Flavonoid biosynthesis and Arabidopsis genetics: more good music

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In this issue of Journal of Experimental Botany (pages 1505–1517), Ishihara et al. report the identification of a gene responsible for the production of flavonol 3-O-gentiobioside 7-O-rhamnoses by elegantly tickling the ivories of Arabidopsis genetics and genetic resources combined with straightforward metabolite analysis: it is a model case of functional evaluation.

In plants and within a given plant species. However, only the decoration of these aglycones with various carbohydrate side chains and further chemical modification provides the full flavonoid range.

Again, genetics in combination with biochemistry, metabolite analyses and, in particular, gene co-expression patterns led to the identification of several UDP-carbohydrate-dependent glycosyltransferases (UGTs) conjugating flavonoids with different carbohydrates at different positions (Jones et al., 2003; Yonekura-Sakakibara et al., 2008, 2012). Nevertheless, there are still unresolved cases, one of them being the production of the Arabidopsis accession-specific flavonol 3-O-gentiobioside 7-O-rhamnoses (F3GG7Rs), comprising the flavonols kaempferol, quercetin or isorhamnetin with the specific carbohydrate decoration 3GG7R, which are most probably derived through glycosylation from flavonol 3-O-glucoside 7-O-rhamnoside (F3G7R) precursors.

A novel flavonol glycosyltransferase

Ishihara et al. (2016) have now identified a gene responsible for this final step in the production of F3GG7Rs. Previous reports that the accessions Ler and Nö-0, but not Col-0 and Cvi, contain F3GG7Rs were extended to a collection of 81 accessions, of which just half were F3GG7R-producers. The Ler F3GG7R trait was inherited in a dominant manner in a cross with the F3GG7R-lacking Col-0. Linkage analysis using an F3GG7R-metabotropic, 95-member-sized Ler × Col recombinant inbred (RI) population as
well as recombination events within the originally identified interval using a further 200 additional RI lines eventually revealed the locus responsible in a small 87 kb region on chromosome 1. No obvious candidate, such as a UGT gene, was located in that interval; however, genome-wide association mapping of the F3GG7R metabotype of the 81 accessions confirmed the RI linkage mapping and eventually identified a single-nucleotide polymorphism leading to a premature stop codon in the Col-0 allele of BETA GLUCOSIDASE 6 (BGLU6). In contrast, Ler contains a fully functional BGLU6 gene and F3GG7R production was generally associated with functional BGLU6 alleles.

Transcriptional co-expression analysis has been shown to be a valuable tool in the study of flavonol biosynthesis (see above); thus, the association of BGLU6 with this pathway further supported its likely involvement in F3GG7R biosynthesis. Nevertheless, this finding is remarkable at first sight, since the underlying expression data were primarily derived from the F3GG7R-deficient Col-0 harbouring only the BGLU6 pseudogene. However, the promoter sequences of the Ler and Col-0 alleles are highly similar and thus transcriptional co-regulation was not affected by either functional (Ler) or non-functional (Col) gene transcripts. On the other hand, Ishihara et al. (2016) point out based on published RNA-Seq data that the expression level of functional BGLU6 alleles was about twofold higher than the transcription from BGLU6 alleles leading to transcripts harbouring the premature stop codon. The reason for this negative impact on the abundance of the non-functional transcript (either transcription or stability of the mRNA) is not clear, but it may be an interesting future issue in relation to the pseudogenization of gene copies.

After this genetic free-form jazz, the scales were still completed successfully: luckily, BGLU6-targeting insertion lines in two F3GG7R-accumulating accessions, Ler and Ws-4, were available and both led to the loss of F3GG7R production. Conversely, the F3GG7R-deficient Col-0 gained the ability to synthesize F3GG7R after genetic transformation with a functional Ler BGLU6 gene fragment.

Genetics leading the way

Biochemical proof by an in vitro enzymatic activity test could not be provided by Ishihara et al. (2016), since expression of the recombinant BGLU6 protein failed in several systems. However, genetics has provided overwhelming evidence that BGLU6 is indeed responsible for F3GG7R formation. This not only adds another piece of information about the complex formation of flavonol glycosides in Arabidopsis, but also provides strong evidence that acyl-carbohydrates utilized as sugar donors by beta-glucosidases, such as the putative beta-glucosidase BGLU6, are involved in flavonol glycosylation in addition to the well-known UDP-carbohydrate donors used by UGTs. This extends recent reports on beta-glucosidases being involved in Arabidopsis anthocyanin glycosylation (Miyahara et al., 2013).

Nevertheless, the identification of this new molecular player being responsible for producing the accession-specific F3GG7R flavonol glycosides could not provide clues to a specific physiological or ecological role. The same is mostly true for the plethora of specifically decorated flavonoids. Most probably, only genetics will be able to lead the way to unraveling such functional relations between specific flavonoid glycosides and particular processes and functions (Yin et al., 2014). More music expected.

Key words: Arabidopsis thaliana, flavonoid, flavonol glucosyltransferase, glycoside hydrolase-type, natural variation, whole-genome association mapping.

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