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PLANT SCIENCE

Maize resistance to witchweed through changes in strigolactone biosynthesis

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Maize (Zea mays) is a major staple crop in Africa, where its yield and the livelihood of millions are compromised by the parasitic witchweed Striga. Germination of Striga is induced by strigolactones exuded from maize roots into the rhizosphere. In a maize germplasm collection, we identified two strigolactones, zealactol and zealactonoic acid, which stimulate less maize germination than the major maize strigolactone, zeaclonalactone. We then showed that a single crytchome P450, ZmCYP706C37, catalyzes a series of oxidative steps in the maize-strigolactone biosynthetic pathway. Reduction in activity of this enzyme and two others involved in the pathway, ZmMAX1b and ZmCLAM1, can change strigolactone composition and reduce Striga germination and infection. These results offer prospects for breeding Striga-resistant maize.

Food security is a growing challenge in the face of climate change and increasing food needs (1). Maize (Zea mays) is one of the most important staple crops in the world, especially in Africa. There, its yield is compromised by the parasitic witchweed Striga hermonthica and Striga asiatica. Damage from these Striga species threatens the livelihood of millions of people, particularly in sub-Saharan regions (fig. S1) (2, 3). Striga seeds lay dormant in soil until their germination is triggered by strigolactones (SLs), signaling compounds exuded by the roots of plants, including maize. The first known SL, strigol, was discovered in the 1960s in the root exudates of cotton (4). In addition to having been co-opted as a cue for root-parasitic plants, SLs serve as host signals for beneficial arbuscular mycorrhizal fungi (AMF) and are plant hormones with developmental roles (5–9).

Thus far, more than 35 different SLs have been discovered, all containing the conserved D-ring (Fig. 1A) (10–12). The canonical SLs include two groups, the “strigol-type” and “orobanchol-type,” whereas noncanonical SLs lack the A-, B-, and/or C-rings (10–12). Plants usually exude a blend of different SLs, and composition of the root exudate can vary greatly between and sometimes also within plant species. Many of the SLs display substantial differences in their biological activity, such as the induction of AMF hyphal branching and parasitic plant germination (9, 13–15). The biological importance of SL blends is far from understood, but in sorghum (Sorghum bicolor), a change in SLs from 5-deoxysterogl to orobanchol decreased Striga germination and increased field resistance (16).

The mechanisms of SL biosynthesis have only been partially elucidated. Three enzymes—DWARF 27 (D27) and two carotenoid cleavage dioxygenases (CCDs), CCD7 and CCD8—catalyze the conversion of β-carotene to carotolactone (CL) (Fig. 1A) (17, 18). In Arabidopsis (Arabidopsis thaliana), CL is oxidized to form carlasaconic acid (CLA) by a cytochrome P450 (CYP) monooxygenase, CYP71A11, encoded by MoreAuxiliaryGrowth1 (MAX1) homolog AtMAX1 (19). Arabidopsis has a single copy of this MAX1, whereas maize has three homologs, and rice has five (18, 20). Although both the Arabidopsis AtMAX1 and the maize ZmMAX1b form CLA from CL, the rice MAX1 homologs, OsS900 and OsS1400, instead convert CL to 4-deoxysterohanochol (4DO) and orobanchol, respectively (18, 21). Dicots also form orobanchol, but from CLA rather than CL, and with a different cytochrome P450, CYP722C. A homolog of this CYP722C can also produce 5-deoxysterol from CLA (22, 23).

Maize roots exude at least six SLs, two of which have been structurally identified as zeaclonalactone and zeappyralactone (Fig. 1A) (24–26). However, the identities of the other four SLs remained elusive, as well as the biosynthetic differences between the six and their individual roles in Striga germination. In this study, we reveal natural variation in the maize SL blend, identify three new maize SLs, elucidate the entire maize SL biosynthetic pathway, and show that changes in the composition of the SL blend correspond to differences in Striga germination and infection. These findings create a pathway for reducing the notorious agricultural problem of Striga infection through breeding maize for favorable SL composition.

Natural variation in strigolactone production by maize

To assess the extent of variation in the production of SLs by maize, we grew a collection of maize genotypes, sampled their root exudate, and analyzed SLs with multiple reaction monitoring (MRM) liquid chromatography–tandem mass spectroscopy (LC/MS/MS) (Fig. 1B and figs. S2 and S3) (24, 25). Quantities of exuded SLs varied among these lines (Fig. 1B and fig. S3). Moreover, one of the genotypes, NP2222, displayed a distinctive SL profile, lacking detectable levels of all but two SLs, an unknown SL and designated compound 5 (Fig. 1B and fig. S3). Compound 5 was previously noted in maize root exudate (24), but its low abundance and chemical instability hampered structural characterization. Therefore, on the basis of nuclear magnetic resonance (NMR) spectra and retrosynthetic analysis (24, 27–29), we postulated structures and subsequently synthesized compound 5 as well as the other unknown SL (figs. S4 to S12). The synthetic products were identical to the natural ones in maize root exudate and were designated zeactal (compound 5) and zeactonoic acid (ZA) (the other unknown SL) (figs. S9 and S12). Bioassay of Striga germination showed that both zeactal and ZA were less inductive than zeactone (Fig. 1C), an outcome that highlights how strongly minute differences in SL structure can alter their biological activity. These findings are further supported by work on sorghum (16). To unravel the mechanistic basis for these differences in SL blends, we revealed the biosynthetic pathway of maize SLs.

Three maize genes encode the carotene biosynthetic pathway

Through homology, we identified the maize orthologs D27, CCD7, and CCD8, which catalyze the formation of CL from β-carotene in other plant species (tables S1 and S2). To confirm ZmCCD8 function, we analyzed root exudate of two independent zmeccd8 mutants (in W22 and Mo17 backgrounds) (30). Zeactone was not detected, although it was the major SL in

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Identification of gene candidates for carlactone conversion

On the basis of the structures of the maize SLs identified thus far (Fig. 1A and fig. S2) (24–26), we postulated the involvement of a methyl transferase and several CYPs in the pathway downstream of CL. Several bioinformatic approaches were combined to select candidate genes for further functional characterization.

Mutual Rank (MR)–based global gene coexpression analysis (34, 35) showed that of the three maize MAXI homologs, only ZmMAXIb tightly coexpressed with ZmCCD8 (fig. S15), making it the strongest candidate for the next biosynthetic step. Analysis of root exudate from a zmmaxxa zmmaxxe double mutant (supplementary materials) showed wild-type levels of zealactone, thus excluding both homologs from being the biosynthetic genes we sought (fig. S13B). Earlier research also demonstrated that ZmMAXIb (GRMZM2G023952) converts CL to CLA more efficiently than does ZmMAXIa (GRMZM2G018612) or ZmMAXII (GRMZM2G070508) (18). The amounts of CL in leaf extracts decreased after cofiltration of ZmMAXIb with ZmD27, ZmCCD7, and ZmCCD8 in N. benthamiana, (Fig. 2A), confirming that ZmMAXIb uses CL as a substrate (18). However, only traces of the expected product, CLA, were detected in this expression system (Fig. 2B and fig. S14B). To resolve this enigma, N. benthamiana extracts were analyzed with LC–quadrupole time-of-flight (QTOF)–MS. Prominent peaks of CLA-hexose and CLA-dihexose conjugates were detected in samples expressing the maize CL pathway genes together with ZmMAXIb. These conjugates were lacking in control samples and other gene combinations (Fig. 2C and table S4). Similar conjugation has been demonstrated for the transient production of other acidic compounds with N. benthamiana (36, 37).

For selection of remaining candidate genes, we combined three approaches: (i) MR–based coexpression with ZmCCD8 and ZmMAXIb as baits (fig. S15), (ii) coexpression modules in MaizeGGM2016 (38), and (iii) differential gene expression in a zmccd8 mutant (Fig. 2D). For the latter, we assumed that SL pathway genes downstream of CCD8 would be transcriptionally regulated in the zmccd8 mutant (39). The ZmCCD7, ZmCCD8, and ZmMAXIb genes clustered together in MaizeGGM2016 module 091, suggesting that the 32 other genes in this module were candidates for the missing pathway genes (table S5). In the roots of zmccd8 seedlings, 1301 genes were differentially expressed (DEGs) (less than or equal to twofold change, false discovery rate (FDR) < 0.05) compared with the B73 wild type (tables S5 and S6). These three approaches shared a seven-gene overlap (Fig. 2D and table S2) in which three [GRMZM2G033126, GRMZM2G158342, and GRMZM2G023952 (ZmMAXIb)] formed a putative gene cluster on chromosome 3 (Fig. 2D and E, and fig. S15) (39). Genes homologous to these also cluster in other Poaceae species (fig. S16), but the functional importance is unknown. So too is the identity of SLs produced by some of these species, such as switchgrass.

ZmCLAMT1 is a carlactonic acid methyltransferase

Because SLs zealactone and zeapyrrolactone are methyl esters, their proposed precursor has been methyl carlactonoate (MeCLA) (24). Thus, we sought a methyltransferase gene that causes the formation of MeCLA from CLA. We bioinformatically identified a top candidate (GRMZM2G033126) (Fig. 2, D and E), which
Fig. 2. Identification of gene candidates for maize strigolactone biosynthesis. (A and B) Representative MRM-LC/MS/MS chromatograms of carlactone (CL), [M+H]+ m/z 303 > 97 (A), and carlactonoic acid (CLA), [M-H]− m/z 331 > 113 (B), in N. benthamiana leaf samples transiently expressing maize strigolactone (SL) precursor pathway genes. (C) Untargeted metabolomics to identify CLA conjugates in N. benthamiana leaf samples, m/z 539.21: CLA + hexose + formic acid − H2O; m/z 701.26: CLA + 2 hexose + formic acid − H2O (D) Venn diagram of candidate gene numbers from several analyses: module091 from maizeGGM, genes differentially expressed in zmccd8 roots (compared with wild type), and the top 100 genes coexpressed with ZmCCD8 and ZmMAX1b (34, 35). (E) Putative SL biosynthetic gene cluster on chromosome 3 consisting of ZmCLAMT1, ZmMAX1b, and ZmCYP706C37, adapted from screenshot from UCSC Genome Browser on Z. mays (B73 RefGen_v3) Assembly (zm3) (http://genome.ucsc.edu) (39). (F) Representative chromatograms of methylcarlactonoic acid (MeCLA), [M+H]+ m/z 347 > 97, in N. benthamiana leaf samples. STD, standard; EV, empty vector infiltrated control sample. CL pathway, maize carlactonoic biosynthetic pathway genes, ZmDeZ7, ZmCCD7, and ZmCCD8. CLA pathway, CL pathway genes + ZmMAX1b. MeCLA pathway, CLA pathway genes + ZmCLAMT1. Bars indicate mean ± SEM.

Successfully produced MeCLA in N. benthamiana when transiently expressed together with genes for the maize CLA pathway (Fig. 2F). We therefore identified GRMZM2G033126 as a carlactonoic acid methyltransferase gene and named the enzyme ZmCLAMT1 (Fig. 1A). The maize gene is an ortholog of At4g36470, which was recently found to encode a carlactonoic acid methyltransferase CLAMT in Arabidopsis (40, 41).

ZmCYP706C37 catalyzes formation of several maize strigolactones

The other candidate genes were coinflected by different combinations of precursor-pathway genes. Coinfiltration of ZmCYP706C37 (GRMZM2G158342) (42) by those encoding the MeCLA pathway decreased levels of MeCLA, indicating that this CYP can use MeCLA as a substrate (fig. S17A) and produce zealactol (Fig. 3A and fig. S2). To check for other possible biosynthetic pathways, we also coexpressed ZmCYP706C37 with genes encoding the CL pathway enzymes. This combination resulted in production of zealactol (Fig. 4A and fig. S17B).

Formation of both zealactone and zealactol involves complex rearrangement of the SL A ring and, for zealactol, a hydroxylation at C19 as well. To exclude the possibility of endogenous enzymes from N. benthamiana contributing to these complex conversions, we expressed ZmCYP706C37 in yeast, isolated its microsomes, and analyzed product formation with different substrates (Figs. 3B and 4B). This approach confirmed that ZmCYP706C37 can convert MeCLA to zealactone and CL to zealactol (Fig. 1A).

To form zealactone from MeCLA, ZmCYP706C37 must catalyze several consecutive oxidative reactions with 3-hydroxy-MeCLA and 3-oxo-MeCLA as putative intermediates (Figs. 1A and 3C). The latter two compounds were previously synthesized as intermediates in the total synthesis of heliolactone (43). We used them here as substrates in our ZmCYP706C37-expressing yeast-microsome assay, and both were successfully converted to zealactone (Fig. 3D). We developed an MRM method for detection of these compounds (fig. S2) and identified them as intermediate products in the conversion of MeCLA to zealactone (fig. S18). Moreover, analysis of maize root exudate revealed that 3-oxo-MeCLA is also a natural maize SL previously referred to as compound 6 (fig. S19 and Fig. 1A) (24). These results demonstrate that a single enzyme, ZmCYP706C37, can catalyze the many oxidative steps necessary for the conversion of MeCLA to zealactone that were previously hypothesized to require several enzymes (Figs. 1A and 3C) (24).

For additional insight into the parallel biosynthetic pathway of CL to zealactol, we further analyzed samples from N. benthamiana and yeast microsome assays with untargeted metabolomics and MR-LC/MS/MS. This process revealed another putative intermediate, 3-oxo-19-hydroxy-CL (compound 7) (Fig. 1A and figs. S2 and S20 and table S7). LC-QTOF-MS analysis showed that the accurate mass of compound 7 is consistent with its putative structure (fig. S20). On the basis of these data, we included compound 7 as an intermediate in the postulated steps required to convert CL to zealactol (Fig. 4C and fig. S21).
Fig. 3. Zealactone biosynthesis. (A) Representative MRM–LC/MS/MS chromatograms of zealactone, [M+H]⁺ m/z 377 > 97, in N. benthamiana leaf samples. (B and D) Representative MRM–LC/MS/MS chromatograms of zealactone from in vitro assays with yeast microsomes expressing ZmCYP706C37 or empty vector (EV) with methyl carlactonoate (MeCLA), 3-hydroxy-MeCLA, or 3-oxo-MeCLA as substrate. (C) Proposed enzymatic conversion of methyl carlactonoate (MeCLA) to zealactone.

Moreover, agroinfiltration of the CL pathway genes with ZmCYP706C37 and ZmMAX1b resulted in production of ZA, a result also confirmed with LC-QTOF-MS (Fig. 4, C and D, and fig. S22).

Last, analysis of root exudate from a zmcyp706c37 mutant [EMS4-045ad8, stop-codon gained (fig. S23A)] showed no detectable levels of zealactol, ZA, zealactone, or three other SLs derived from the latter (fig. S23B) (44). Although 3-oxo-MeCLA was detectable in the mutant exudate, it was present at a much lower level than in that of the wild type. Instead, CLA and MeCLA accumulated in the mutant exudate, whereas they are absent in the wild type exudate (fig. S23, C and D). Together, these data support our functional characterization of ZmCYP706C37.

Biosynthetic control of the maize strigolactone blend

To determine how the different maize SLs are biosynthetically related, we applied 3-hydroxy-MeCLA, 3-oxo-MeCLA, and zealactol to seedlings...
were used as positive and negative control, respectively. (Emerged Striga biosynthesis (ZmMAX1b together with ZmCLAMT1 (Fig. 1A and fig. S25). Application of zealactone to by ZmMAX1b and fig. S26B). This line produces zealactone in fluridone-resistant plants led to the formation of zealactone and zealactone-derived SLs, whereas the level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C).

Next, we sought mechanisms underlying the distinctive maize SL profile of NP2222 (Fig. S26). This line produces zealactone in fluridone-resistant plants led to the formation of zealactone and zealactone-derived SLs, whereas the level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C). The level of zealactol was higher than in the W22 control (Fig. 5C).

Conclusions

We have shown that two parallel SL biosynthetic pathways operate in maize and that both pathways produce the major maize SL, zealactone. Changes in flux through these pathways can alter the maize SL profile by shifting the balance between zealactone and zealactol.
plus ZA. Zealactol and ZA induce much less Striga germination, thus imparting a strong reduction in Striga infection to genotypes that exude more zealactol and ZA than zealactone. Future research should investigate whether these changes in the SL blend affect colonization by AM fungi, which was not observed for lgs sorghum (16). Our results offer a perspective for breeding Striga resistance through modification of the SL blend in maize and thus potentially reducing the devastating effects of this parasitic weed in Africa.

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