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Evolutionarily conserved phenylpropanoid pattern on angiosperm pollen

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The male gametophyte of higher plants appears as a solid box containing the essentials to transmit genetic material to the next generation. These consist of haploid generative cells that are required for reproduction, and an invasive vegetative cell producing the pollen tube, both mechanically protected by a rigid polymer, the pollen wall, and surrounded by a hydrophobic pollen coat. This coat mediates the direct contact to the biotic and abiotic environments. It contains a mixture of compounds required not only for fertilization but also for protection against biotic and abiotic stressors. Among its metabolites, the structural characteristics of two types of phenylpropanoids, hydroxycinnamic acid amides and flavonol glycosides, are highly conserved in Angiosperm pollen. Structural and functional aspects of these compounds will be discussed.

Diversity and specificity of phenylpropanoids

The general phenylpropanoid pathway is present in all higher plants. It dates back to the evolution of the earliest enzymatic steps from phenylalanine to hydroxycinnamic acids 450 million years ago [1]. During subsequent evolution within the plant kingdom, adaptive processes and molecular mechanisms resulted in an unsurpassed diversification and structural variation of the aromatic building blocks by a plethora of mainly oxidative and conjugating steps including ligation, glycosylation, and methylation, to name the most prominent ones [2,3]. The combinatorial potential of a single organism usually exceeds our expectations, with sometimes only vague ideas of functional justification. This complexity becomes already apparent in the model organism *Arabidopsis thaliana* (*Arabidopsis*), not necessarily known for coloration of leaves or attractive petals. Its major reddish leaf anthocyanin A11, observed under stress, is composed of four sugars, two aryl groups, and one acyl group attached to a cyanidin core structure [4]. In addition, about 25 different flavonol glycosylates were identified in *Arabidopsis* flowers by liquid chromatography–mass spectrometry (LC–MS) techniques [5]. Structural complexity and diversity usually increase with specialization and functionalization in organs of other flowering species [6].

The overall diversification of phenylpropanoids in a single species may seem overwhelming, but careful analysis of individual organs reveals a much simpler pattern of specialized metabolites that is not only characteristic for such organs or cell types but which is also sometimes conserved beyond the species level throughout the plant kingdom. The universal appearance of hydroxycinnamic acid amides (HCAA) and flavonol α-1,2-linked diglycosides (sophorosides) on the pollen wall surface discussed in this article are prominent, but apparently overlooked examples of organ specificity beyond the species level. Although the recent development of molecular tools facilitates the annotation of metabolites, enzymes, and pathways resulting in this specificity, precise functions of many of these unique and conserved chemical structures and their evolutionary significance or roles in fitness are either missing or often blurry. To cite the famous chemist Erwin Chargaff (1905–2002): 'Science is wonderfully equipped to answer the question 'How?' but it gets terribly confused when you ask the question 'Why?' The conserved HCAA and flavonol sophoroside pattern deposited onto in the polymeric pollen wall and covering the mature gametophyte is one of these intriguing examples that illustrate our lack of knowledge on the precise function of specialized metabolites and will be discussed in the following.

Sporopollenin and the tryphine are essential parts of the pollen wall

Angiosperm gametophytes are protected by a complex, rigid, and very durable polymer, the pollen wall synthesized by the tapetum, the inner layer of the anther locule [7]. This single cell layer is a biosynthetically highly-active and specialized compartment with the unique task of providing metabolites and structural components to the protective shell of the maturing gametophyte [8]. The rigid polymeric part of the pollen wall, also termed sporopollenin, is composed of several layers of heterogeneous aliphatic and aromatic building blocks covalently bound by ether and ester bonds. The usually sculptured exine on top of a less-structured intine is very substantial [9,10]. Harsh chemical degradation of the pollen wall was only of limited value in resolving its monomeric composition [11], whereas recent genetic evidence was

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able to dissect the contributions of aliphatic and aromatic building blocks from fatty acid and the polyketide biosynthetic pathways [10,12–14].

The polymer is covered by a hydrophobic mix of compounds consisting of various aliphatic and aromatic structures including waxes, lipids, sterols, sugars, and proteins, termed pollen coat, tryphine, or pollen kit [9]. This mixture provides the actual interface between the pollen and the surrounding environment, and contains components essential for pollen recognition and successful fertilization [15,16]. Among the non-polymerized and soluble aromatic constituents deposited on the surface, two specific types of phenylpropanoids, flavonol glycosides and HCAAs, were already identified as being characteristic and highly abundant in pollen of many species three decades ago [11,17]. Since then a universal but distinct signature of both phenylpropanoid classes was reported from evolutionarily distant angiosperm families such as Brassicaceae, Fagaceae, Oleacease, and Iridaceae: the characteristic presence of 3-*O*-sophorosides in the case of flavonols, and the presence of tris-substituted spermidine conjugates in the case of HCAAs (Figure 1). Early biosynthetic and functional hypothesis were published in the 1990s followed by 15 years of virtual silence and lack of significant scientific novelties or functional relevance. Only in the past 5 years have simultaneous and rapid breakthroughs by several research groups resulted in the identification of a set of tapetum-specific genes associated with the formation of both types of phenylpropanoids characteristic of (or specific to) the pollen coat. The corresponding biosynthetic enzymes resulting in formation of both types of compounds have been characterized in detail, and first datasets are now available which address functional aspects of these specialized pollen metabolites.

Flavonol α-1,2-linked 3-*O*-sophorosides are universally found in the tryphine

Since the earliest identification of flavonol sophorosides (Figure 1) as the major constituent in the pollen of Juglandaceae, Fagaceae, Betulaceae, and Oleaceae [18], the

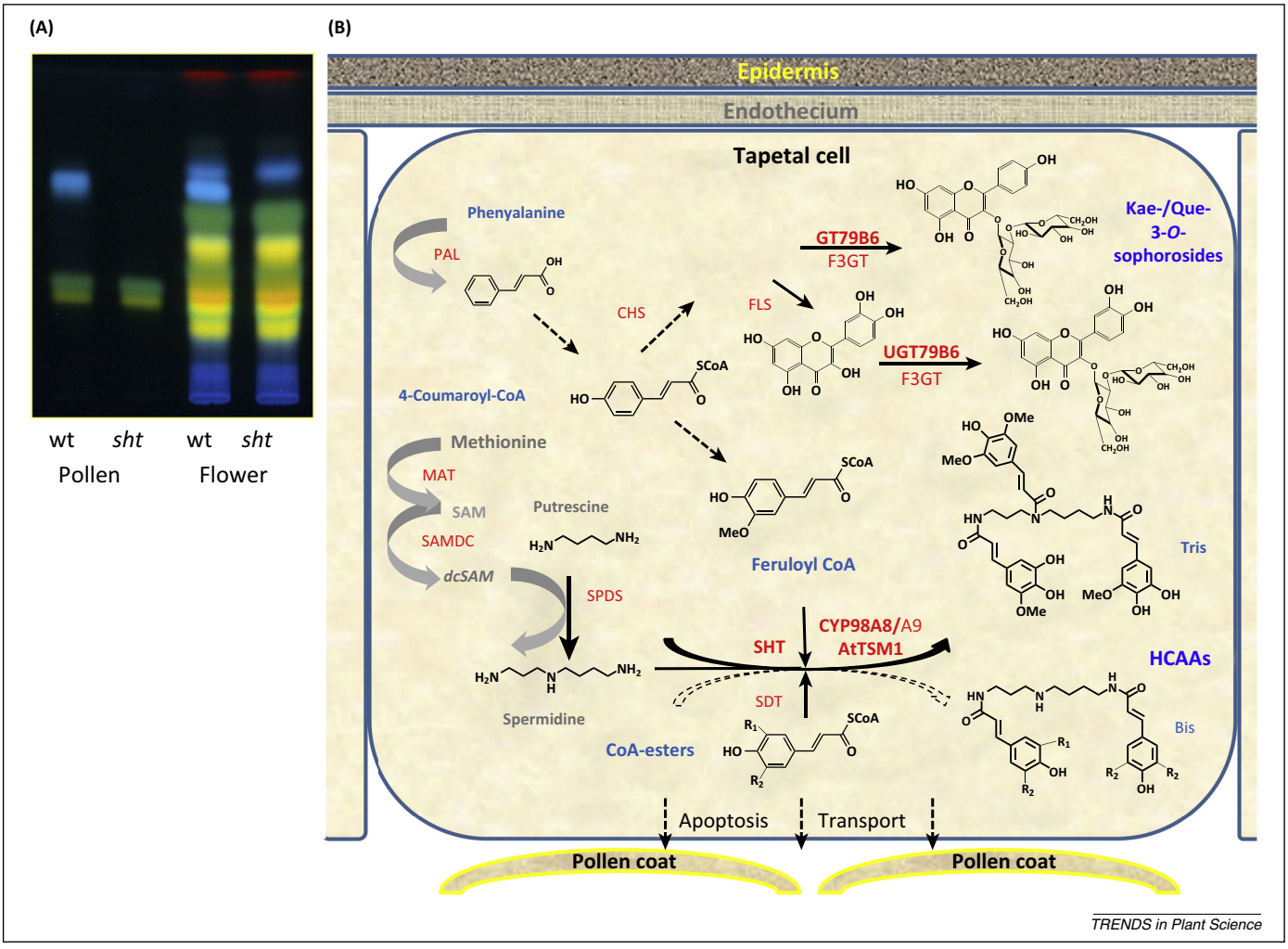

Figure 1. **(A)** High performance thin layer chromatography (HPTLC)-based phenylpropanoid fingerprints of pollen and flower buds from wild type (wt) and *sht* (spermidine hydroxycinnamoyltransferase-deficient) *Arabidopsis*. Compounds were derivatized with Naturstoffreagent A to better differentiate between HCAAs (blue), kaempferol (greenish), and quercetin (yellow) 3-*O*-sophorosides in pollen. Notice the simple pattern of pollen specific flavonoids compared to the complex pattern of flavonoids in flower buds. **(B)** Schematic illustration of the main pathways contributing to the pattern of soluble phenylpropanoids of the pollen coat. Relevant enzymes are marked red; those catalyzing tapetum-specific reactions are in bold. Abbreviations: AtTSM1, *Arabidopsis thaliana* tapetum-specific methyltransferase 1; CHS, chalcone synthase; F3GT, flavonol 3-*O*-glucosyltransferase; FLS, flavonol synthase; MAT, methionine adenosyltransferase; PAL, phenylalanine ammonia lyase; SDT, spermidine disinapoyltransferase; SAMDC, *S*-adenosyl-L-methionine decarboxylase; SHT, spermidine hydroxycinnamoyl transferase; SPDS, spermidine synthase. Kae/Que-3-*O*-sophorosides; kaempferol/quercetin-3-*O*-sophorosides. R1/R2, -OH or -OMe.