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Sticky business: the intricacies of acylsugar biosynthesis in the Solanaceae

Eloisa Vendemiatti · Lillian Nowack · Lazaro Eustáquio Pereira Peres · Vagner A. Benedito · Craig A. Schenck

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Abstract Plants display tremendous chemical diversity. Like all organisms, they possess a core set of metabolites for growth and development. However, plants are notorious for their specialized chemical repertoire. Biologically active specialized metabolites enable plants to interact with their environment and provide humans with diverse medicines. Specialized metabolites are derived from core metabolites, often using enzymes that evolved from core pathways in a lineage-specific manner. Biochemical understanding of plant specialized metabolic pathways provides insight into the evolutionary origins of chemical diversity and tools for engineering the production of biologically active metabolites. Acylsugars are a class of specialized metabolites occurring widely in the Solanaceae and other plant families where they contribute to fitness. Although assembled from simple core metabolic precursors, sugars and acyl chains, tremendous acylsugar structural diversity is observed across the Solanaceae family. Enzymes that catalyze the esterification of acyl chains to sugar cores have been well characterized from phylogenetically diverse species, and their biochemical diversity contributes to acylsugar structural variation. The upstream metabolic pathways that provide the acyl chain precursors also contribute to acylsugar structural variation. Yet, biochemical and genetic understanding of these upstream biosynthetic pathways is less well known. Here, we focus on recent advances in acyl chain biosynthesis and elongation pathways, the subcellular distribution of acylsugar biosynthesis, and how biochemical innovations in acylsugar biosynthesis contribute to structural diversity specifically focusing on Solanaceae-type acylsugars.

Keywords Acylsugars · Specialized metabolites · Trichome · Core metabolism · Tomato · Defense · Solanaceae

Introduction

Plant specialized metabolism emerged in response to challenges in the terrestrial environment. Specialized metabolism encompasses structurally diverse molecules that enhance plant fitness in harsh environments

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E. Vendemiatti · V. A. Benedito
Division of Plant & Soil Sciences, Davis College of Agriculture, Natural Resources and Design, West Virginia University, Morgantown, WV, USA

L. Nowack · C. A. Schenck (✉)
Department of Biochemistry, Interdisciplinary Plant Group, University of Missouri, Columbia, MO, USA
e-mail: caschenck@missouri.edu

L. E. P. Peres
Department of Biological Sciences, College of Agriculture 'Luiz de Queiroz' (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil

(Lacchini and Goossens 2020; Weng et al. 2021). Evolutionary events such as gene and genome duplication followed by sub and neofunctionalization of core metabolic pathways have enabled specialized metabolic diversity (Weng et al. 2012; Weng 2014; Moghe and Last 2015; Fan et al. 2019; Schenck and Last 2020; Conneely et al. 2022; Rieseberg et al. 2023). In nature, plants use these metabolites to interact with other organisms and to cope with stress (e.g., attract beneficials or pollinators, deter herbivores, and reduce water loss). Humans have co-opted and modified plant chemical diversity to serve as flavors, fragrances, and roles in nutrition, medicine, and industry (McConkey et al. 2000; Wang et al. 2008; Zuzarte et al. 2010; Caissard et al. 2012; Andre et al. 2016; Xiao et al. 2016; Tissier 2018; Fiesel et al. 2022). In this manner, specialized metabolites play a vital role in shaping the interdependencies and diversity in the plant kingdom. A greater understanding of specialized metabolism provides insight into the evolution of biochemical novelty and tools to facilitate the engineering of biologically active metabolites (Weng et al. 2012, 2021; Chalvin et al. 2020).

The Solanaceae family consists of around 3,000 species adapted to diverse environments worldwide. The family includes agronomically important plants, such as petunia, tobacco, potato, tomato, peppers, and eggplant, and provides a framework for understanding the emergence of novel traits. The cultivated tomato has been used as a model to investigate key developmental pathways, such as compound leaf architecture (Hareven et al. 1996), sympodial flowering (Pnueli et al. 1998), fleshy fruit development (Quinet et al. 2019; Fenn and Giovannoni 2021; Gan et al. 2022; Trubanová et al. 2022), climacteric ripening (Klee and Giovannoni 2011; Kou et al. 2021; Yang et al. 2021; Liu et al. 2022), and glandular trichome development (Goffreda et al. 1990; Vendemiatti et al. 2017; Chalvin et al. 2020). In addition, the Solanaceae offers insights into the evolution of many specialized metabolites, such as alkaloids, flavonoids, terpenes, and acylsugars (Fan et al. 2019; Sonawane et al. 2020; Fiesel et al. 2022).

Acylsugars are specialized metabolites comprising acyl chains attached to sugar cores (Fan et al. 2019). They are widely distributed across the Solanaceae, but similar structures have been observed in many other plant families (Kruse et al. 2022a; Moghe et al. 2023a). The acyl chains of Solanaceae-type acylsugars are attached to the hydroxyl groups of sugar cores via an ester linkage (Fan et al. 2019). Although derived from simple core metabolite precursors—acyl chains and sugars—acylsugars exhibit remarkable structural diversity at intra-species, genus, and family-wide levels (Goffreda et al. 1990; Vendemiatti et al. 2017, 2024; Chalvin et al. 2020). Acylsugars are synthesized in glandular trichomes, facilitating the identification of pathway-specific genes using comparative approaches (Fan et al. 2019, 2020; Feng et al. 2021; Leong et al. 2020; Lou et al. 2021; Moghe et al. 2017; Schenck et al. 2022). However, acylsugars were recently detected from root exudates in tomato (Kerwin et al. 2024; Korenblum et al. 2020). Acylsugars have significant biological activity, acting as deterrents against small insects and microbes and conferring protection against desiccation (Luu et al. 2017; Feng et al. 2021). A better understanding of the acylsugar pathway could guide breeding efforts aimed at increasing acylsugar content for enhanced crop resilience (Maluf et al. 2010; Luu et al. 2017; Lotina-Hennsen et al. 2013; Feng et al. 2021; Mutschler et al. 2023).

Acylsugar biosynthesis has been well-studied in tomato, its wild relatives, and other Solanaceae species. These studies have focused on acylsugar acyltransferase (ASAT) enzymes that add acyl chains to sugar cores. ASAT gene loss and gain events, substrate promiscuity, and tissue localization are key molecular innovations that enabled acylsugar structural diversity across the family (Schilmiller et al. 2008, 2010, 2012, 2015, 2016; Fan et al. 2016, 2019, 2020; Leong et al. 2019, 2020, 2022; Kerwin et al. 2024; Lybrand et al. 2020; Lou et al. 2021; Schenck et al. 2022; Moghe et al. 2017; Nadakuduti et al. 2017). However, another major contributor to acylsugar structural variation is the upstream biosynthetic and elongation pathways leading to diverse acyl chains. Yet, much less is known about the pathways upstream of ASATs. For clarity and consistency with a previous classification scheme for the acylsugar pathway (Moghe et al. 2023a), we divide acylsugar biosynthesis into three phases: (1) the *upstream* pathway, which encompasses enzymes responsible for acyl chain biosynthesis and elongation, (2) the *core* pathway, which involves ASATs that add acyl chains to the sugar core, and (3) the *downstream* pathway, which contains enzymes that cleave acyl chains or glycosidic linkages creating breakdown products or enhancing structural diversity (Leong et al. 2019; Schilmiller et al. 2016). This review focuses on recent advances in biochemical knowledge of the upstream pathway, subcellular distribution of acylsugar biosynthesis, and phylogenetic distribution of acylsugar structural variation across the Solanaceae.

The ecological function of acylsugars

Glandular trichomes are widespread in the plant kingdom, occurring in at least 30% of land plants and are the primary site of acylsugar biosynthesis (Schuurink and Tissier 2020; Chalvin et al. 2020). Glandular trichomes function as specialized metabolite factories important in biotic and abiotic stress response (Hauser 2014). Among the plethora of trichome-synthesized specialized metabolites, acylsugars are prevalent in the Solanaceae family and contribute to defense against herbivores and microbes, with potential roles in mitigating abiotic stresses.

Acylsugars offer direct and indirect protective effects, benefiting the host and neighboring plants (Tagawa and Watanabe 2021) through diverse defense mechanisms. As physical defenses, the adhesive properties of acylsugars function as insect traps, impeding movement, oviposition, and feeding (Van Dam and Hare 1998; Hare 2005; Voigt et al. 2007; Luu et al. 2017; Vendemiatti et al. 2024). Moreover, acylsugars can serve as a chemical defense due to their toxic properties that repel insects and even label them for recognition by natural predators (Goffreda et al. 1989; Puterka et al. 2003; Wagner 2004; Weinhold and Baldwin 2011). Additionally, acylsugars can enhance plant resistance against various pathogens, including fungi and bacteria (Kato and Arima 1971; Marshall and Bullerman 1986; Chortyk et al. 1993; Luu et al. 2017; Ben-Mahmoud et al. 2019). Typically, acylsugar-producing plants synthesize a blend of acylsugars with varying numbers of acylations and chain lengths (Table 1). The function of producing diverse acylsugars is not well known, though there is evidence to support that greater chemical diversity is an adaptive advantage (Richards et al. 2015). However, evidence suggests that longer acyl chains may offer superior protection against certain biotic pressures (Chortyk et al. 1993, 1996).

Acylsugar exudates may also shield plants against abiotic stresses such as heat and drought (Scalabrin et al. 2015; Feng et al. 2021). It is plausible that these metabolites form a protective barrier on the epidermis of aerial organs of the plant alongside the physical presence of acylsugar-producing trichomes (*e.g*. type-IV trichomes in tomatoes), which also increase in density under high temperatures or water scarcity (Galdon-Armero et al. 2018; Säbel et al. 2023). This barrier could improve the reflection of sunlight radiation, heat dissipation, and lower water transpiration (Perez-Estrada et al. 2000; Scalabrin et al. 2015).

Acylsugars detected in root exudates of tomato and *Brassica rapa* (Wu et al. 2013; Kerwin et al. 2024; Korenblum et al. 2020) opens new potential biological functions of these compounds belowground. This discovery suggests that acylsugars may be used against soil pests – *e.g*., nematodes, fungi, and root-feeding insects, providing defense against subterranean threats. Alternatively, acylsugars may feed beneficial soil flora and fauna – *e.g.,* mycorrhizal fungi, earthworms, and bacteria, fostering a more ecologically balanced and sustainable soil ecosystem.

Acylsugar structural diversity across the *Solanaceae*

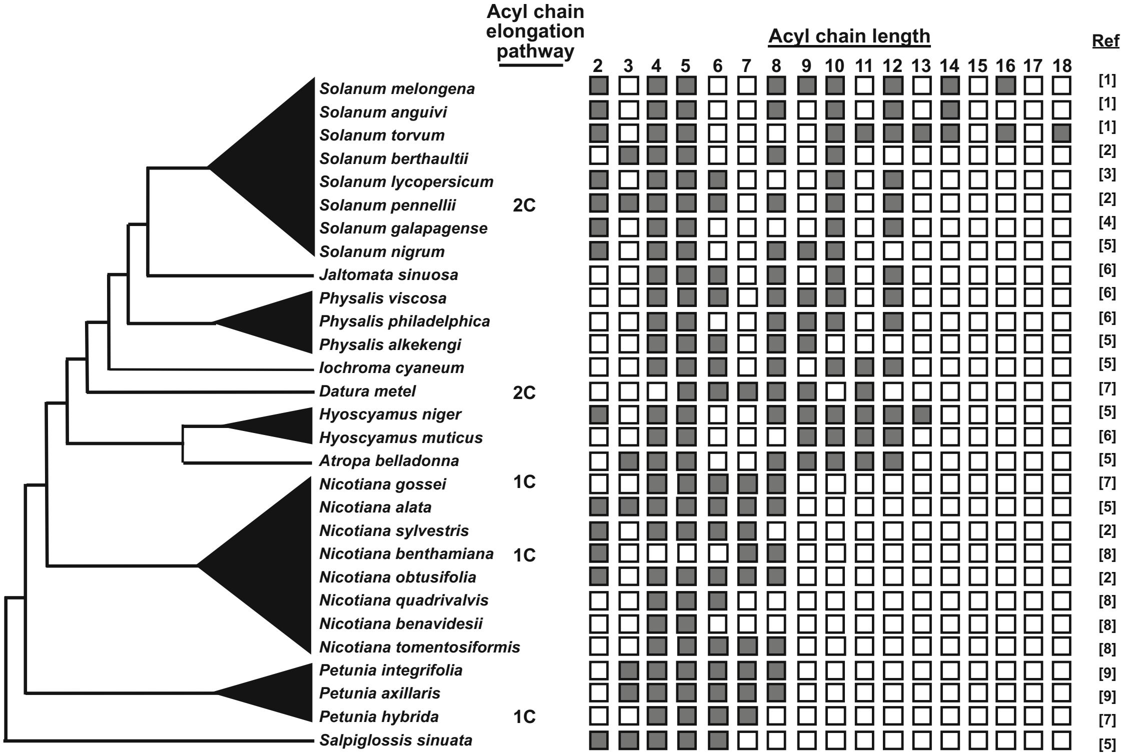
Despite the seemingly straightforward constituents of acylsugars (sugar and acyl chains), remarkable acylsugar structural diversity is observed across the Solanaceae. Acylsugars are synthesized through the esterification of acyl chains of varying lengths and branching patterns to hydroxyl groups at several positions on the sugar cores. Although the sugar cores are typically sucrose or glucose, inositol has also been detected in restricted Solanaceae lineages (Leong et al. 2020; Fiesel et al. 2024). The primary distinguishing feature among acylsugars lies in the number of acylations on the sugar core and the length of these acyl chains, ranging from 2 to 18 carbons. In cultivated tomato, tri- or tetra-acylated sucroses with chains of 2, 4, 5, 10, and 12 carbons have been detected (Kim et al. 2012). Acylsugar screening from species distributed across the family revealed that specific acyl chain lengths are phylogenetically restricted (Fig. 1). Early diverging genera such as *Nicotiana*, *Petunia,* and *Salpiglossis* tend to exhibit shorter acyl chains, with a maximum of 8 carbons (Moghe et al. 2017; Fan et al.

Table 1 Examples of acylsugar protection and the types of acyl chains involved in each process

Host species	Stress type	Acyl chain length	Effect	References
Datura wrightii	*Manduca sexta*	C6–C9	Lower consume rate and slower development	Van Dan and Hare (1998)
Pyrus communis	*Cacopsylla pyricola*	C8 and C10	High mortality	Puterka et al. (2003)
N. tabacum	*Myzus nicotianae*			
Malus domestica	*Tetranychus urticae*			
N. langsdorffii	Heat shock	C4 and C5	Protection to heat shock	Scalabrin et al. (2015)
N. attenuata	*Fusarium brachygibbosum*	C5 and C6	Lower spore germination	Luu et al. (2017)
	Alternaria sp.			
	Manduca sexta		Reduced growth	
S. pennellii	TSWV	C10	Lower inoculation	Ben-Mahmoud et al. (2019)
	Frankliniella occidentalis	C4, C11, C12	Reduction in oviposition	
S. pennellii S. habrochaites	*Bemisia tabaci*	C5 and C11	Lower survival rate	Kortbeek et al. (2021)
N. benthamiana	Desiccation	C2, C7, C8	Reduced the water content and increased leaf temperature in transgenic plants with low AS	Feng et al. (2021)
	Myzus persicae		Lower survival rate, lower weight gain, and/or lower reproduction	
	Bemisia tabaci			
	Macrosiphum euphorbiae			
	Helicoverpa zea			
	Macrosiphum euphorbiae			
	Trichoplusia ni			
N. benthamiana	*Spodoptera litura*	C2, C7, C8	Mortality and transgenerational fitness costs	Wang et al. (2022)
S. lycopersicum	*Bemisia tabaci*	C4, C5, C10, C12	Reduction in oviposition	Vendemiatti et al. (2024)
	Manduca sexta		Reduced growth	
	Septoria lycopersici		Delayed inoculation and resistance at the exponential growth	

2020; Schenck et al. 2022; Matsuzaki et al. 1989). Whereas genera more closely related to *Solanum* (e.g., *Jaltomata*, *Physalis*, *Iochroma*, *Datura*, *Atropa*, and *Hyoscyamus*) accumulate acylsugars with acyl chains longer than 8 carbons (Maldonado et al. 2006; Kroumova et al. 2016; Moghe et al. 2017; Fan et al. 2020). The phylogenetic distribution of acyl chain lengths implies underlying metabolic differences. Further, acylsugar screening of species at key phylogenetic positions can provide additional insight into the acyl chain length distribution.

The acylation of the acylsugar core is orchestrated in the apical cells of trichome glands by a group of ASAT enzymes, which are classified within Clade III of the BAHD acyltransferase family (Moghe et al. 2023b). ASATs operate sequentially, where the product of one enzymatic reaction serves as the substrate for the subsequent (D'Auria 2006; Schilmiller et al. 2012; Fan et al. 2016). For example, in cultivated



	Acyl chain elongation pathway	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Ref
Solanum melongena		■	□	■	■	□	□	■	■	■	□	■	□	■	□	■	□	□	[1]
Solanum anguivi		■	□	■	■	□	□	■	□	■	□	■	□	■	□	□	□	□	[1]
Solanum torvum		■	□	■	■	□	□	□	□	■	■	■	■	■	□	■	□	■	[1]
Solanum berthaultii		□	■	■	■	□	□	■	□	■	□	□	□	□	□	□	□	□	[2]
Solanum lycopersicum		■	□	■	■	■	□	□	□	■	□	■	□	□	□	□	□	□	[3]
Solanum pennellii	2C	■	■	■	■	■	□	■	□	■	□	■	□	□	□	□	□	□	[2]
Solanum galapagense		■	□	■	■	□	□	□	□	■	□	■	□	□	□	□	□	□	[4]
Solanum nigrum		■	□	■	■	□	□	■	■	■	□	□	□	□	□	□	□	□	[5]
Jaltomata sinuosa		□	□	■	■	■	□	■	□	■	□	■	□	□	□	□	□	□	[6]
Physalis viscosa		□	□	■	■	■	□	■	■	■	□	■	□	□	□	□	□	□	[6]
Physalis philadelphica		□	□	■	■	□	□	■	■	■	□	■	□	□	□	□	□	□	[6]
Physalis alkekengi		□	□	■	■	■	□	■	■	□	□	□	□	□	□	□	□	□	[5]
Iochroma cyaneum		□	□	■	■	■	□	■	□	■	■	■	□	□	□	□	□	□	[5]
Datura metel	2C	□	□	□	■	■	■	■	■	□	■	□	□	□	□	□	□	□	[7]
Hyoscyamus niger		■	□	■	■	□	□	■	■	■	■	■	■	□	□	□	□	□	[5]
Hyoscyamus muticus		□	□	■	■	□	□	□	■	■	■	■	□	□	□	□	□	□	[6]
Atropa belladonna		□	■	■	■	□	□	■	■	■	■	■	□	□	□	□	□	□	[5]
Nicotiana gossei	1C	□	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[7]
Nicotiana alata		■	■	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[5]
Nicotiana sylvestris		■	□	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	[2]
Nicotiana benthamiana	1C	■	□	□	□	□	■	■	□	□	□	□	□	□	□	□	□	□	[8]
Nicotiana obtusifolia		■	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[2]
Nicotiana quadrivalvis		□	□	■	■	■	□	□	□	□	□	□	□	□	□	□	□	□	[8]
Nicotiana benavidesii		□	□	■	■	□	□	□	□	□	□	□	□	□	□	□	□	□	[8]
Nicotiana tomentosiformis		□	□	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[8]
Petunia integrifolia		□	■	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[9]
Petunia axillaris		□	■	■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	[9]
Petunia hybrida	1C	□	□	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	[7]
Salpiglossis sinuata		■	■	■	■	■	□	□	□	□	□	□	□	□	□	□	□	□	[5]

Fig. 1 Phylogenetic distribution of acylsugar acyl chain lengths. A Solanaceae phylogenetic tree based on Särkinen et al., 2013 was used to map acylsugar acyl chain lengths. Filled boxes indicate presence of an acyl chain of the indicated carbon length, whereas empty boxes indicate absence. Acyl chain elongation pathways were mapped onto the phylogeny based on precursor feeding studies from Kroumova and Wagner 2003, 2C indicates a two-carbon fatty acid-like elongation pathway and 1C indicates a one-carbon elongation pathway. The source of acylsugar acyl chain lengths is indicated in the reference (ref) column, where 1-Fiesel et al. 2024, 2-Kroumova 2016, 3-Ghosh et al. 2014, 4-Vendemiatii et al. 2024, 5-Moghe et al. 2017, 6-Fan et al. 2020, 7-Kroumova and Wagner 2003, 8-Schenck et al. 2022, and 9-Liu et al. 2017

tomato, four ASATs act sequentially to add acyl chains at specific hydroxyl groups: SlASAT1 produces a monoacylated sucrose at position R4 on the pyranose ring, which serves as a precursor for SlASAT2, which in turn adds another acyl chain to produce diacylated sucrose (Fan et al. 2016). SlASAT3 and SlASAT4 catalyze the last two acylations (Fan et al. 2019; Schilmiller et al. 2012). Details about the acylsugar core pathway and macro and microevolutionary processes enabling acylsugar diversity have been recently investigated and reviewed (Fan et al. 2019; Landis et al. 2021). The remarkable diversity observed in acylsugars is partially due to ASAT gene loss/gain events and neofunctionalization. This leads to modified substrate affinities and enzyme promiscuity and can even change the order of the enzymatic reactions (Schilmiller et al. 2012, 2016; Fan et al. 2017; Schenck et al. 2022). Understanding ASAT diversity at the structural level, particularly active site residues involved in acyl chain length recognition can provide tools to manipulate acylsugar composition with potential implications for increased defense against pests. Additionally, other core metabolism gene co-option events have contributed to acylsugar diversity including an invertase that gained trichome expression and can cleave acylsucroses into acylglucoses in some *Solanum* lineages (Leong et al. 2020; Lou et al. 2021).

Acyl chain biosynthesis, elongation, and substrate-feeding studies

Unlike the well-characterized ASATs of the core phase of acylsugar biosynthesis, the upstream pathway, consisting of acyl chain synthesis and elongation, is poorly understood. In this phase, core metabolism becomes intricately intertwined with specialized metabolism within the trichomes. A significant contributor to the acylsugar structural diversity observed across the Solanaceae family is the variation in acyl chain length, branching pattern, and modifications of the acyl chains. Acyl chain lengths vary from 2 to 18 carbons (Fig. 1) and can be unbranched (n), branched at the terminal carbon (iso, i), or subterminal carbon (anteiso, ai). Additionally, hydroxylated acyl chains have recently been detected in some *Solanum* species (Fiesel et al. 2024; Ji et al. 2023). This acyl chain structural diversity suggests underlying biosynthetic complexity.

The acyl chain precursors for acylsugars are derived from the branched-chain amino acids (BCAA) and converted into their subsequent acyl-CoAs by the branched-chain α-keto acid dehydrogenase complex (BCKDH) (Kroumova and Wagner 2003) (Fig. 2). Transcriptomics studies show that some BCKDH components are highly enriched in trichomes, and knockdown of trichome-enriched BCKDH components in two *Nicotiana* species and *S. pennellii* confirmed the role of BCKDH in acylsugar biosynthesis (Luu et al. 2017; Slocombe et al. 2008; Mandal et al. 2020). However, the substrates for the BCKDH complex are the deaminated α-keto acids and not the BCAAs themselves; it remains to be determined whether BCAAs are first synthesized and then deaminated by branched-chain aminotransferases (BCAT) or the α-keto acid is used before completion of amino acid synthesis. Some BCAT homologs show trichome-enrichment in tomato and other species, suggesting there could be a role for BCATs in acylsugar biosynthesis (Mandel et al., 2020).

Transcriptomics studies identified trichome-enriched BCAA genes in several species (Slocombe et al. 2008; Mandal et al. 2020; De Sousa Cardoso et al. 2021), and biochemical and genetic studies support the role of some trichome-enriched BCAA biosynthetic enzymes in acylsugar biosynthesis. In tomato, a trichome-enriched isopropyl malate synthase (IPMS), typically involved in leucine biosynthesis, was identified with a functional role in acylsugar biosynthesis (Ning et al. 2015). Three genes coding for acetolactate synthase (ALS, also known as acetohydroxyacid synthase, AHAS) were identified in *N. tabacum*. Of these, one showed trichome-enrichment, whose targeted disruption using CRISPR/Cas9 gene editing led to a significant reduction in acylsugars (Chang et al. 2020). Only a limited number of trichome-enriched BCAA biosynthetic genes have a demonstrated role in acylsugar biosynthesis: it remains unclear if acyl chain precursors are fully synthesized within the trichomes or if some amount of the BCAA precursor supply is provided by other tissues and imported into the trichomes. BCAAs, however, only provide acyl chains of 4 and 5 carbons (Fig. 2), and longer acyl chains are observed across the Solanaceae (Fig. 1), suggesting acyl chain elongation is required.

Precursor feeding studies have been used to investigate elongation pathways and, in some instances, have provided inconsistent findings (Walters and Steffens 1990; Van Der Hoeven and Steffens 2000; Kroumova and Wagner 2003). This is likely because different acyl chain elongation pathways are present across the Solanaceae. The two elongation pathways identified are: the two-carbon and the one-carbon mechanisms (Kroumova and Wagner 2003). The two-carbon mechanism is analogous to fatty acid biosynthesis and has been reported for the wild tomato *S. pennellii* and *Datura metel* (Kroumova and Wagner 2003), however precursor feeding studies have not been reported in cultivated tomato. The one-carbon elongation pathway, similar to a portion of the citric acid cycle, is present in *Petunia* and *Nicotiana* (Kroumova and Wagner 2003; Slocombe et al. 2008; Wang et al. 2022). Both pathways extend the acyl chains through iterative elongation cycles; with one-carbon elongation using acetyl-CoA and two carbon elongation using malonyl-ACP. Interestingly, species proposed to use the two-carbon elongation pathway accumulate acyl chains longer than 8 carbons compared with species that use the one-carbon elongation, which only accumulate acyl chains of up to 8 carbons (Fig. 1). This suggests that biochemical limitations may exist regarding the lengths of acyl chains produced from distinct elongation pathways.

Recent studies on acyl chain elongation

Genetic and biochemical evidence supports the roles of distinct elongation pathways across the Solanaceae. Fatty acid synthase components, such as β-ketoacyl-ACP synthases (KAS) and β-ketoacyl-ACP reductase (KAR), are highly enriched in the trichomes of species

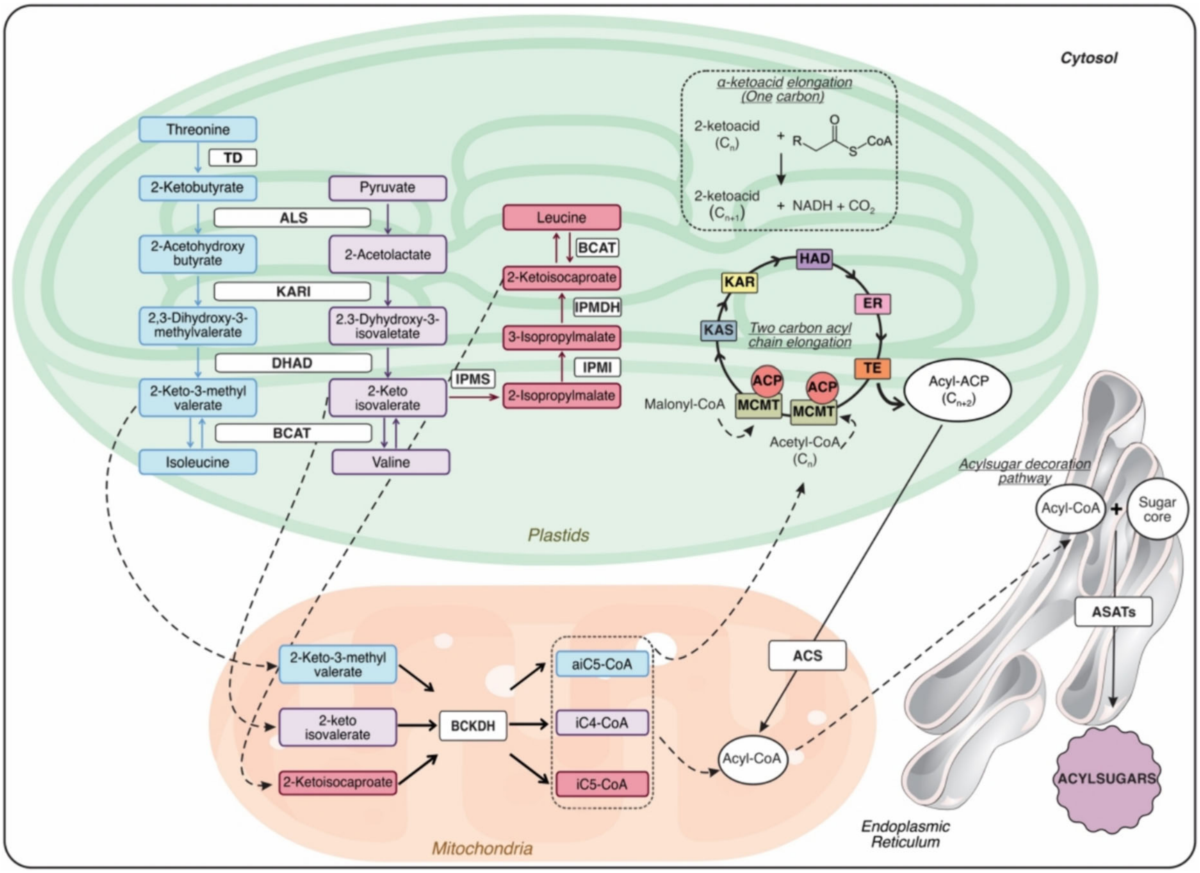


Fig. 2 Subcellular distribution of acylsugar biosynthesis. Branched-chain amino acids (BCAA) are the acyl chain precursors for acylsugars. Short branched acyl-CoAs can be elongated through 1 or 2-carbon elongation cycles depending on the species. Acylsugar biosynthesis is distributed across multiple subcellular compartments. ACP: Acyl carrier protein; ACS: Acyl-CoA synthetase; ALS: Acetolactate synthase; BCAT: Branched-chain aminotransferase; BCKDH: Branched-chain α-ketoacid dehydrogenase; DHAD: Dihydroxy-acid dehydratase; ER: enoyl-ACP reductase; FAS: Fatty acid synthase; HAD: β-hydroxyacyl ACP dehydratase; IPMDH: Isopropylmalate dehydrogenase; IPMI: Isopropylmalate isomerase; IPMS: Isopropylmalate synthase; KAR: β-ketoacyl ACP reductase; KARI: Ketol-acid reductoisomerase; KAS: β-ketoacyl synthase; MCMT: Malonyl-CoA: ACP malonyltransferase; TD: Threonine deaminase; TE: Thioesterase

that use the two-carbon elongation pathway (Ji et al. 2023; Mandal et al. 2020; Slocombe et al. 2008). Knockdown of a trichome-enriched *KAR* gene in *S. pennellii* using VIGS reduced the amount of long chain-containing acylsugars, which require elongation. In contrast, the amounts of acylsugars containing short chains that require no elongation remained constant and, in some cases, increased (Ji et al. 2023). Knockdown of trichome-enriched KAS homologs, which catalyze the condensation of malonyl-ACP or acetyl-CoA with acyl chains, in wild tomato (*S. pennellii*) resulted in reduced long-acyl chains (Slocombe et al. 2008; Mandal et al. 2020). The knockout of acyl-CoA synthetase in cultivated and wild tomato led to the elimination of long-chain-containing acylsugars and a modest decrease in acylsugars with short-branched chains (Fan et al. 2020). Thus, acyl-CoA synthetase may perform the last step in activating the acyl chains to their CoAs, which are the substrates of ASATs. Labeling, genetic, and biochemical studies support the role of trichome-enriched two-carbon elongation pathway in Solanaceae species that produce long acyl chains, yet more work is needed to understand this pathway fully. The one-carbon chain elongation pathway present in early diverging Solanaceae lineages remains to be fully characterized. Gene expression studies in *N. benthamiana* show that BCAA and BCKDH genes are highly enriched in trichomes, but not two-carbon chain elongation components (Slocombe et al. 2008). It should be noted that studies investigating acyl chain elongation pathways have been conducted in limited species, and there is likely biochemical diversity in acyl chain elongation pathways across the Solanaceae as has been observed for the core portion of acylsugar biosynthesis.

Although feeding studies indicate that BCAA serve as the precursors to both short and elongated branched acyl chains, many species produce straight long acyl chains. There are a few possibilities for how straight long chains are biosynthesized. First, it is possible, albeit unlikely, that straight chains are synthesized as branched acyl chains, and the branch is later removed. Second, during BCAA catabolism, when threonine is converted to isoleucine, the 2-keto 3-methyl valerate could also be used by BCKDH, resulting in a 5-carbon straight-chain that could be elongated by the mechanisms described above. Only odd-numbered straight acyl chains would be observed in species that use a two-carbon elongation pathway, and 10, 12, and 14-carbon straight acyl chains are observed in *Solanum* species (Ghosh et al. 2014; Fiesel et al. 2022). Third, straight chains could be synthesized using the fatty acid biosynthesis pathway with straight-chain precursors, such as acetyl-CoA and malonyl-ACP, similar to canonical fatty acid biosynthesis (Li-Beisson et al. 2013). Many components of acyl chain biosynthesis and elongation remain to be characterized genetically and biochemically. However, gene expression studies have identified many candidate genes, including BCAA, BCKDH, membrane transporters, and transcription factors, that are likely candidates for involvement in acylsugar biosynthesis and exudation (Mandal et al. 2020; Ji et al. 2023).

Subcellular distribution of acylsugar biosynthesis

Acylsugar biosynthesis, including acyl chain biosynthesis, elongation, sugar core esterification, breakdown, and exudation from the trichomes, have likely been co-opted from various core metabolic pathways. This suggests that different portions of acylsugar biosynthesis are possibly distributed across different subcellular compartments. Limited acylsugar biosynthetic steps have been determined through localization studies including *NtALS*, *NtASATs*, and *SlACS*. Fluorescent protein labeling studies have shown that NtALS is localized to the plastids, NtASATs to the endoplasmic reticulum, and SlACS to the mitochondria (Chang et al. 2020, 2022; Fan et al. 2020). The remaining acylsugar biosynthetic steps remain to be determined. Yet, if we assume trichome-enriched acylsugar biosynthetic enzymes are localized to the same locations of core metabolic pathways, a distributed pathway begins to emerge (Fig. 2). BCAA biosynthetic enzymes are likely confined to the plastids (Binder 2010). The BCAAs may be converted into acyl-CoAs by the mitochondrial BCKDH complex (Mooney et al. 2002). Subsequently, since most fatty acid biosynthesis occurs in plastids, the acyl chain elongation pathways may also be confined to plastids (Li-Beisson et al. 2013). The possibility of two-carbon elongation pathways occurring in plastids is also supported by the presence of these organelles in tomato type-IV trichome cells (Vendemiatti et al. 2017, 2022), where acylsugar assembly occurs. One-carbon elongation components are also likely plastidic, but experimental evidence is lacking. Acyl-CoA synthetase, which activates the acyl chain to its CoA following elongation, localizes to the mitochondria, so there appears to be a requirement for transport from the plastids to mitochondria at multiple steps in the acylsugar biosynthesis (Fan et al. 2020). Then, acyl-CoAs are esterified to sugar cores in the apical cell of the glandular trichome by ER-localized ASATs (Chang et al. 2022). The wide array of enzymes involved in the acylsugar biosynthesis from BCAA biosynthesis, elongation pathways, and ASATs underscores the complexity of metabolite trafficking between organelles and the secretion of end products from trichomes.

Acylsugar production and accumulation varies across species. In some wild species, including tomato and tobacco accessions, acylsugars accumulate up to 15–20% leaf dry weight (Fobes et al. 1985). To sustain acylsugar biosynthesis at a high level locally in the trichomes, the sugar cores are probably produced in plastids and/or the cytosol within mesophyll cells and then exported to the glandular trichomes (Ruan 2014). Although trichomes are photosynthetic, the bulk of the carbon comes from sucrose in the leaf (Balcke et al. 2017). Additionally, the sucrose transporter *SlSUT1* from tomato is highly expressed in the trichomes (Weise et al. 2008), indicating a role in sugar import.

Whereas two ASATs from *N. tabacum* have been shown to localize to the ER through the transient expression of GFP-tagged proteins in *N. benthamiana* leaves (Chang et al. 2022). Thus, acyl-CoAs produced in the mitochondria and sugars likely synthesized in the mesophyll must be transported to the ER of the trichome apical cell to be assembled into acylsugars by ASATs. Once assembled, acylsugars are secreted by the glandular trichome and remain on its surface. The metabolic coordination between the trichome cells and the membrane transporters involved with sugar translocation, inter- and intracellular metabolite trafficking, and acylsugar efflux remains unknown. Several transporters show trichome-enrichment and could participate in acylsugar transport. For example, ATP-binding cassette (ABC) transporters may play a crucial role in inter and intracellular trafficking of substrates or as acylsugar effluxers at the tip of glandular trichomes (Mandal et al. 2020; Ji et al. 2023). The subcellular organization and transport of acylsugars and precursors are unknown and ripe for future studies. A comprehensive understanding of acylsugar biosynthesis, transport, and regulation at the metabolic and genetic levels could pave the way for optimizing acylsugar biosynthesis for enhanced production.

Striking similarity between acylsugar and capsaicinoid biosynthesis pathways

Although capsaicinoids and acylsugars belong to different chemical classes and do not seem structurally similar on the surface, they emerge from similar biosynthetic pathways. Capsaicinoids are a class of alkaloids restricted to the *Capsicum* genus of Solanaceae that impart a spicy flavor to foods that evolved to deter mammalian herbivory and fungal infections (Naves et al. 2019). Capsaicinoids consist of elongated BCAAs (valine or leucine) and a cyclic moiety—vanillyamine derived from phenylalanine. Although capsaicin is the predominant capsaicinoid (Aza-González et al. 2011), differences in acyl chain lengths provide structural variation similar to that observed in acylsugars. Like acylsugar biosynthesis, elongated acyl-CoAs are attached to the acyl acceptor by a BAHD acyltransferase (Pun1) during capsaicinoid biosynthesis (Stewart et al. 2005), which was recently shown to synthesize capsaicinoids in yeast (Muratovska et al. 2022). Acyl-CoA chains of acylsugars are attached to hydroxyl groups, creating ester linkages, whereas with capsaicinoids, acyl-CoAs are attached to the amine of vanillyamine through an amide linkage (Stewart et al. 2005). Despite ASATs and Pun1 acylating on different functional groups, they are evolutionarily related. ASATs and Pun1 group within the same BAHD clade (III), whereas most BAHDs that attach acyl chains to amines are found within BAHD clade IV (Kim et al. 2014; Kruse et al. 2022b; Moghe et al. 2017, 2023b). This implies the unique trait of accepting elongated acyl-CoAs and modifying specialized metabolites may have emerged specifically within the Solanaceae followed by divergence of enzyme activity resulting in Pun1 and ASATs using different acyl-CoA acceptor substrates.

Analogous to acylsugar acyl chains being elongated by a fatty acid-like two-carbon elongation mechanism before esterification to the sugar core, capsaicinoid acyl chains can also be elongated possibly by a similar mechanism. Expression of acylsugar biosynthetic genes is enriched in glandular trichomes (Ji et al. 2023), whereas capsaicinoid biosynthesis occurs in the placental tissues within the developing fruit (Aluru 2003; Kim et al. 2014; Stewart et al. 2005; Mazourek et al. 2009; Arce-Rodríguez and Ochoa-Alejo 2019). Interestingly, fatty acid synthesis components are highly enriched in the placental tissue of pepper (Aluru 2003; Kim et al. 2014), suggesting that *Capsicum* and acylsugar-producing species have co-opted the same biochemical machinery for synthesizing structurally distinct specialized metabolites. Likely, gene duplication events followed by changes in gene expression and/or enzyme activities have enabled the evolution of these biosynthetic pathways. While capsaicinoids appear to be a monophyletic trait restricted to certain *Capsicum* species (Blum et al. 2003; Tewksbury et al. 2006), acylsugars have not been reported in this genus (Moghe et al. 2017), though rigorous sampling across *Capsicum* has not been reported. Given the shared biosynthetic machinery, this may suggest that plants have the capacity to make capsaicinoids or acylsugars but not both, which poses a further challenge to the proposition of bioengineering tomato to produce capsaicinoids (Naves et al. 2019). While a binary switch from acylsugars to capsaicinoid biosynthesis is an exciting evolutionary scenario, further taxonomic sampling and biochemical characterization is required to support this hypothesis. Further in-depth comparative analyses of the capsaicin and acylsugar biosynthetic pathways will enhance our comprehension of the evolutionary divergence of acyltransferase activities from a shared ancestral enzyme and how tissue-specific expression enables specialized chemical diversity (Fig. 3).

Connection of acylsugars and related compounds outside of the *Solanaceae*

Acylsugars are not restricted to the Solanaceae and have been detected in taxonomically distinct plant families, such as Convolvulaceae, Martyniaceae, Rubiaceae, and Caryophyllaceae (Asai et al. 2010, 2011; 2012; Asai and Fujimoto 2010; Dalsgaard et al. 2006; Grellet Bournonville et al. 2020; Kruse et al. 2022a; Moghe et al. 2023a; Płachno et al. 2009; Wu et al. 2013). All these families belong to the Superasterids clade of eudicots, with the Caryophyllaceae forming a distinct branch. This points to an ancient pathway that appeared early in the Magnoliopsida class (dicots) and later diverged, or that acylsugars independently evolved by convergently assembling the catalytic components to produce chemically related structures. The major differences between Solanaceae-type acylsugars from the acylsugars in other clades is that the acyl chains of Solanaceae-type acylsugars are attached via ester linkages (R-COO-R'), whereas acyl chains of acylsugars in other families can be attached by ester or ether (R-O-R') linkages and that the acyl chains sometimes adopt a cyclic nature outside the Solanaceae (Asai et al. 2011; Kruse et al. 2022a).

Biosynthetic pathways for acylsugars from outside the Solanaceae remain largely unknown, likely

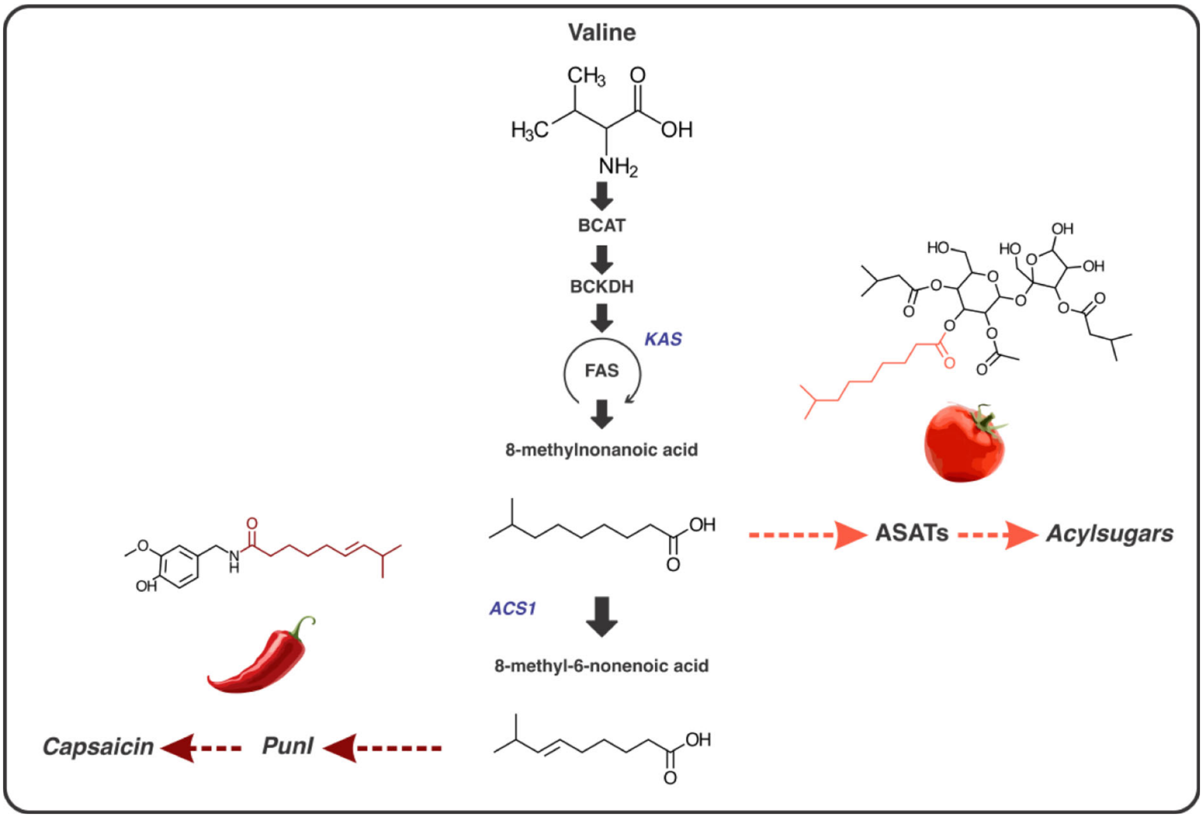


Fig. 3 Co-option of similar biochemical pathways to produce structurally distinct metabolites in the Solanaceae. Capsaicinoids and acylsugars are derived from similar biochemical pathways including BCAA, fatty acid elongation and homologous acyltransferase enzymes (Pun1 in capsaicinoid biosynthesis and ASATs in acylsugar biosynthesis). ACS: Acyl-CoA synthetase; BCAT: Branched-chain aminotransferase; BCKDH: Branched-chain α-ketoacid dehydrogenase; FAS: Fatty acid synthase; KAS: β-ketoacyl-ACP synthase

because there is not an extreme tissue specificity as observed in Solanaceae-type acylsugar biosynthesis, making pathway elucidation via comparative omics approaches challenging. It is likely that similar enzyme and transporter families have evolved in these diverse lineages to facilitate acylsugar metabolism and may provide unique insight into the convergent evolution of structurally related compounds across dicots. Although our understanding about the biological activity of these compounds is still incipient, it is thought that they can be directly related to the plant defense system. They can act, for example, as triggers to defense genes and cell apoptosis, cytotoxins, microbiological and α-glucosidase inhibitors, and traps for herbivores (Asai et al. 2010; Almeida-Souza et al. 2016; Grellet Bournonville et al. 2020; Maharani et al. 2022; Badawy et al. 2023). This way, Solanaceae-type acylsugars and acylsugar from other lineages have similar biological activities, bringing a broad spectrum of protection for the host.

Conclusions

The diverse pathways that have been co-opted into specialized metabolism in the Solanaceae illustrates the evolutionary ingenuity that plants display in adapting to the challenges of their environments. This review underscores the significant role of glandular trichomes as biochemical factories, producing a diverse array of acylsugars that serve as defenses against biotic and abiotic stresses. The biosynthetic pathways producing acylsugars across the Solanaceae and capsaicinoids within the *Capsicum* genus, are examples of the dynamic evolutionary processes that have shaped plant specialized metabolism. Through a detailed examination of the acylsugar biosynthetic pathway, this review highlights the complex network of enzymes that contribute to the structural diversity of these compounds. The ASATs demonstrate remarkable evolutionary plasticity, showcasing how gene and genome duplications, coupled with neofunctionalization, can lead to metabolic novelty. Furthermore, the parallels between acylsugar and capsaicinoid biosynthesis provide a compelling case study for the co-option of a common ancestral pathway and lineage-specific alterations leading to distinct metabolite classes. The discovery of acylsugars in taxonomically diverse plant families opens new avenues for understanding the evolutionary convergence of metabolic pathways and their impact on plant fitness.

Although Solanaceae-type acylsugars are particularly well studied plant specialized metabolites, much is yet to be discovered about the evolution and application of acylsugars. Further biochemical investigation of taxonomically diverse Solanaceae species and placement of metabolic traits within a phylogenetic context will continue to provide evolutionary insight into how plant chemical diversity emerges (Schenck and Busta 2021). Future studies should also focus on identification of key missing steps in the pathway and transport mechanisms that are inevitably involved in moving precursors, intermediates, and final products around and outside of the cell (Fig. 2). Additionally, using synthetic biology to engineer acylsugar production in other species, including plants and microbes, will provide insight into limitations of acylsugar biosynthesis in other species and allow production of biologically active metabolites in non-hosts.

In summary, the study of acylsugars and their biosynthesis not only enriches our knowledge of plant biology but also holds promise for applications in crop improvement and protection. These insights lay the groundwork for future research into the many ways plants produce and utilize specialized metabolites to navigate their ecological niches. As we advance our understanding of these pathways, we will unlock potential strategies for crop bioengineering. Enhancing the production of acylsugars through targeted breeding or genetic manipulation can lead to crops with improved resistance to pests and environmental stresses, thus contributing to sustainable agricultural practices and food security.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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