple mutants (*yuc Q*) displayed resistance to ethylene in roots. The five *YUC* genes form two distinct clades in the *YUC* phylogenetic tree (6). As shown in Fig. 2 *A* and *B*, the *yuc Q* mutants were strongly resistant to the treatment of 1-aminocyclopropane-1-carboxylic-acid (ACC), an ethylene biosynthesis precursor. In the presence of 10 μM ACC, root elongation in WT *Arabidopsis* seedlings was almost completely inhibited (Fig. 2*A*). In contrast, the *yuc Q* mutants displayed healthy and elongated roots (Fig. 2*A*). The ethylene resistance of the *yuc Q* roots was much stronger than the resistance of *wei8 tar2-1* double mutants, which are known to confer ethylene resistance in roots (Fig. 2 *A* and *B*). Our data showed that *YUC* genes also play an important role in root responses to ethylene.

We previously analyzed shade avoidance responses of the *yuc Q* mutants, because *YUC8* seemed to be induced by shade conditions (9). However, the *yuc Q* mutants did not show obvious defects in shade avoidance responses under our conditions (9). Our previous experiments had clearly shown that *YUC1*, *YUC2*, *YUC4*, and *YUC6* are the predominant *YUC*s functioning in aerial parts of *Arabidopsis* (6). Unfortunately, the *yuc1 yuc4* double null mutants are completely sterile, making analysis of *yuc1 yuc4* in shade avoidance responses unfeasible. We used a weak allele of *yuc1* (*yuc1-163*), which showed vascular and floral defects when combined with *yuc4* mutant. The *yuc1-163 yuc4* could generate a small amount of seeds that allowed us to analyze the shade avoidance response. As shown in Fig. 2*C*, under shade conditions, hypocotyls of WT plants were much elongated. In contrast, hypocotyls of *sav3-1* were only one-half as long as those hypocotyls of WT plants. The *yuc1-163 yuc4* was clearly shade-resistant (Fig. 2*C*), indicating that *YUC* genes also participate in shade avoidance responses.

Inactivation of *TAA1*/*TIR2* caused root resistance to the auxin transport inhibitor NPA (11) (Fig. 2*D*). NPA leads to auxin accumulation in root tips and causes root tip swelling in WT plants (Fig. 2*D*). We also investigated whether *YUC* genes play a role in mediating root responses to NPA treatments. Like the *taa* mutants *wei8 tar2-1*, the *yuc Q* mutants did not have a swollen root tip (Fig. 2*D*), indicating that *yuc Q* mutants were also resistant to NPA treatments.

Our data showed that the three main characteristic phenotypes associated with *taa* mutants were also observed in *yuc* mutants. Interestingly, *Arabidopsis* plants use the same *TAA* genes in both the shade avoidance and root responses to ethylene and NPA. By contrast, *Arabidopsis* uses two different sets of *YUC* genes for shade avoidance and root responses to ethylene and NPA. *YUC1* and *YUC4* were the main *YUC*s used in shade avoidance responses, and *Arabidopsis* relied primarily on *YUC3*, *YUC5*, *YUC7*, *YUC8*, and *YUC9* for responses to ethylene and NPA in roots.

Characteristic *yuc* Phenotypes Were Mimicked by Inactivating *TAA* Genes. We previously showed that *yuc* mutants displayed dramatic genetic interactions with known auxin mutants *pin1*, *pid*, and *npy1* (10, 17, 18). We investigated whether *taa* mutants could mimic the synergistic genetic interactions observed between *yuc* and known auxin mutants.

When *yuc1 yuc4* was combined with *pin1*, the development of true leaves in the triple mutants was completely abolished (10). The no-leaf phenotypes could also be observed when *yuc1 yuc4* were grown on media containing NPA (10) (Fig. 3*A*). Clearly, the development of true leaves in both *wei8 tar2-1* and *wei8 tar2-2* was completely abolished by NPA, whereas under the same

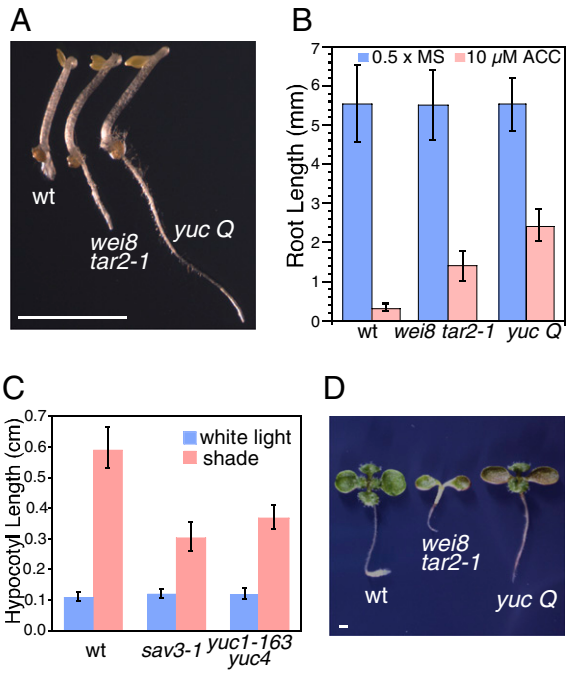

Fig. 2. Inactivation of *YUC* genes phenocopied the *taa* mutants. (*A*) The *yuc3 yuc5 yuc7 yuc8 yuc9* quintuple mutants (*yuc Q*) displayed strong resistance to ethylene in roots. (*B*) Measurements of root length. Both *wei8 tar2-1* and *yuc Q* had longer roots in the presence of 10 μM ACC. (*C*) The *yuc1-163 yuc4* double mutants displayed defects in shade avoidance similar to the defects observed in *sav3-1*/*taa1*. (*D*) The *yuc Q* displayed resistance to the auxin transport inhibitor NPA. Roots of WT plants became swollen, but both *wei8 tar2-1* and *yuc* quintuple mutants had normal root tips. Error bars refer to SD. (Scale bar: 1 mm.)

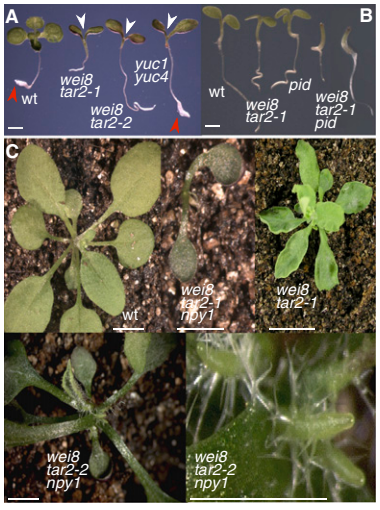

Fig. 3. Mutations in *TAA* genes could mimic *yuc* mutants. (*A*) Inactivation of *WEI8* and *TAR2* caused the same phenotypes as those of *yuc1 yuc4* in aerial parts in the presence of NPA. NPA completely abolished the initiation of true leaves in both *taa* and *yuc* mutants (white arrows). Note that *yuc1 yuc4* was not resistant to NPA in the root, whereas *wei8 tar2* was resistant to NPA in roots (red arrows). (*B*) The triple mutants of *wei8 tar2-1 pid* completely deleted cotyledons. (*C*) Mutations in *npy1* enhanced *wei8 tar2* mutants. The *wei8 tar2 npy1* developed pin-like inflorescences and failed to make flowers. (Scale bar: *A* and *B*, 1 mm; *C*, 1 cm.)

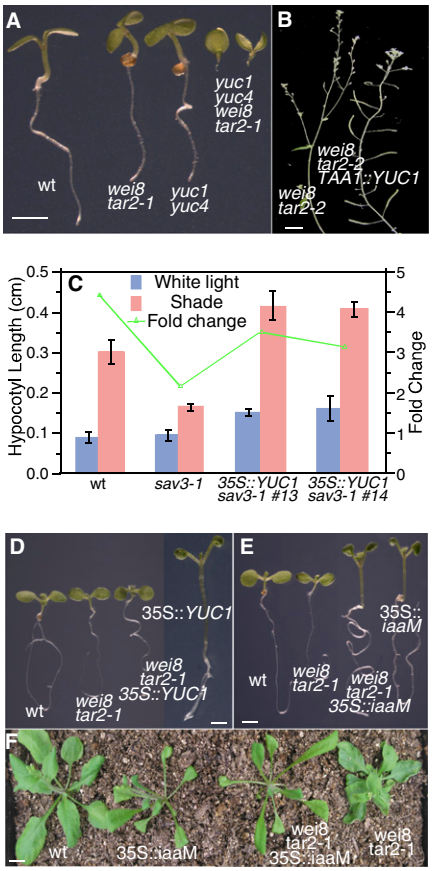

Fig. 4. Genetic interactions between *YUCs* and *TAAs* in *Arabidopsis*. (*A*) Synergistic interactions between *wei8 tar2-1* and *yuc1 yuc4*. The *yuc1 yuc4 wei8 tar2-1* failed to make hypocotyls and roots. (Scale bar: 2 mm.) (*B*) Expression of *YUC1* cDNA under the control of *TAA1* promoter partially rescued the sterile phenotypes of *wei8 tar2-2*. (Scale bar: 1 cm.) (*C*) Overexpression of *YUC1* cDNA using the 35S promoter partially rescued the shade avoidance defects in *sav3-1*. Error bars refer to SD. (*D*) Auxin overproduction phenotypes of *35S::YUC1* are suppressed in *wei8 tar2-1* background. (Scale bar: 1 mm.) (*E*) Overexpression of iaaM in *wei8 tar2-1* caused auxin overproduction in both WT and *wei8 tar2-1* backgrounds. (Scale bar: 1 mm.) (*F*) The adult phenotypes of *wei8 tar2-1* were also partially suppressed by 35S::iaaM. (Scale bar: 1 cm.)

conditions, WT seedlings developed true leaves (Fig. 3*A*). Interestingly, roots of both *yuc1 yuc4* and WT plants were swollen (Fig. 3*A*), whereas *wei8 tar2* mutants were resistant to NPA in roots (Figs. 2*D* and 3*A*). Our data showed that *taa* mutants could phenocopy *yuc1 yuc4* in terms of the development of true leaves in the presence of NPA (Fig. 3*A*). The NPA experiments also showed that *YUC1* and *YUC4* were the main *YUC*s in regulating the development of aerial parts, but *YUC*1 and *YUC*4 were not the main *YUC*s in root responses to NPA.

We previously showed that both *YUC1* and *YUC4* were important for cotyledon development (17, 18). Inactivation of both *YUC1* and *YUC4* in the *pid* mutant background leads to a complete deletion of cotyledons (17, 18). When we combined *wei8 tar2-1* with *pid*, the resulting triple mutants also failed to develop any cotyledons (Fig. 3*B*), showing that both the *YUC*s and *TAA*s play similar roles in cotyledon development.

Another well-characterized genetic interaction between *yuc* and known auxin mutants is the enhancement of *yuc1 yuc4* double mutants by *npy1* (17). The *yuc1 yuc4 npy1* triple mutants completely eliminated the formation of flowers but still developed pin-like inflorescences (17). Simultaneous inactivation of *TAA1*/*WEI8*, *TAR2*, and *NPY1* also abolished the formation of flowers (Fig. 3*C*). The *wei8 tar2-2 npy1* developed many pin-like inflorescences (Fig. 3*C*). Combining the strong mutants *wei8 tar2-1* with *npy1* led to even stronger phenotypes (Fig. 3*C*). Both the *yuc1 yuc4 npy1* and *wei8 tar2 npy1* mutants developed similar pin-like inflorescences, but the *wei8 tar2 npy1* had more severe phenotypes. The observed differences could be caused by the fact that additional *YUC* genes are involved in inflorescences development (6). In fact, the juvenile plants of *wei8 tar2-1* were similar to those plants of *yuc1 yuc2 yuc4 yuc6* quadruple mutants (6, 8). Our data showed that the *taa* mutants could mimic all of the *yuc* phenotypes.

Genetic Interactions Between *yuc* and *taa* Mutants. We directly tested whether there were genetic interactions between *yuc* and *wei8 tar2* mutants. We chose *yuc1 yuc4* and *wei8 tar2-1*, because both double mutants displayed strong developmental phenotypes (6, 8). The *yuc1 yuc4 wei8 tar2* quadruple mutants did not make any hypocotyls and roots, a phenotype that was not observed in either *yuc1 yuc4* or *wei8 tar2-1* (Fig. 4*A*). Interestingly, the *yuc1 yuc4 wei8 tar2* quadruple phenotypes were very similar to those phenotypes of *yuc1 yuc4 yuc10 yuc11* and *wei8 tar1 tar2-1* (8, 10).

We expressed *YUC1 cDNA* under the control of the *TAA1* promoter (9) in *wei8 tar2-2* background. The *wei8 tar2-2* double mutants were weaker than *wei8 tar2-1* but still sterile (Fig. 4*B*). Expression of *YUC1* partially rescued the sterile phenotype of *wei8 tar2-2* (Fig. 4*B*).

We also overexpressed *YUC1* cDNA using the *Cauliflower mosaic virus* (CaMV) 35S promoter in the *taa1*/*sav3-1* background. Overexpression of *YUC1* in *sav3-1* leads to longer hypocotyl, a characteristic phenotype associated with auxin overproduction (6, 7) (Fig. 4*C*). Overexpression of *YUC1* also partially suppressed the shade avoidance phenotypes of *sav3-1* (Fig. 4*C*).

When we introduced *35S::YUC1* construct into *wei8 tar2-1* by transforming *wei8 tar2-1*$^{+/-}$ plants, we did not observe the typical auxin overproduction phenotypes in *wei8 tar2-1* plants (Fig. 4*D*). Because the *tar2-1* is a T-DNA line, we investigated whether *35S::YUC1* was silenced in the *wei8 tar2-1* background. We found that the expression levels of *YUC1* in *35S::YUC1 wei8 tar2-1* were higher than in WT (Fig. S1), suggesting that overproduction of auxin by *35S::YUC1* is dependent on active *TAA* genes.

***YUC*s and the iaaM Gene Behaved Differently in *wei8 tar2* Mutants.** We previously showed that expression of the bacterial auxin biosynthesis gene iaaM rescued *yuc* mutant phenotypes (6, 7). Expression of iaaM also rescued the shade avoidance phenotypes of *sav3-1* (9). We investigated whether iaaM could rescue the developmental defects of *wei8 tar2* mutants. As shown in Fig. 4*E*, overexpression of iaaM led to the typical auxin overproduction phenotypes in both WT and *wei8 tar2* mutants. The iaaM gene also partially rescued the defects of *wei8 tar2* phenotypes at juvenile and adult stages (Fig. 4*F*). Our work shows that *YUC*s and iaaM genes probably use different mechanisms for auxin biosynthesis.

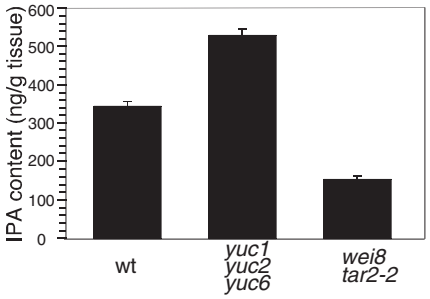

Fig. 5. IPA levels in auxin biosynthesis mutants. Inactivation of *yuc1 yuc2 yuc6* caused IPA accumulation, whereas the *wei8 tar2-2* mutants had less IPA than WT plants. Error bar refers to SD.

Both *YUC*s and *TAA* Genes Affected the Homeostasis of IPA. We directly measured the IPA contents in WT plants, *yuc* mutants, and *taa* mutants. Because the strong *yuc* and *taa* mutants have dramatic developmental defects, we chose *yuc1 yuc2 yuc6* triple mutants and *wei8 tar2-2* double mutants to analyze the IPA contents. The *yuc1 yuc2 yuc6* mutants are similar to *wei8 tar2-2*. Both the *yuc1 yuc2 yuc6* and *wei8 tar2-2* are sterile and have similar sizes. As shown in Fig. 5, the IPA levels in *wei8 tar2-2* were reduced dramatically. In contrast, the *yuc1 yuc2 yuc6* had elevated levels of IPA (Fig. 5). Our data indicate that *TAA* genes are involved in the production of IPA, whereas *YUC* genes are involved in metabolism of IPA (Fig. 1*B*).

Discussion

Both YUCs and TAAs were reported to participate in Trp-dependent auxin biosynthesis. Here, we have shown that the YUCs and TAAs participate in the same auxin biosynthesis pathway and that YUCs work downstream of TAAs. Our data indicate that TAAs are responsible for making IPA from Trp and that YUCs are required for converting IPA to IAA.

We conclude that YUCs and TAAs participate in the same pathway to convert Trp into IAA. First, inactivation of *YUC* genes caused the same phenotypes as those phenotypes observed in *taa* mutants (Fig. 2). Second, all of the *yuc* phenotypes were mimicked by *taa* mutants or *taa* mutant combinations (Fig. 3). The *yuc1 yuc4 pid* and *wei8 tar2-1 pid* displayed the exact same no cotyledon phenotypes (Fig. 3). The fact that the *yuc* and *taa* mutants had similar phenotypes in every aspect of growth and developmental processes that we have analyzed is indicative that both gene families participate in the same pathway. Alternatively, *YUCs* and *TAAs* may participate in parallel auxin biosynthesis pathways. The similar phenotypes observed in *yuc* and *taa* mutants could simply be a reflection of decreased auxin levels in the mutants. The latter interpretation is consistent with the observation that *yuc1 yuc4* and *wei8 tar2* enhanced each other (Fig. 4). However, *yuc1 yuc4* mutants are not null for *YUC* functions because of the existence of other *YUC*s. The *wei8 tar2* mutants are not null for *TAA* activity either. Therefore, the synergistic genetic interactions between *yuc1 yuc4* and *wei8 tar2* are also compatible with the interpretation that YUCs and TAAs participate in the same pathway. The hypothesis that YUCs and TAAs are in the same pathway is also supported by the studies on *YUC* overexpression lines. Overexpression of *YUC1* in WT background leads to dramatic auxin overproduction phenotypes (Fig. 4). However, the *YUC1* overexpression phenotypes were weakened in the *taa1/sav3* background (Fig. 4). We did not observe the long hypocotyl phenotypes associated with *YUC1* overexpression in *wei8 tar2-1*, suggesting that *YUC1* overexpression-mediated auxin overproduction is dependent on *TAA* functions. We were concerned that perhaps hypocotyls of *wei8 tar2-1* simply could not elongate. However, when we overexpressed iaaM in *wei8 tar2-1*, the auxin overproduction phenotypes were clearly observed (Fig. 4).

We place YUCs downstream of TAAs in auxin biosynthesis. We discovered that the *taa* mutants made less IPA than WT, but *yuc* mutants accumulated much more IPA (Fig. 5). A logic interpretation is that TAAs make IPA from Trp and that YUCs participate in the conversion of IPA to IAA. This interpretation is also consistent with our findings that overexpression of *YUC1* partially rescued the shade avoidance phenotypes of *sav3-1* and that expression of *YUC1* partially rescued *wei8 tar2-2* (Fig. 4). The conversion of the residual IPA in the weak *taa* mutants was accelerated in *YUC* overexpression lines, thus making more IAA to partially rescue the weak *taa* mutant phenotypes.

Although all of the genetic data indicate that *YUC*s and *TAA*s participate in the same auxin biosynthetic pathway, we have been puzzled by the fact that *yuc* mutants did not have lower levels of IAA than WT plants, whereas *taa1* alone had a dramatic reduction in IAA levels (8, 9). It is puzzling, because *yuc* mutants such as *yuc1 yuc2 yuc4 yuc6* quadruple mutants had much more severe phenotypes than the phenotypes of *taa1/sav3* (6, 8, 10). We hypothesize that the paradoxical results may be partially caused by the nonenzymatic conversion of IPA to IAA during the process of IAA measurement given that *yuc* mutants accumulate much more IPA (Fig. 5). It is well-known that IPA is very labile in aqueous solutions and that IPA is easily converted to IAA in vitro nonenzymatically (19). It will be necessary to redo the IAA analysis in the *yuc* mutants by removing IPA first.

It is evident that TAAs convert Trp to IPA by removing the amino group from Trp. However, it is not obvious how YUCs participate in IPA metabolism. We propose that YUCs convert IPA to IAA using a mechanism analogous to the mechanism of lactate monooxygenases, which convert lactate to acetic acid and CO_2 (20, 21). Lactate is first converted to pyruvate by transferring the two electrons to the flavin cofactor in lactate monooxygenase. The reduced flavin binds oxygen and subsequently reacts with pyruvate to release CO_2 and acetic acid. YUCs probably use NADPH to reduce the flavin cofactor. After the flavin is reduced, the resulting FADH2 can bind oxygen and convert IPA to IAA.

Materials and Methods

The *wei8*, *tar2-1*, and *tar2-2* mutants were described in ref. 8, and *sav3-1* was reported in ref. 9. The *npy1* mutant used in this study was the *npy1-2* T-DNA allele (SALK-108406) (17). Genotyping of the *npy1* mutant has been described (17). The *yuc1* and *yuc4* mutants were described in ref. 6. The *yuc3*, *yuc5*, *yuc7*, *yuc8*, and *yuc9* quadruple mutants were described in ref. 9. The *pid* allele was the SALK-049736 line as previously reported (17). Methods for genotyping the various mutants used in this work were described previously (6, 8, 9, 17).

Shade avoidance assay was conducted according to the procedures described previously (9). Ethylene responses were measured using 4-d-old dark-grown seedlings. Root length was measured using the National Institutes of Health image software.

Methods for IPA analysis are shown in *SI Methods*.

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