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Understanding the chemical language mediating maize immunity and environmental adaptation

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Summary

Diverse networks of specialized metabolites promote plant fitness by mediating beneficial and antagonistic environmental interactions. In maize (*Zea mays*), constitutive and dynamically formed cocktails of terpenoids, benzoxazinoids, oxylipins, and phenylpropanoids contribute to plant defense and ecological adaptation. Recent research has highlighted the multifunctional nature of many specialized metabolites, serving not only as elaborate chemical defenses that safeguard against biotic and abiotic stress but also as regulators in adaptive developmental processes and microbiome interactions. Great strides have also been made in identifying the modular pathway networks that drive maize chemical diversity. Translating this knowledge into strategies for enhancing stress resilience traits has the potential to address climate-driven yield losses in one of the world's major food, feed, and bioenergy crops.

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I. Introduction

Predicted maize (*Zea mays*) harvest losses nearing 30–40% annually underscore the severe impact of increasing pest, disease, and climate pressures on agricultural productivity (Bailey-Serres *et al.*, 2019; Savary *et al.*, 2019; Yactayo-Chang & Block, 2023). As a major staple food, animal feed, and bioenergy crop, reduced maize production directly impacts global food and energy security,

disproportionately affecting densely populated and food-deficient regions (Wani *et al.*, 2023). Considering these multifactorial environmental stressors, understanding the molecular mechanisms that govern maize innate immunity and stress resilience is imperative to enable breeding and engineering strategies.

Analogous to a language, plants employ a myriad small molecule specialized metabolites to communicate with their environment. These compounds are often of narrow taxonomic distribution, underly tight spatiotemporal and environmental regulation, and have critical functions in environmental interactions that enable

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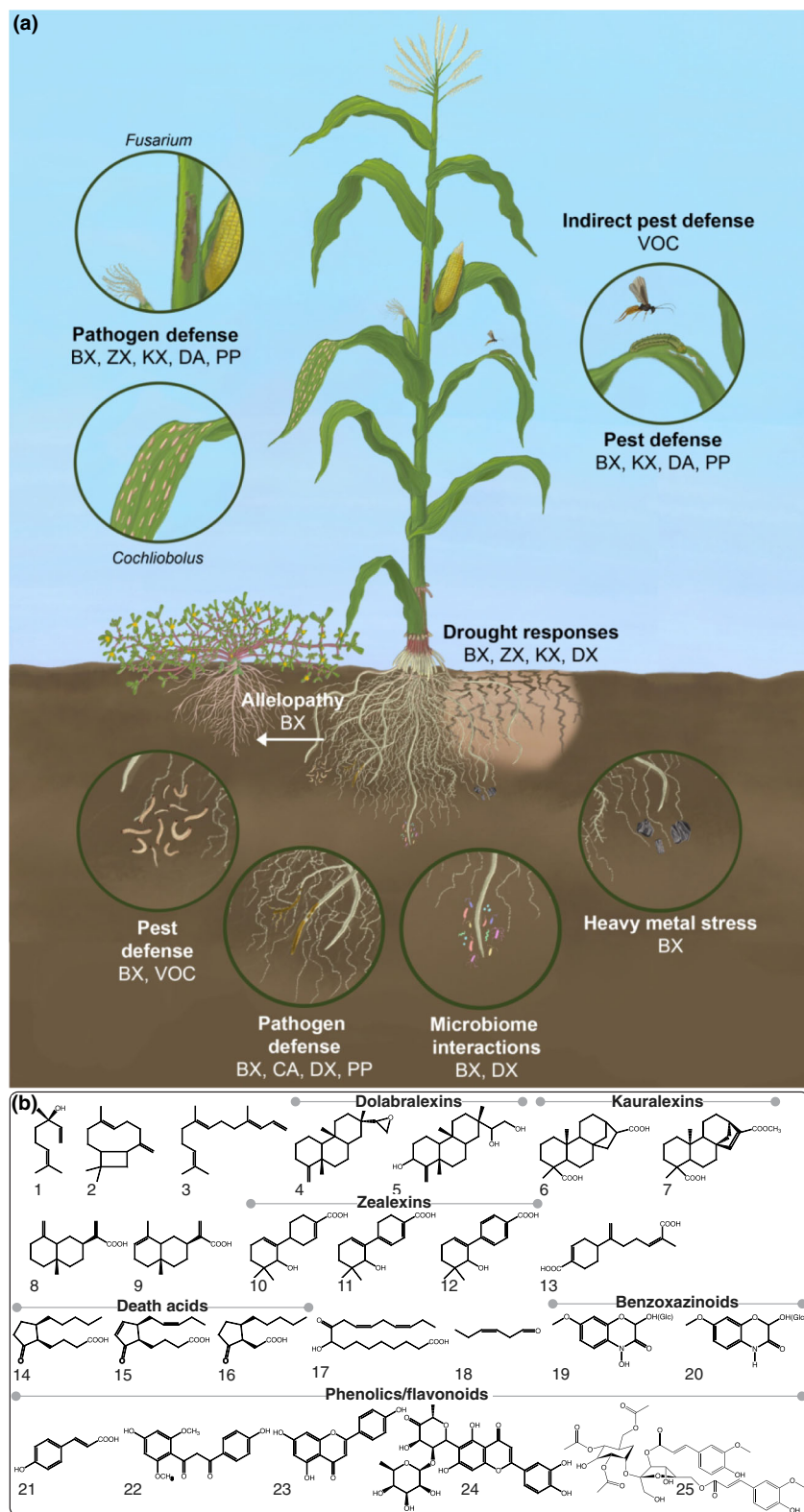


Fig. 1 Diversity and function of maize specialized metabolites. (a) Maize (*Zea mays*) specialized metabolites serve multiple functions beyond well-defined phytoalexin bioactivities that include allelopathy, regulation of abiotic stress responses and microbiome interactions, and contribution to adaptive, developmental processes that are to be fully understood. (b) Representative structures of major maize specialized metabolites. BX, benzoxazinoids; CA, costic acids; DA, death acids; DX, dolabralaxins; KX, kauralexins; PP, phenylpropanoids; VOC, volatile organic compounds (terpenoids, phenolics, green leaf volatiles); ZX, zealexins. 1, linalool; 2, β -caryophyllene; 3, TMTT; 4, epoxydolabrene; 5, trihydroxydolabrene; 6, kauralexin A2; 7, kauralexin B2; 8, α -costic acid; 9, zealexin A3; 10, zealexin B3; 11, zealexin C3; 12, zealexin D2; 13, DA⁰-4:0; 14, DA⁰-4:1; 15, DA⁰-2:0; 17, 9-hydroxy-10-oxo-12,15-octadecadienoic acid; 19, DIMBOA; 20, HMBOA; 21, *p*-coumarate; 22, xilonenin; 23, apigenine; 24, maysin; 25, smiglaside.

plants to thrive under changing ecological conditions (Gershenson & Dudareva, 2007). Maize specialized metabolites that serve as core components mediating environmental interactions include terpenoids, benzoxazinoids, oxylipins, and phenylpropanoids

(Christensen *et al.*, 2015; Zhou *et al.*, 2018; Block *et al.*, 2019; Fig. 1a,b). In addition to well-studied phytoalexin functions in pest and pathogen defense, recent studies suggest roles of maize specialized metabolism in abiotic stress responses and cooperative

interorganismal interactions (Schmelz *et al.*, 2014; Yactayo-Chang & Block, 2023). Fueled by advanced multi-omics, genetics, and synthetic biology technologies, the past decade of research has transformed our understanding of the diversity, biosynthesis, and function of maize specialized metabolites. Collectively, these efforts illustrate that genome evolution through repeated gene duplication and functional divergence has given rise to enzyme families composed of mechanistically related yet functionally distinct members (Fig. 2a,b; Zi *et al.*, 2014; Ding *et al.*, 2019, 2020). By integrating these enzyme modules into dynamic pathway networks, plants produce diverse, stress-responsive metabolite blends (Zerbe & Bohlmann, 2015; Lacchini & Goossens, 2020). This review highlights recent progress in our understanding of the modular biosynthesis and function of specialized metabolites that govern maize environmental interactions.

II. Maize specialized metabolites play multifaceted roles in plant defense, signaling, and development

Maize is impacted by various environmental factors, with insect pests, pathogens, drought, heat, and poor soil quality being major causes of reduced yield (Savary *et al.*, 2019; Yang *et al.*, 2023). As new metabolic pathways are being discovered, a functional landscape of interconnected long-distance and local defensive and cooperative interactions and other regulatory mechanisms emerges.

Identified in maize and other monocot and dicot plants in the 1950s, the functions of indole-derived benzoxazinoids in allelopathic interactions and in countering pests and microbial diseases are well-defined (Zhou *et al.*, 2018; Erb & Kliebenstein, 2020; Florean *et al.*, 2023; Fig. 1a,b). Recent studies demonstrated broader benzoxazinoid functions in mediating the uptake and chelation of heavy metals, directly affecting crop nutrition (Hu *et al.*, 2018; Caggia *et al.*, 2024). Furthermore, signaling functions of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in pathogen- and insect-elicited callose deposition have been shown (Yang *et al.*, 2023). Maize benzoxazinoids also influence developmental processes, including root/shoot growth and root architecture, via modulating hormonal signaling pathways and root-microbiome interactions (Kudjordjie *et al.*, 2019; Erb & Kliebenstein, 2020; Cadot *et al.*, 2021; Thoenen *et al.*, 2023). Notably, variable levels of benzoxazinoid exudation across maize lines impact the rhizosphere and endosphere microbiome (Wang *et al.*, 2022). Recent field studies of benzoxazinoid-deficient maize lines showed that root-exuded benzoxazinoids reduce adverse plant-soil feedback, leading to yield increases in wheat and possibly other crops (Gfeller *et al.*, 2024).

Although clear overlap exists, benzoxazinoids serve as primary chemical defenses in maize seedlings, while other metabolite groups dominate at later developmental stages. Particularly, a diverse array of specialized terpenoids are key components of maize immunity and stress resilience (Fig. 1a,b). Emission of volatile terpenoid blends, including linalool, 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), and β -caryophyllene, mediates indirect herbivory defenses by attracting parasitoids or predators of invading insect pests (Yactayo-Chang & Block, 2023). Such tritrophic interactions

are not limited to airborne metabolites. For instance, β -caryophyllene released from roots aids in recruiting predatory nematodes in response to Western corn rootworm (*Diabrotica virgifera*) infestation (Degenhardt *et al.*, 2009). Additionally, α/β -costic acids, a group of sesquiterpenoid acids first identified in *Eupatorium capillifolium* (Rao & Alvarez, 1981), have been demonstrated to confer pathogen and insect pest resistance in maize stems and roots (Christensen *et al.*, 2016; Ding *et al.*, 2017). Beyond these metabolites also occurring in other species, maize produces several groups of species-specific terpenoids, which act as direct pathogen and pest defenses at the site of attack (Schmelz *et al.*, 2014). Kauralexin diterpenoid acids serve as feeding deterrents to impede insect pests such as the European corn borer (*Ostrinia nubilalis*) and function as potent antibiotics against major pathogens, including species of *Fusarium*, *Cochliobolus*, *Rhizopus*, *Colletotrichum*, and *Aspergillus* (Schmelz *et al.*, 2011; Ding *et al.*, 2019). Likewise, maize-specific sesquiterpenoid acids, termed zealexins, confer quantitative resistance to several maize pathogens in stems and roots (Huffaker *et al.*, 2011; Ding *et al.*, 2020). Notably, the antibiotic potency against different pathosystems differs across structurally distinct terpenoids. For example, the pathogen-induced accumulation and antibiotic efficacy of A- and B-series kauralexins varies in the interaction with different pathogens (Schmelz *et al.*, 2011). Likewise, zealexins A1, 3, and 4 exhibit antibiotic activity, whereas zealexin A2 (which solely differs in the position of the hydroxy group) is inactive (Huffaker *et al.*, 2011; Ding *et al.*, 2020). Similarly, dolabralixin diterpenoids show pathogen-elicited root accumulation and exudation in select maize lines, and the dolabralixins, epoxydolabranol, and trihydroxydolabrene exhibit strong antifungal activity *in vitro* (Mafu *et al.*, 2018). However, a dolabralixin-deficient mutant (*Zmksl4*) showed no pathogen-susceptible phenotype yet featured an altered root-to-shoot ratio and root architecture, suggesting that not only benzoxazinoids but also terpenoids contribute to root-microbe interactions (Huang & Osbourn, 2019; Murphy *et al.*, 2021, 2023). Two observations support this hypothesis. First, the diterpenoid-deficient *anther ear 2* (*Zman2*) mutant shows an altered rhizosphere microbiome in field studies (Murphy *et al.*, 2021). Second, dolabralixins accumulate in plants grown in field soil but are largely absent in potting soil, pointing toward the presence of the microbiome or soil pathogens as a factor in diterpenoid accumulation (Murphy *et al.*, 2023). It then appears plausible that the drought-induced accumulation of root terpenoids is associated with terpenoid-mediated root-microbe interactions (Vaughan *et al.*, 2015; Mafu *et al.*, 2018). Zealexin production in response to elevated atmospheric carbon dioxide (CO₂) levels further highlights the multifaceted defensive roles of maize terpenoids (Vaughan *et al.*, 2015).

Lipid-derived metabolites form a third major chemical defense layer in maize (Fig. 1a,b). This includes green leaf volatiles, such as the widely distributed (*Z*)-3-hexenal, which are derived from membrane lipids and function alongside volatile terpenoids and phenolics in mediating long-distance insect herbivory defenses (Christensen *et al.*, 2013; Yactayo-Chang *et al.*, 2022; Yuan *et al.*, 2023). In addition, formed from the same lipid precursors, cyclopent(a)none oxylipins, termed maize death acids, and other

oxylipins, such as 9-hydroxy-10-oxo-12,15-octadecadienoic acid, accumulate locally in response to tissue wounding caused by pest and pathogen attack and serve as potent phytoalexins, impeding the

growth of herbivores such as *Helicoverpa zea* and fungal pathogens including major *Aspergillus* and *Fusarium* maize pathogens (Christensen *et al.*, 2015, 2016; Huang *et al.*, 2023). Notably,

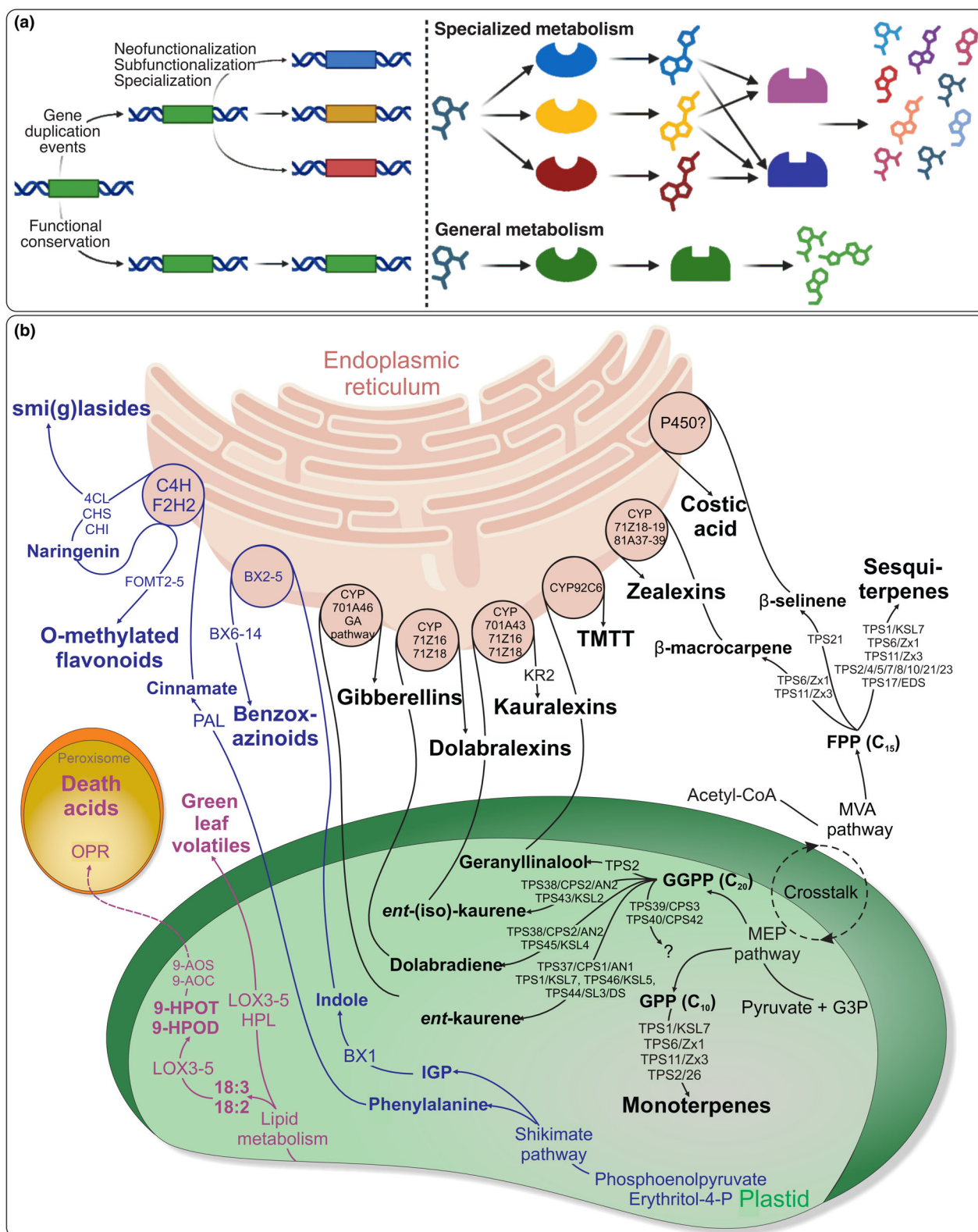


Fig. 2 Evolution and biosynthesis of maize specialized metabolites. (a) Genome evolution via repeated gene duplication events and subsequent gene functional divergence has given rise to large, functionally diverse gene families of specialized metabolism (left). Interaction of these enzymes as part of modular pathway networks drives the chemical diversity of specialized metabolism (right). (b) The biosynthesis of major maize specialized metabolites from common precursors of general metabolism required modular pathway networks that can span several cellular compartments. 4CL, 4-cinnamate ligase; AOC, allene oxide cyclase; AOS, allene oxide synthase; BX, benzoxazinoid; C4H, cinnamate-4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; CYP, cytochrome P450 monooxygenase; FOMT, flavonoid O-methyl transferase; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; HPL, hydroperoxide lyase; HPOD, 13S-hydroperoxy-9(Z),11(E)-octadecadienoic acid; HPOT, 13S-hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid; IGP, imidazoleglycerol phosphate; KR, kauralexin reductase; LOX, lipoxygenase; MEP, methyl erythritol phosphate; MVA, mevalonate; OPR, oxo-phytyldienoate reductase; PAL, phenylalanine ammonium lyase; TMTT, (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene; TPS, terpene synthase.

cyclopent(a)none oxylipins also occur in other crop species, including potato and sunflower, where they have predicted below-ground defensive functions (Hamberg, 2000; Grechkin *et al.*, 2007).

Recent discoveries also have expanded the diversity of phenolic compounds involved in maize–environmental interactions. This includes O-methylated flavonoids such as xilonenin, and more common flavonoids such as apigenin feature antifungal bioactivities and C-glycosyl flavones (maysins) and coumarates act as herbivory defenses (Casas *et al.*, 2016; Block *et al.*, 2020; Förster *et al.*, 2022b). Moreover, multi-omics and quantitative trait loci studies identified previously unrecognized acetylated diferuloylsucrose metabolites, termed smiglasides, with probable antifungal activity against *F. graminearum* and other pathogens (Zhou *et al.*, 2019).

III. Modular metabolic networks drive the evolution of complex maize chemical defenses

The divergence of maize specialized metabolism through continuous genome evolution is evident in large, functionally diverse enzyme families catalyzing various scaffold-forming and modifying reactions that generate chemical diversity (Fig. 2a). For instance, the expansion of the terpene synthase (TPS) and cytochrome P450 monooxygenase (P450) gene families has given rise to the diversity of specialized terpenoid metabolites (Chen *et al.*, 2011; Murphy & Zerbe, 2020; Jia *et al.*, 2022; Fig. 2a,b). Derived from duplication and subfunctionalization of ancestral bifunctional diterpene synthases (diTPSs), monofunctional class II and class I diTPSs, producing conserved gibberellin (GA) phytohormones and various specialized diterpenoids, will have served as a genetic reservoir for the expansion of the TPS family (Jia *et al.*, 2022). This continued gene duplication paired with rapid neofunctionalization, and often, high substrate- and/or product specificity of these enzyme families lead to diverse TPS-catalyzed mono-, sesqui-, and diterpene scaffolds that can undergo numerous functional modifications facilitated by P450s and other modifying enzymes (Fig. 2b). Maize research in the past few years has highlighted an intricate terpenoid-metabolic network that provides catalytic redundancies at key pathway nodes to safeguard against deleterious mutations, while utilizing enzyme promiscuity of downstream reactions to increase product diversity (Ding *et al.*, 2020). As one example, the entry point to major diterpenoid pathways is controlled by two catalytically redundant class II diterpene synthases (diTPS), ZmTPS37/CPS1/AN1 and ZmTPS38/

CPS2/AN2, that form the common precursor *ent*-copalyl diphosphate (*ent*-CPP) (Murphy & Zerbe, 2020). Although some crosstalk may occur, differential expression ensures that ZmTPS37/CPS1/AN1 supplies GA metabolism, whereas ZmTPS38/CPS2/AN2 feeds stress-inducible kauralexin and dolabrallexin biosynthesis (Fig. 2b). Subsequent branching of the kauralexin and dolabrallexin pathways is achieved through two additional class I diTPSs, ZmTPS43/KSL2 and ZmTPS45/KSL4, which form the committed *ent*-iso-karene and dolabradene precursors of kauralexins and dolabrallexins, respectively (Mafu *et al.*, 2018; Ding *et al.*, 2019). Downstream of these pathway partitions, a group of functionally promiscuous P450s, including CYP701A43, CYP71Z16, and CYP71Z18, catalyzes substrate-dependent oxygenations to yield the range of bioactive kauralexins and dolabrallexins (Mafu *et al.*, 2018; Ding *et al.*, 2019). Such combined metabolic control through biochemical pathway separation and differences in spatiotemporal and stress-elicited expression minimizes the dysregulation of GA and derived specialized defense pathways and coordinate growth and defense processes (Ding *et al.*, 2019). Notably, the catalytic promiscuity of CYP71Z16 and CYP71Z18 goes beyond diterpenoid metabolism as both enzymes, alongside recently identified P450s of the CYP81A family, bring about the oxygenation and desaturation of β -macrocarpene in zealexin biosynthesis (Ding *et al.*, 2020). Costic acids are formed via α/β -selinene as sesquiterpenoid scaffold with the involved P450s yet to be elucidated (Ding *et al.*, 2017). Contrasting these multi-enzyme pathways, maize contains at least 35 TPSs that directly convert the common geranyl diphosphate (GPP) or farnesyl diphosphate (FPP) precursors into a diverse bouquet of volatile mono- and sesqui-terpenoids such as linalool and β -caryophyllene involved in above-ground and volatile defenses. Despite the rapid advances in pathway discovery, it stands to reason that our map of the maize terpenoid network remains incomplete. The identification of ZmTPS39/CPS3 and ZmTPS40/CPS4 as diTPSs producing (+)-CPP and the double isomer 8,13-CPP as alternate diterpenoid precursors and the presence of yet uncharacterized TPSs suggest that additional pathways with predictable defensive functions exist in maize (Murphy *et al.*, 2018).

Similar to the evolution of specialized terpenoids from GA metabolism, death acids have likely evolved from ancestral phytohormone metabolism, in this case (JA) biosynthesis (Christensen *et al.*, 2015, 2016). Although the precise pathways en route to death acids remain elusive, their cyclopent(a)none oxylipin scaffold suggests a biosynthetic origin from linoleic acid and

linolenic acid via lipase-catalyzed cleavage from plastidial membranes (Fig. 2b). In contrast to JA metabolism that proceeds via regio-specific dioxygenation at C-13 of linolenic acid by 13-lipoxygenases (LOX) and downstream-acting C-13-specific allene oxide synthase (AOS), allene oxide cyclase (AOC), and 12-oxo-phytodienoic acid reductase (OPR) enzymes, death acid biosynthesis requires fatty acid dioxygenation at C-9. Indeed, several such LOX enzymes (ZmLOX3-5) producing 9-hydroperoxides have been identified (Christensen *et al.*, 2015, 2016). Moreover, recent studies of a *lox10opr2* maize mutant showed reduced JA-mediated defenses but increased death acid accumulation, suggesting regulatory crosstalk in addition to biochemical pathway separation to fine-tune JA- and death acid-mediated defense mechanisms (Huang *et al.*, 2023). Other LOX enzymes, including ZmLOX10 and ZmLOX5, have been shown to function in producing green leaf volatiles and the -ketol 9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid (9,10-KODA), respectively, thus marking branch points in the biosynthesis of volatile and local lipid-derived chemical defenses (Christensen *et al.*, 2013; Yuan *et al.*, 2023).

Numerous biochemical and genetic studies have generated a comprehensive characterization of benzoxazinoid metabolism (Zhou *et al.*, 2018). Although the benzoxazinoid pathway appears not to

result from a direct duplication of ancestral phytohormone metabolism as shown or predicted for terpenoids and death acids, its root in the shikimate pathway and use of indole as a core intermediate overlap with the formation of tryptophan and downstream auxin biosynthesis (Fig. 2b). Indole formation represents the branchpoint toward benzoxazinoid biosynthesis, with indole-3-glycerol phosphate lyase (benzoxazinless 1, BX1) as a primary catalyst (Zhou *et al.*, 2018). While two alternate enzymes, indole-3-glycerol phosphate lyase1 (IGL1) and tryptophan synthase α -subunit (TSA1), may provide some genetic redundancy, no primary function in benzoxazinoid metabolism is known (Frey *et al.*, 2000; Wisecaver *et al.*, 2017). Downstream of indole, a network of functional modifications catalyzed by the P450s of the CYP71C subfamily (BX2-5), dioxygenases (BX6, BX13), UDP-glucosyltransferases (BX8-9), and *O*-methyltransferases (BX7, BX10-12, BX14), then completes the biosynthesis of known benzoxazinoids (Zhou *et al.*, 2018). Interestingly, recent studies suggest that, rather than deriving from general metabolism, some maize BX-metabolic *O*-methyltransferases evolved from related *O*-methyltransferases involved in flavonoid metabolism (Förster *et al.*, 2022a). The identification of *O*-methyltransferases (ZmFOMT2-5) with roles in producing antifungal *O*-methyl flavonoids such as xilonenin further supports this hypothesis (Förster *et al.*, 2022a,b).

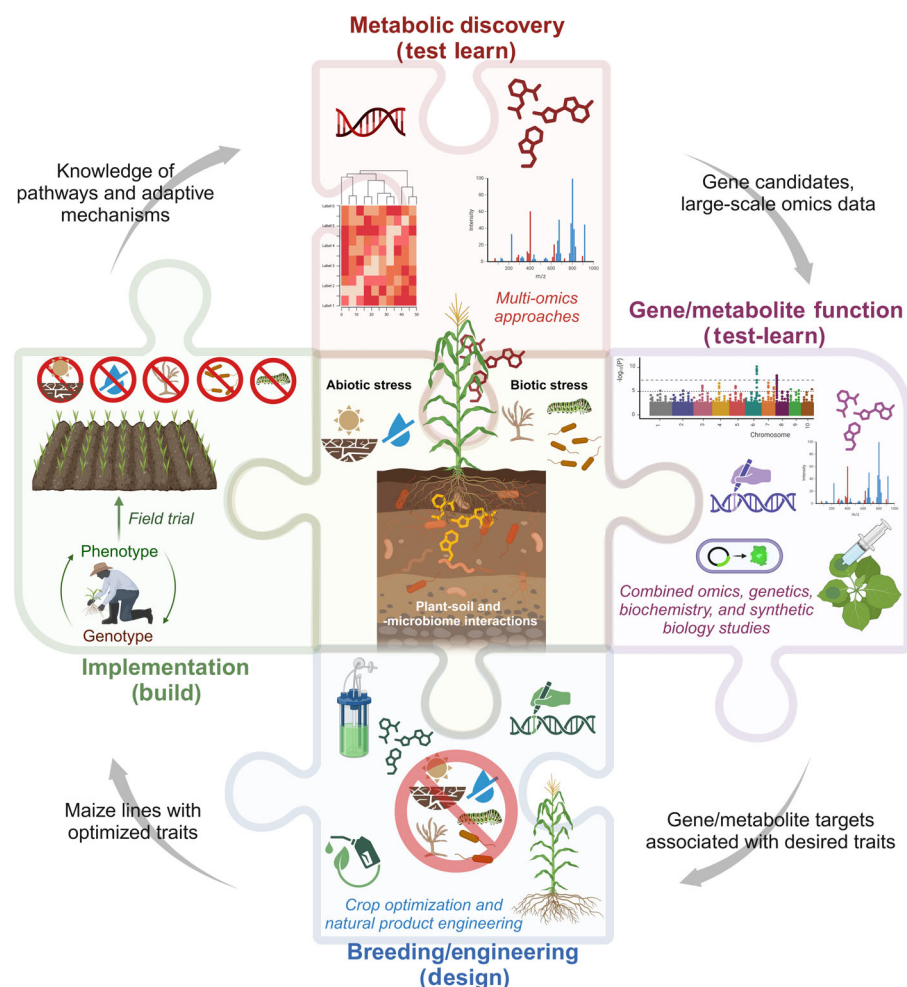


Fig. 3 Integrating today's multi-omics, genetics, biochemistry, and synthetic biology technologies offers unprecedented opportunities to solve the puzzle underlying the diversity, biosynthesis, regulation, and function of specialized metabolites in maize immunity and environmental adaptation. Leveraging this knowledge through precision breeding and crop engineering can generate crops with enhanced yield and climate resilience. Likewise, synthetic biology and metabolic engineering advances enable the production of natural and new-to-nature metabolites for use as biocides, biofuels, and other high-value chemicals. Effective translation of such integrative approaches into agricultural applications requires the collaborative efforts of plant biologists, agronomists, bioengineers, breeders, and farmers. The illustration was created with BioRender.com.

IV. Conclusions

The above examples highlight the chemical ingenuity of plants in using dynamic metabolic networks to adapt to their ecological niche. Genome evolution of ancestral general metabolism (often phytohormone) pathways has provided the required genetic reservoir for the evolution of common and species-specific, multifunctional metabolites, enabling plants to respond to various environmental conditions and metabolic needs (Erb & Kliebenstein, 2020). Despite much progress in decoding maize specialized metabolism, many open questions remain. What are the mechanisms-of-action underlying metabolite bioactivities? How are different chemical defense layers regulated in response to combined biotic and abiotic stressors? What drives chemical diversity across maize genotypes? Ultimately, how can this knowledge be translated into strategies for developing maize varieties with improved yield-defining and resilience traits? Applying modern omics approaches to explore the remarkable genetic and phenotypic plasticity inherent in maize cultivars is likely to uncover more bioactive metabolites and their biosynthetic and gene regulatory networks that can support breeding efforts (Fig. 3; Christensen *et al.*, 2018; Medeiros *et al.*, 2021; Murphy *et al.*, 2023; Mishra *et al.*, 2024). Advances in maize genetic transformation paired with the establishment of genetically diverse maize collections, including isogenic lines deficient in specific metabolites, will accelerate the elucidation of biosynthetic pathways and the largely unknown regulatory networks that govern maize chemical defenses. The modular pathway architecture underlying maize specialized metabolism can further be leveraged through synthetic biology and genome editing technologies. For instance, reconstituting different pathway modules in microbial or plant host systems facilitates the engineering of various natural and new-to-nature metabolites (Owen *et al.*, 2017; Calgato-Kozina *et al.*, 2020). These approaches can be employed to assess the structure–activity relationships of natural defense compounds and the engineering of compounds with new or enhanced bioactivities. Although the impact of modifying defense pathways *in planta* on maize resilience is yet to be explored, applying this strategy via genome editing has the potential to generate more resistant varieties as exemplified by the overexpression of the maize P450 CYP71Z18 in rice, which resulted in the accumulation of new terpenoids and increased pathogen resistance (Shen *et al.*, 2019). Increased crop yields through benzoxazinoid-mediated reduction in adverse plant–soil feedback further shows applications beyond enhancing direct stress defenses (Gfeller *et al.*, 2024). Integrating these transformative approaches offers new avenues to accelerate the development of crop varieties that can withstand current and future climate pressures while reducing agricultural footprint. Critically, to realize this vision, we need to close the gap between research outcomes and sustainable and economically viable agricultural solutions, which will require interdisciplinary efforts with stakeholders, including agronomists, bioengineers, breeders, and farmers.

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Competing interests

None declared.

Author contributions

PZ conceived of the study. PZ, AEC and FY jointly wrote the manuscript. FY and AEC contributed equally to this work.

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