Maize resistance to witchweed through changes in strigolactone biosynthesis


DOI
10.1126/science.abq4775

Publication date
2023

Document Version
Final published version

Published in
Science

License
Article 25fa Dutch Copyright Act (https://www.openaccess.nl/en/in-the-netherlands/you-share-we-take-care)

Citation for published version (APA):
Maize resistance to witchweed through changes in strigolactone biosynthesis

C. Li1, L. Dong1, J. Durairaj1, J.-C. Guan3, M. Yoshimura4,6, P. Quinodoz5, R. Horber5, K. Gaus5, J. Li7, Y. B. Setotaw7, J. Qi5, H. De Groot6, Y. Wang2, B. Thombiason6, K. Floková9, A. Walmsley1, T. V. Charnikhova3, A. Chojnacka1, S. Correia de Lemos2,10, Y. Ding11, D. Skibbe12, K. Hermann5, C. Screpanti5, A. De Mesmaeker5, A. Menkir13, M. Medema2, A. D. J. Van Dijk2, J. Wu7, K. E. Koch3, H. J. Bouwmeester1a

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 germination collection, we identified two strigolactones, zeacalanol and zeacalanolic acid, which stimulate more Striga germination than the major maize strigolactone, zeaxanthin. We then showed that a single cytochrome 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 ZmCLAMT1, can change strigolactone composition and reduce Striga germination and infection. These results offer prospects for breeding Striga-resistant maize.

PREVIOUS PAGE

PLANT SCIENCE

Maize roots exude at least six SLs, two of which have been structurally identified as zealactone and zeapyranolactone (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 spectrometry (LC/MS/MS) (Figs. 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 zealactol (compound 5) and zealaconic acid (ZA) (the other unknown SL) (figs. S9 and S12). Bioassay of Striga germination showed that both zealactol and ZA were less inductive than zeaxanthol (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 carlactone 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 zmccd8 mutants (in W22 and Mo17 backgrounds) (30). Zeaxanthol was not detected, although it was the major SL in...
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 MAX1 homologs, only ZmMAX1b tightly coexpressed with ZmCCD8 (fig. S15), making it the strongest candidate for the next biosynthetic step. Analysis of root exudate from a zmmax1a zmmax1b 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 ZmMAX1b (GRMZM2G023952) converts CL to CLA more efficiently than does ZmMAX1a (GRMZM2G0158657) or ZmMAX1c (GRMZM2G070508) (18). The amounts of CL in leaf extracts decreased after cofiltration of ZmMAX1b with ZmD27, ZmCCD7, and ZmCCD8 in N. benthamiana, (Fig. 2A), confirming that ZmMAX1b 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 ZmMAX1b. 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 ZmMAX1b 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 ZmMAX1b genes clustered together in MaizeGGM2016 module 091, suggesting that the 92 other genes in this module were candidates for the missing pathway genes (table S5). In the roots of zmcd8 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 GRMZM2G070508 (GRMZM2G023952) (ZmMAX1b) formed a putative gene cluster on chromosome 3 (Fig. 2, D 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 carlactonoic acid methyltransferase

Because SLs zealactone and zeapyranolactone are methylesters, 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...
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 carlactonic 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 carlactonic acid methyltransferase CLAMT in Arabidopsis (40, 41).

ZmCYP706C37 catalyzes formation of several maize strigolactones

The other candidate genes were co-infiltrated by different combinations of precursor-pathway genes. Co-infiltration 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. S1A) and produce zealactone (Fig. 3A and fig. S2). To check for 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. S16). 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 MRM-LC-MS/MS. This process revealed another putative intermediate, 3-oxo-19-hydroxy-CL (compound 7) (fig. S24) and 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).
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 carlactonate (MeCLA), 3-hydroxy-MeCLA, or 3-oxo-MeCLA as substrate. (C) Proposed enzymatic conversion of methyl carlactonate (MeCLA) to zealactone.

Fig. 4. Zealactol and zealactonoic acid biosynthesis. (A) Representative MRM–LC/MS/MS chromatograms of zealactol, [M+H⁺/m/z 331 > 97, in N. benthamiana leaf samples. (B) Representative MRM–LC/MS/MS chromatograms of zealactol from in vitro assays with yeast microsomes expressing ZmCYP706C37 or empty vector (EV) with carlactone (CL) as substrate. (C) Reactions from CL to zealactol and ZA catalyzed by ZmCYP706C37 and ZmMAX1b. Structure in square brackets is putative. (D) Representative MRM–LC/MS/MS chromatograms of ZA, [M+H⁺/m/z 363 > 249, in N. benthamiana leaf samples. STD, standard; EV, empty vector control. CL pathway, maize carlactone biosynthetic pathway genes, ZmD27, ZmCCD7, and ZmCCD8.

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.
To analyze biological consequences of the different SL profiles, several maize lines were selected for *Striga* germination and infection assays. The NP2222 root exudate induced much lower germination than that of NK Falkone. Results were consistent with their respective SL profiles and differences in germination-inducing activity of the individual SLs (Figs. 1C and 5A and fig. S26D). CML52 and NC358, both with high proportions of zealactol and ZA, induced significantly less *Striga* germination than did CML69 and Ky21, which produced mostly zealactone despite similar total SL peak areas (Figs. 1C and 5A, and fig. S27, A and B). These differences were also reflected in a *Striga* infection assay with a containerized system, in which *Striga* emergence was less for low-zealactone genotypes (Fig. 5B). In addition to their SL blend, these lines may have other genetic differences that could affect these results. However, we also analyzed a gene-suppression mutant of *ZmMAX1b* (transposon insertion in a W22 background) (fig S28, A and B). This mutant exuded significantly less izealactone and zealactone-derived SLs, whereas the level of zealactol was higher than in the W22 control (Fig. 5C). The *zmmax1b* mutant also induced less *Striga* germination and emergence (Fig. 5E). Results confirm that a change in activity of specific SL biosynthetic enzymes in maize can change the SL composition and confer *Striga* resistance. Although the underlying mechanisms are completely different, these findings resemble those of *Igs* sorghum (I6) and present a promising prospect for *Striga* resistance breeding in maize. The *zmmax1b* mutant did not exhibit a branching phenotype, in contrast to *znccd8* (fig S28C). Also, *zmccp706c37*, which is located parallel to or downstream of *ZmMAX1b*, did not display an obvious branching phenotype either. This all suggests that the downstream SLs are not nor precursors of the branching inhibiting hormone and are therefore safe breeding targets that will not result in unwanted pleiotropic effects.

**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 of another commercial line, NK Falkone, which were treated with fluridone, an inhibitor of SL biosynthesis (A5). Each of these three compounds complemented zealactone production (fig. S24A), confirming that they can serve as biosynthetic precursors for zealactone. Combined transient expression of *ZmMAX1b* and *ZmCLAMT1* in *N. benthamiana* leaves and subsequent infiltration of zealactol also showed that the latter can be converted to zealactone by *ZmMAX1b* together with *ZmCLAMT1* (Fig. 1A and fig. S25). Application of zealactone to fluridone-treated plants led to the formation of zeapyranolactone and two other maize SLs, designated compounds 3 and 4, suggesting that zealactone is their precursor (Fig. 1A and fig. S24, B to D) (24).

Next, we sought mechanisms underlying the distinctive maize SL profile of NP2222 (fig. S26). This line produces zealactone in fluridone-treated seedlings, as does NK Falkone, but only from MeCLA and 3-oxo-MeCLA, not from zealactol (figs. S24A and S26A), suggesting inactivity of MAX1b and/or CLAMT1. As previously noted, ZA accumulated in the root exudate of NP2222 (Fig. 1B and fig. S26D), indicating dysfunction of CLAMT1. Zealactol added to either NK Falkone or NP2222 was converted to ZA, showing that ZmMAX1b is active in NP2222 (fig. S26, B and C). Inspection of the CLAMT1 sequence in a proprietary NP2222 genome database revealed a large insertion in the second exon of this gene, and reverse transcriptase polymerase chain reaction (RT-PCR) showed that regions flanking the insertion were not transcribed (fig. S26E). These collective data indicate dysfunction of CLAMT1 in NP2222.
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 Igs 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.

REFERENCES AND NOTES

35. S. Stelflug et al., Plant Genome 9, e2015004.0025 (2016).
42. Y. Li, K. Wei, BMC Plant Biol. 20, 93 (2020).

ACKNOWLEDGMENTS

We acknowledge S. Al Babili from King Abdullah University of Science and Technology and D. Wieck-Reichhart from the University of Strasbourg for helpful discussions, as well as L. Hagemann from Syngenta for his support in NMR analyses and interpretation.

Funding: This work was funded by the China Scholarship Council (CSC) PhD scholarship 201706300041 (C.L.), the European Research Council (ERC) Advanced grant CHEMCOMRHIZO 670211 (H.J.B.), the Dutch Research Council (NWO/OCW) Gravitation program Harnessing the second genome of plants (MiCRop) 024.004.014 (H.J.B.), the Marie Curie fellowship NEMHATCH 793795 (L.D.). K.E.K and J.G. acknowledge funding from the US National Science Foundation (NSF) Plant Genome Research Program (PGRP) 1421300 and 1746105.

AUTHOR CONTRIBUTIONS

C.L., L.D. and H.J.B. conceived and designed the project. C.L. discovered and characterized the candidate genes, grew the plants, collected and analyzed the root exudate, cloned the genes, performed agroinfiltration, yeast microsome assay and plant compound treatment assays, and coordinated the project; K.F., T.V.C. and A.C. developed LC-MS methods and helped with SL analysis; T.V.C., J.D., and A.D.J.V.D. helped to establish the biosynthesis mechanisms; I.G. and K.E.K. developed and provided maize seeds (ZAMC08, zmcyp706c37 and zmmax1azmmax1c) and analyzed RNA-seq and related data; B.T. and L.D. supported the metabolomics analysis; M.Y., K.G., A.D.M. synthesized zealactol and provided zealactone, 3-hydroxy-MeCLA, and 3-oxo-MeCLA; P.Q., R.H., and A.D.M. synthesized zealoactonic acid; J.L., Y.B.S., and R.H. wrote the manuscript, with contributions from other authors.

Competing interests: M.H.M. is a consultant to Corteva Agriscience, but that company was not involved in this work. All the other authors declare that they have no competing interests.

Data and materials availability: The maize mutants zmcc08 and zmmax1azmmax1c were obtained via a material transfer agreement (MTA) with the University of Florida Board of Trustees. The RNA-seq data of zmcc08 and Z73 root tissues are available in the NCBI database (BioProject PRJNA757767) under accession numbers SRR15613590, SRR15613591, SRR15613593, SRR15613595, and SRR15613597. All the other data are presented in the main text and in the Supplementary Materials.

License information: Copyright © 2023 the authors. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abb4775

Materials and Methods

Figs. S1 to S28

Tables S1 to S8

References (46–70)

View/request a protocol for this paper from Bio-protocol.

Submitted 12 April 2022; accepted 30 November 2022.

10.1126/science.abb4775