Trends in
Plant Science

CellPress

Spotlight

Caffeoylputrescine-hexenal-mediated nonhost resistance against leafhoppers

Rajinikanth Mohan,[1] Sara Spells,[1] Daowen Wang,[2,*] and Zheng Qing Fu[3,*]

Despite its critical role in repelling damaging insects, our understanding of nonhost resistance against herbivores remains very limited. Recently, Bai et al. identified a novel caffeoylputrescine-green leaf volatile (GLV) compound in wild tobacco plants that confers nonhost resistance to Empoasca leafhoppers through high-throughput multi-omics analyses.

Even though many pathogens and pests could potentially cause severe diseases and damages to their host plants, plants in nature often appear healthy. In fact, due to the contribution of nonhost resistance, most plants are naturally resistant to most pathogens and pests. Nonhost resistance represents a major barrier, which repels damaging biotic stressors. Unfortunately, at least partially due to lack of field studies, our current understanding on this very important topic, especially nonhost resistance to herbivores, remains very limited.

Large-scale omics studies have been gradually adopted to unveil important traits in plants. Recently, an exciting paper was published as the cover story in Science. Through natural history-guided omics, Bai et al. identified a novel caffeoylputrescine-GLV compound in wild-type tobacco that confers nonhost resistance to Empoasca leafhoppers [1].

Bai et al. took innovative approaches to identify defense chemicals that are associated with nonhost resistance of wild tobacco plants (Nicotiana attenuata) against Empoasca leafhoppers [1]. The authors generated 650 (26 × 25) recombinant inbred lines (RILs) from 26 parent lines. Bai et al. adopted a forward-genetics strategy to identify genes and metabolites that are induced in 1907 individual plants from the replicated 674 RILs following treatments simulating herbivory. They then built a multi-omics dataset based on high-throughput genomic, transcriptomic, and metabolomic analyses. To dissect the genetics and metabolomic responses of these RILs to Empoasca leafhoppers, the authors focused on the jasmonic acid (JA) pathway to construct a co-association network built from correlations among metabolomes, transcriptomes, phytohormones, and SNPs. JA plays a key role in the resistance of plants, including the genus Nicotiana, against herbivores [2]. Previously, it has been shown that Empoasca leafhoppers use their mouthparts to probe plant leaves to find appropriate food source [3]. If JA-mediated defense is activated, Empoasca leafhoppers leave the plant without causing any damage. However, if the JA pathway is disrupted, these plants become defenseless and will be consumed by Empoasca leafhoppers. The authors discovered that the transcription factor MYB8 controls the production of induced phenolamides such as N-feruloylputrescine and N-caffeoylputrescine. A set of genes, including MYC2 and JAZi, in the JA pathway that positively or negatively correlate with leafhopper numbers and damage were also identified. Excitingly, subsequent structural studies revealed a previously unobserved defense metabolite, which is identified as a caffeoylputrescine-hexenal compound (CPH) (Figure 1A). The half-life of CPH, which is likely responsible for Empoasca resistance, is only 22 h. As identified in this study, CPH is synthesized by a three-pronged pathway in plants (Figure 1A). Caffeoyl-CoA is first synthesized via the phenylpropanoid pathway, then reacts with putrescine synthesized through the polyamine pathway, and is followed by the addition of (Z)-3-hexenal, which is produced from fatty acids by the GLV pathway [4]. This study revealed a new mechanism for the biosynthesis of the insect-repellent CPH in plants through the convergence of herbivore-triggered JA, polyamine, and GLV pathways.

Reverse genetics revealed that MYC2, MYB8, and JAZi coregulate the defense of wild-type tobacco against leafhoppers (Figure 1A) [1]. In irMYC2 (RNAi silenced MYC2), irMYB8, and ovJAZi (overexpression of JAZi) plants, the production of CPH is dramatically reduced (Figure 1B). Consequently, these plants are significantly more susceptible to Empoasca leafhoppers than the empty vector-transformed control plants.

To find out whether CPH indeed confers plant resistance to Empoasca leafhoppers, Bai et al. performed feeding assays. Empoasca decipiens fed with CPH showed close to 100% mortality, while the control diet did not have any adverse effect on E. decipiens [1]. Strikingly, after Bai et al. engineered the CPH biosynthetic pathway into broad bean (Vicia faba) and a wild tomato species (Solanum chilense), these compatible host plants became lethal host plants for Empoasca leafhoppers, confirming that CPH is responsible for leafhopper resistance (Figure 1C). While incubation of CP and (Z)-3-hexenal with purified His-tagged polyphenol oxidase NaPPO1 or NaPPO2 protein yields CPH in vitro, the production of CPH in planta requires an additional berberine bridge enzyme-like 2 (BBL2) gene (Figure 1A).

The authors proposed two possible mechanisms underpinning the crucial roles of CPH in the nonhost resistance against leafhoppers [1]. First, the rapid polymerization of nucleophilic and electrophilic groups in

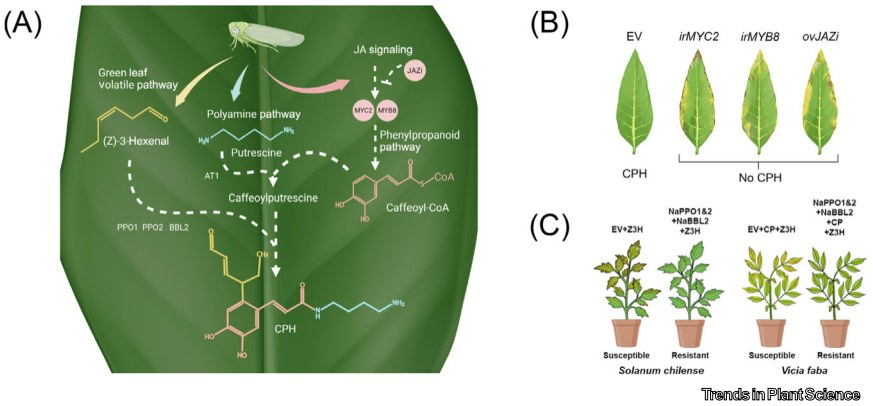

Figure 1. Engineering herbivory resistance by exploiting a three-pronged biosynthetic pathway involved in nonhost resistance. (A) Elucidation of the pathway involved in the synthesis of a caffeoylputrescine-green leaf volatile compound, CPH, in Nicotiana attenuata against Empoasca leafhoppers. The leafhopper-triggered jasmonate (JA) pathway involving the positive regulators, MYC2 and MYB8 transcription factors, and the negative regulator, JAZi repressor, leads to the synthesis of the novel compound CPH by activating the phenylpropanoid pathway. The caffeoyl-CoA synthesized by this pathway is combined with the polyamine pathway product putrescine, through the activity of hydroxycinnamoyl-coenzyme A: putrescine acyltransferase (AT1) to produce caffeoyl putrescine (CP). CP is condensed with the oxylipin pathway/green leaf volatile pathway-derived (Z)-3-hexenal through the action of polyphenol oxidases (PPO1, PP2) and berberine bridge enzyme-like (BBL2) to produce CPH. (B) CPH is required for nonhost resistance to Empoasca leafhoppers. N. attenuata plants express an empty vector (EV) that can produce CPH display resistance to the leafhopper, whereas plants with reduced JA signaling through RNAi inverted repeat-mediated knockdown of MYC2 (irMYC2) and MYB8 (irMYB8), as well as plants overexpressing the repressor JAZi (ovJAZi), show insect damage characterized by chlorosis and browning. (C) Synthetic biology-mediated engineering of herbivory resistance in heterologous plants. Solanum chilense (Solanaceae) and the distantly related broad bean (Vicia faba) (Fabaceae), both of which do not normally produce CPH, are susceptible to Empoasca leafhoppers. Expressing the enzymes involved in the final steps of CPH production provides these plants with resistance to Empoasca leafhoppers as long as the missing substrates are provided. The figure was created with the software BioRender (BioRender.com). Abbreviations: NaBBL2, N. attenuata berberine bridge enzyme-like 2; NaPPO1&2, N. attenuata polyphenol oxidases 1 and 2; Z3H, (Z)-3-hexenal.

CPH could block the mouthpart of invading leafhoppers. Second, the alpha, beta-unsaturated aldehyde may crosslink leafhopper proteins. Further studies will be required to test these two hypotheses. Because CPH is lethal to leafhoppers, it remains to be determined whether CPH has any detrimental effects on human beings and animals.

In total, there are more than 25 000 species and over 3200 genera of leafhoppers [5]. Leafhoppers can not only cause damage to crops, but also transmit viruses, phytoplasmas, and bacteria. For example, potato leafhopper (Empoasca fabae) in the same genus of Empoasca is a serious agricultural pest [6]. Bai et al. made a breakthrough discovery by showing that CPH confers nonhost resistance to E. decipiens [1]. It remains to be determined whether CPH is responsible for nonhost resistance to other genera or species of leafhoppers as well as to herbivores other than leafhoppers. In this paper, Bai et al. discovered that CPH, which is related to the JA pathway, plays a key role in nonhost resistance against a sucking insect E. decipiens. It is well known that the JA pathway is activated by chewing insects [2]. Several open questions remain. Does CPH contribute to nonhost resistance against both chewing insects and sucking insects? Is there a major difference between the mechanisms underlying nonhost resistance against chewing insects and sucking insects?

A better understanding of nonhost resistance to pathogens and pests not only provides promising tools for plant protection, but also offers great opportunities for studying how adapted pathogens and herbivores overcome nonhost resistance and cause harm to plants. Despite the fact that many studies related to nonhost resistance against plant pathogens have been published [7,8], we still have limited understanding of the key metabolites associated with nonhost resistance to most plant pathogens. High-throughput omics approaches that are deployed in this study could be adopted to identify crucial genes and metabolites that are involved in nonhost resistance against many other important pathogens and pests, including the potato late blight oomycete pathogen Phytophthora infestans, the rice blast fungal pathogen Magnaporthe oryzae, root-knot nematodes, sucking insects, such as aphids, and chewing insects, such as cotton bollworms (Helicoverpa armigera) [9]. It is worthwhile mentioning that large-scale omics approaches could also be promising tools for the study of nonhost resistance against psyllids, which act as vectors for citrus greening disease or huanglongbing. Currently, there is no cure for this disease.

The JA pathway plays an essential role in plant resistance against herbivores and necrotrophic pathogens, and the salicylic acid (SA) pathway is required for plant defense against biotrophic pathogens and hemi-biotrophic pathogens [10]. In this study, Bai et al. found that CPH associated with the JA pathway plays a critical role in nonhost resistance against Empoasca leafhoppers. It will be interesting to find out whether a JA-associated metabolite is also associated with nonhost resistance to necrotrophic pathogens. Likewise, an SA-induced chemical may be closely related to nonhost resistance against biotrophic and hemi-biotrophic pathogens.

In conclusion, the authors in this groundbreaking study demonstrate how the marriage of cutting-edge genetics, multi-omics, and genetic engineering can be

used effectively to not only understand nonhost resistance in nature but also highlight the potential of nonhost resistance as a promising tool to engineer durable resistance. Climate change can increase the proliferation of insect herbivores like leafhoppers and through their large numbers and quick generation times, they have the potential to transform from being opportunistic passersby to damage-causing pests [11]. In this scenario, it is incumbent on us to arm crop plants; strategies like the one described by Bai et al. have the potential to turn host plants into nonhosts [1], bringing down the financial and environmental cost of agriculture through reduced input and pesticide use.

Acknowledgments

The work is supported by the grant from National Science Foundation (IOS-1758994) to Z.Q.F. and the Helping Today grant from Erie Community Foundation (2022) to R.M. We thank Johannes Stratmann for critical reading of this manuscript.

Declaration of interests

The authors declare no competing interests.

[1]Department of Biology, Mercyhurst University, Erie, PA 16546, USA
[2]State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy and Center for Crop Genome Engineering, Henan Agricultural University, Longzi Lake Campus, Zhengzhou 450046, China
[3]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

*Correspondence:
dwwang@henau.edu.cn (D. Wang) and
zfu@mailbox.sc.edu (Z.Q. Fu).

https://doi.org/10.1016/j.tplants.2022.05.009

Published by Elsevier Ltd.

References

1. Bai, Y. et al. (2022) Natural history-guided omics reveals plant defensive chemistry against leafhopper pests. Science 375, eabm2948
2. Wang, L. and Wu, J. (2013) The essential role of jasmonic acid in plant–herbivore interactions – using the wild tobacco Nicotiana attenuata as a model. J. Genet. Genomics 40, 597–606
3. Kallenbach, M. et al. (2012) Empoasca leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. Proc. Natl. Acad. Sci. U. S. A. 109, E1548–E1557
4. Scala, A. et al. (2013) Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. Int. J. Mol. Sci. 14, 17781–17811
5. Hanchipura Mallesh, M.S. et al. (2020) DNA barcoding and phylogenetic analysis of leafhoppers associated with aster yellow disease on China aster, marigold and chrysanthemum. Mitochondrial DNA A DNA Mapp. Seq. Anal. 31, 64–72
6. Taylor, R.A.J. and Shields, E.J. (2018) Revisiting potato leafhopper, Empoasca fabae (Harris), migration: implications in a world where invasive insects are all too common. Am. Entomol. 64, 44–51
7. Panstruga, R. and Moscou, M.J. (2020) What is the molecular basis of nonhost resistance? Mol. Plant Microbe Interact. 33, 1253–1264
8. Senthil-Kumar, M. and Mysore, K.S. (2013) Nonhost resistance against bacterial pathogens: retrospectives and prospects. Annu. Rev. Phytopathol. 51, 407–427
9. Wang, N. (2019) The citrus huanglongbing crisis and potential solutions. Mol. Plant 12, 607–609
10. Spoel, S.H. et al. (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. U. S. A. 104, 18842–18847
11. Skendžić, S. et al. (2021) The impact of climate change on agricultural insect pests. Insects 12, 440