Commentary

All roads lead to Rome: alternative biosynthetic routes in plant specialised metabolism

About 500 million years ago, land plants emerged from an aquatic algal ancestor (Morris *et al.*, 2018). Understanding the nature of the evolutionary adaptations that allowed these first colonisers to survive the challenges of life on land is a core question of plant biology. The growing number of sequenced genomes from plant lineages other than angiosperms has enabled unprecedented studies of the evolutionary trajectory towards terrestrialisation. Major adaptations to life on land have included not only visible changes to plant structure and biology – such as roots, vasculature and alternation of generations (Bowles *et al.*, 2022) – but also biochemical strategies to face new abiotic and biotic stresses (de Vries *et al.*, 2021; Rieseberg *et al.*, 2022). Terrestrial plants have evolved a great diversity of specialised compounds that protect them from the challenges of their environment such as drought, increased UVB radiation, temperature fluctuations and pathogen attack (de Vries *et al.*, 2021; Kulshrestha *et al.*, 2022). In this issue of *New Phytologist*, T-T. Zhu *et al.* (2023; pp. 515–531) reveal a novel biosynthetic pathway to one of these stress-tolerance compound groups.

'This biosynthetic route differs from that reported for the production of the structurally similar cannabinoid precursors in Cannabis…*'.*

Each land plant lineage synthesises a distinct set of specialised metabolites, most likely as a consequence of adaptation to their specific ecological niches. Some of these metabolites will be produced by pathways inherited from the last common ancestor of land plants, but many will be made by pathways that arose after lineages diverged. These lineage-specific compounds may be alternative compounds fulfilling the same ecological role or could have unique attributes conferring distinct ecological advantages. Elucidating the genetic basis of such specialised metabolite pathways is important both for understanding plant–environment interactions and for resolving the evolutionary history of plant metabolism. The overlapping presence of

metabolites in itself is not sufficient to deduce common genetic pathways, and there are increasing examples of the same metabolite structures being produced by different pathways as the result of convergent evolution, both among land plants and between plants and fungi (Kulshrestha *et al.*, 2022; Lou *et al.*, 2022).

T-T. Zhu *et al.* (2023) investigated the genetic players in the bibenzyl biosynthetic pathway of the model liverwort species *Marchantia polymorpha*, in which the pathway appears to be a core biotic defence mechanism (Romani *et al.*, 2020). Although bibenzyls are relatively rare in plants and have received little research attention, the core bibenzyl scaffold is key to the production of numerous commercially valuable plant natural products. Bibenzyls have been reported from a small number of species scattered across angiosperm orders, including the Asparagales (specifically, the Orchidaceae), Asterales, Cornales, Rosales (notably, *Cannabis* spp.) and Fabales, as well as in liverwort genera, such as *Marchantia* and *Radula*. Bibenzyl production in these diverse species, therefore, likely represents several convergent evolution events.

T-T. Zhu *et al.* (2023) confirm the involvement and the essential interaction of two core bibenzyl biosynthetic enzymes: a Type III polyketide synthase (PKS; stilbene carboxylate synthase/STCS) and a polyketide reductase (PKR), whose catalytic activity and physical interaction produce the bibenzyl lunularic acid. This biosynthetic route differs from that reported for the production of the structurally similar cannabinoid precursors in *Cannabis* (Boddington *et al.*, 2022), supporting the hypothesis of convergent evolution. Type III PKSs are multifunctional enzymes catalysing the sequential condensation of malonyl-CoA to a CoA-linked starter molecule and subsequent ring cyclisation. These enzymes are powerful drivers of metabolic diversity in plants, as they are able to generate structurally and functionally diverse compounds from a limited pool of precursor molecules through varied numbers of condensations, and by alternative cyclisation reactions of the intermediate, for example, C6-C1 Claisen and C2-C7 aldol cyclisation (chalcone synthase/CHS and stilbene synthase/STS in flavonoid or bibenzyl biosynthesis, respectively) or C5-O-C1 lactonisation. Polyketide synthases have likely had a significant role in facilitating terrestrialisation by providing the precursors for flavonoid and sporopollenin synthesis (Naake *et al.*, 2021), compounds that are essential for UV protection and spore/pollen durability, respectively.

As with many specialised metabolic pathways (Lou *et al.*, 2022), *PKS* genes are thought to have arisen from the duplication of a gene from a primary metabolic pathway (fatty acid biosynthesis), followed by neofunctionalisation (Austin & Noel, 2003; Naake *et al.*, 2021). The *PKS* genes probably originated before the emergence of the last common ancestor of land plants, based on analyses of whole-genome data of 126 species spanning the green
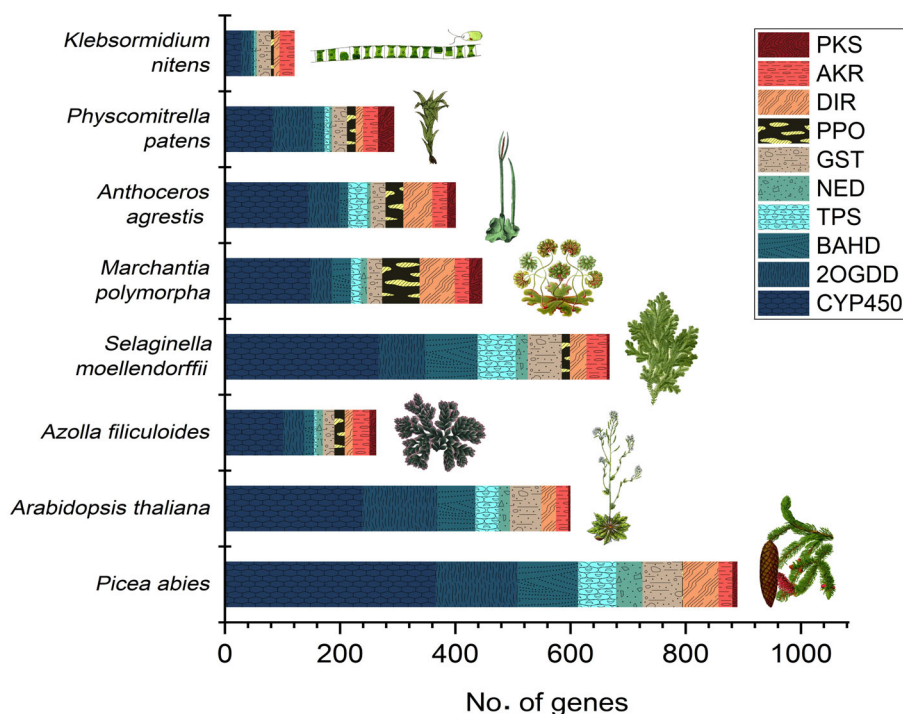
Fig. 1 Gene numbers in enzyme families important in plant specialised metabolism. Gene numbers were compared in eight plant species representing the major lineages of streptophytes: *Klebsormidium nitens* (streptophyte algae); *Physcomitrium patens* (mosses); *Anthoceros agrestis* (hornworts); *Marchantia polymorpha* (liverworts); *Selaginella moellendorffii* (lycophytes); *Azolla filiculoides* (ferns); *Arabidopsis thaliana* (angiosperms); and *Picea abies* (gymnosperms). 2OGDD, 2-oxoglutarate-dependent dioxygenase; AKR, aldo–keto reductase; BAHD, BAHD acyltransferase; CYP450, cytochrome P450; DIR, dirigent protein; GST, glutathione-*S*-transferase; NED, NAD-dependent epimerase/dehydratase; PKS, polyketide synthase; PPO, polyphenol oxidase; TPS, terpene synthase. Sources: Li *et al.* (2018) and our own BLAST searches using publicly available genomes.

lineages, with *STS*s arising multiple times from *CHS* genes (Naake *et al.*, 2021). In *M. polymorpha*, L. Zhu *et al.* (2022) show that the 24 identified *PKS* sequences include a mix of *CHS* and *STCS* genes.

Metabolites synthesised through PKS activity rarely accumulate *in planta* and are usually processed by diverse tailoring enzymes, of which PKRs are one class, increasing polyketide chemical and functional diversity. Few PKRs have been characterised, notably chalcone reductase (Bomati *et al.*, 2005), but new activities have recently been reported from both bryophytes (T-T. Zhu *et al.*, 2023) and angiosperms (Ohno *et al.*, 2022; L. Zhu *et al.*, 2022). PKSs play a crucial role in guiding the metabolic flux during the PKS reaction, stabilising the intermediates formed and preventing them from undergoing nonspecific cyclisation. T-T. Zhu *et al.* (2023) confirm the importance of protein–protein interactions in the coordinated action of a PKS–PKR complex. There is increasing recognition of the importance of such accessory proteins for generating diversity in specialised metabolism (Dastmalchi, 2021), and gene numbers suggest they may be relatively more abundant in bryophytes (Fig. 1).

Polyketide synthases are members of the aldo–keto reductase (AKR) superfamily that is important in oxido-reduction reactions in plants, including specialised metabolism. Phylogenetic analysis on 36 plant species identified 1268 *AKR* genes (Duan *et al.*, 2020), including algae. The number of *AKR* genes appears to be diverse and species-specific in angiosperms, reflecting gene and genome duplication followed by subfunctionalisation (the same activity is retained) or neofunctionalisation (when mutations confer a new activity). L. Zhu *et al.* (2022) suggest that, in *Gerbera hybrida*, PKRs were recruited from the conserved sporopollenin biosynthesis pathway and co-opted to defence-related polyketide biosynthesis. The sporopollenin biosynthesis-associated PKS A/B clade is one of the two early clades of plant PKSs that emerged before the divergence of bryophytes and tracheophytes (vascular plants; Naake *et al.*, 2021).

Studies on the *AKR* gene family support the hypothesis that all land plants started with the same core toolkit of biosynthetic genes, inherited from their last common ancestor, but that gene families adapted and evolved markedly differently according to extrinsic environmental factors and the chances of evolution during the diversification of each lineage. The production of the chemical catalogue of land plants is often catalysed by the protein products of members of large gene families, with diverse neofunctionalisations within families indicating high evolutionary versatility (de Vries *et al.*, 2021). Angiosperms usually contain the largest gene families, followed by other vascular plants, with bryophytes having the smallest. However, this is not universal across the different enzyme types and, in the case of the AKRs, the gene families in lycophytes and bryophytes examined are larger than in some seed plants (Duan *et al.*, 2020).

In Fig. 1, we present gene family numbers across model species for different land plant lineages for 11 of the main enzyme families for specialised metabolism. While gene numbers in the families generally follow the pattern of increasing from bryophytes to lycophytes to seed plants, there are marked variations, with the *AKR*, *PKS*, *Dirigent* and *Polyphenol Oxidase* families being largest in bryophytes and forming a much larger proportion of the total gene numbers of those species. This could reflect gene duplication events that occurred early in the evolution of the bryophyte lineage that generated candidates for neofunctionalisation and retention in the hornworts, liverworts and mosses. However, it is also possible these gene families shrank in relative size during the evolution of seed plants. The comparatively large *Cytochrome P450 monooxygenase*, *UDP-Glycosyltransferase* and *BAHD acyltransferase* gene families of

angiosperms are reflected in the extensive secondary modifications that 'decorate' specialised metabolites in many species.

The increasing availability of plant whole-genome sequences is facilitating an exciting period of research advances for plant specialised metabolism. This includes both the elucidation of new biosynthetic pathways, as is well illustrated by T-T. Zhu *et al.* (2023), and also comparative genomics to infer the possible evolutionary pathways of specialised metabolism across land plants, horizontal gene transfer events and the frequency of biosynthetic gene clusters. However, for evolutionary studies, the accuracy of functional annotation is key. Yet, this is often unreliable when the existing angiosperm data are used to annotate genomes from other major plant lineages. Moreover, the study of T-T. Zhu *et al.* (2023) highlights that, even when genes share high sequence similarity, shared enzymatic function cannot be assumed. Functional data on candidate genes are greatly desirable for such studies, especially for nonseed plants. A limitation is the lack of genome data for lineages such as hornworts, liverworts, lycophytes, ferns and cycads, where we currently have only a few model species from which to draw conclusions. The divergence in the estimated sizes of *Cytochrome P450 monooxygenase* gene families among the three available fern genome sequences illustrates the problems of limited sampling, ranging from 80 in *Salvinia cucullata* (1C = 0.26 Gb) to 309 in *Ceratopteris richardii* (1C = 9.6 Gb; Marchant *et al.*, 2022). Additional high-quality genome sequences from across the phylogenetic diversity of nonseed plants should facilitate both more robust evolutionary proposals and exciting discoveries, particularly on the novel biosynthetic pathways and biological activities of the specialised metabolites of these under-studied major land plant lineages.

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