Insight

Insights into the control of metabolism and biomass accumulation in a staple C₄ grass

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As Sorghum is an important staple food and biofuel crop, Chen et al. (2020) performed an EMS mutagenesis screen in Sorghum bicolor to isolate candidate genes regulating biomass production and grain yield. The mutant by1 showed a drastic reduction in both biomass and yield. The characteristic growth features associated with decreased biomass of by1 were reduced plant height, thinner stems, and smaller, narrower leaves. In by1, internode, leaf blade, and vascular cells were developed normally except that the final cell volume was significantly reduced. The authors conclude that reduced cell expansion was associated with abnormal growth phenotypes of by1, which ultimately resulted in decreased plant biomass. Developmental defects including poorly developed panicles, anthers, and pollen contributed to the final reduction of grain yield in by1. In by1, anthers were thin and small and, moreover, pollen viability was reduced by ~50% and pollen grains appeared shrunken.

To map the causative gene responsible for the by1 phenotype, by1 was outcrossed to Shangzhuang broomcorn. Using a map-based cloning approach, an EMS-induced single single nucleotide polymorphism (SNP; cytosine to thymine) was mapped to the Sobic.002G379600 (BY1) gene, which resulted in a Pro to Leu amino acid substitution (Pro192Leu) in the protein product. The gene BY1 encodes a DAHPS, which catalyses the first reaction in the shikimate pathway. Also, mutations of various shikimate enzymes were identified in the screen for embryo-lethal mutants in Arabidopsis (Tzafrir et al., 2004; Pagnussat et al., 2005). Despite its importance, it is still not completely understood how the shikimate pathway in plants is regulated. Although the existence of feedback regulation by the levels of AAAs and metabolites derived from them has been shown in a few previous studies, the molecular mechanism remains elusive (Maeda and Dudareva, 2012).

The shikimate pathway in chloroplasts is an important link between primary and secondary plant metabolism. It is composed of seven steps, starting with the substrates phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P), produced by the glycolytic and pentose phosphate pathways, respectively. The pathway results in the formation of chorismate, which serves as a precursor molecule for the synthesis of the aromatic amino acids (AAAs); Phe, Tyr, and Trp (Box 1). In plants, AAAs are not only essential building blocks for protein synthesis, but are also precursors for many secondary metabolites including flavonoids, lignin, and hormones (auxin and salicylic acid) (Maeda and Dudareva, 2012).

The shikimate pathway is required for growth and development. The well-known herbicide, glyphosate, inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase—an enzyme involved in the shikimate pathway. Also, mutations of various shikimate enzymes were identified in the screen for embryo-lethal mutants in Arabidopsis (Tzafrir et al., 2004; Pagnussat et al., 2005). Despite its importance, it is still not completely understood how the shikimate pathway in plants is regulated. Although the existence of feedback regulation by the levels of AAAs and metabolites derived from them has been shown in a few previous studies, the molecular mechanism remains elusive (Maeda and Dudareva, 2012).
Box 1. The shikimate pathway in plants

The shikimate pathway converts the substrates phosphoenol pyruvate (PEP) and erythrose-4-phosphate (E4P) into chorismate in seven enzymatic steps. Chorismate is the precursor for aromatic amino acids and many secondary metabolites. The \textit{by1} mutation in Sorghum was mapped to an amino acid substitution in the enzyme catalysing the first committed step in the shikimate pathway, the DAHP synthase (highlighted in red).

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Abbreviations: Phosphoenolpyruvate (PEP), erythrose-4-phosphate (E4P), 3-deoxy-o-arabino-heptulosonate-7-phosphate (DAHP), 3-dehydroquinate (DHQ), 3-dehydroquinate dehydratase/shikimate:NADP oxidoreductase (DHQase/SORase), 5-enolpyruvylshikimate-3-phosphate (EPSP).

Report that the Pro192Leu substitution reduced the BY1 enzyme activity by \(~33\%\). \textit{BY1} function was validated in rice by targeting Loc\_Os07g42960 (a homologue of Sorghum \textit{BY1}) by CRISPR/Cas9 [clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein]. Growth and developmental abnormalities were similar to Sorghum \textit{by1}, and both biomass and yield were also drastically decreased.

Chen \textit{et al.} (2020) analysed the leaf transcriptome and metabolome of the wild type and \textit{by1} to dissect the developmental pathways that were affected in the mutant line. Most of the genes involved in pathways operating upstream of the shikimate pathway (photosynthesis, glycolysis, and the pentose phosphate pathway) and the phenylpropanoid pathway that operates downstream of the shikimate pathway were significantly up-regulated. In contrast, the levels of metabolites in those pathways including PEP, shikimate, Phe, and chalcone were reduced. Interestingly, most of the metabolites with significantly lowered levels were phenylpropanoids, and 19 of 22 flavonoids were less abundant. The authors propose that a positive feedback signal in response to reduced metabolites enhances the expression of genes involved in
the shikimate pathway and its upstream pathways to restore the carbon flux into the shikimate pathway. This is in agreement with previous studies that showed that shikimate pathway genes in response to AAAs and metabolite levels were regulated at the gene expression level rather than at the post-translational level (Maeda and Dudareva, 2012).

The reported developmental defects in the by1 mutant are interesting given that previous works on DAHPS orthologues have also mainly proposed roles in secondary metabolism. In Arabidopsis and cotton, DAHPS expression is induced upon wounding or pathogen infection (Keith et al., 1991; Yang et al., 2015). RNAi silencing of PhiDAHP1 in Petunia, one of the two DAHPS isoforms, leads to a reduction of floral volatile benzoid/phenylpropanoids levels (Langer et al., 2014).

**Future perspectives**

This work is interesting in the context that Sorghum is an important C₄ grass belonging to the NADP-ME subtype. In C₄ plants, PEP is the main carbon acceptor of the initial CO₂ fixation via PEP carboxylase leading to the formation of the four-carbon acid. As the metabolite profiling in the by1 mutant showed low PEP levels, it would be interesting to know how this impacts the carbon flux through the C₄ pathway and ultimately photosynthetic efficiency. Low photosynthetic efficiency may underpin the reduction in biomass and grain yield in the by1 mutant.

Plant growth in by1 could also be affected due to reduced cell expansion. Levels of AAAs including Trp and most of the flavonoids were lowered in by1. The plant hormone auxin, which is a major regulator of plant growth and development, exerts many of the developmental processes through cell division and expansion (Perrot-Rechenmann, 2010), and polar auxin transport is known to control these processes (Michniewicz et al., 2007). Auxin is mainly synthesized from the amino acid Trp (Trp-dependent pathway); however, it can also be produced via a Trp-independent pathway. The branch point of both pathways, indole-3-glycerol phosphate, is an intermediate product of the Trp biosynthesis pathway (Ouyang et al., 2000).

The Arabidopsis, indole-3-glycerol phosphate synthase (IGS) and tryptophan synthase mutants were smaller and free auxin levels were reduced in IGS mutants (Last et al., 1991; Ouyang et al., 2000). Interestingly, it has been shown that flavonoids function as endogenous regulators of polar auxin transport. In the flavonoid-deficient Arabidopsis mutant transparent testa4 (tt4), plant height and inflorescence stem thickness were decreased due to elevated basipetal auxin transport (Brown et al., 2001). Furthermore, the root gravitropic response was delayed in tt4 due to failure in the prompt establishment of the auxin gradient (Buer and Munday, 2004). Some of the auxin efflux transporters (PITs) were mislocalized in flavonoid-deficient mutants (Peer et al., 2004). However, the level of conservation of the mechanism of flavonoids as negative regulators of auxin transport is unknown. Therefore, measuring free auxin levels and studying auxin transport in the by1 mutant might help to dissect the contribution of auxin to the developmental defects observed in by1.

In by1, 50% of the pollen grains were sterile due to a poorly developed pollen wall. What possible mechanisms could be involved? It has been shown that pollen integrity is associated with its viability (Zhang et al., 2007; Ren et al., 2020). The pollen wall is developed in a stepwise process: the microspore mother cell is surrounded by a temporary callose wall, and its timely degradation is important for the release of newly formed microspores from tetrads and formation of exine (outer pollen wall). Pollen wall development and cell integrity were impaired in Arabidopsis ms188 (MYB103) and rice dmd1 (defective microspore development l) knockout mutants that showed a delay in the timely degradation of the callose wall (Zhang et al., 2007; Ren et al., 2020). This ultimately resulted in male sterility. Moreover, flavonoids are known to be involved in pollen development and viability, and their role as reactive oxygen species (ROS) scavengers is crucial in maintaining pollen viability under heat stress conditions (Santiago and Sharkey, 2019).

Therefore, in-depth cytological and transcriptome/proteome/metabolome analyses of anthers and/or pollen would be helpful to understand the developmental and molecular mechanisms behind abnormal anthers and pollen of by1. This would have broader applications in breeding male-sterile hybrids for important agronomic traits.

The amino acid substitution Pro192Leu could affect DAHPS activity in many different ways. The authors propose that the by1 mutation changes the tertiary structure of the protein and this causes a reduction in enzyme activity. Previous work on the Arabidopsis DAHPS enzyme activity showed that DAHPS is subjected to redox regulation via the thioredoxin (TRX)/ferredoxin (Fd) system (Entus et al., 2002). Further research would be needed to validate the change in tertiary structure, and its effect on substrate binding and redox regulation to work out precisely how the mutation compromises enzyme activity.

In summary, Chen et al. (2020) report that reduced activity of DAPHS alters the homeostasis between primary and secondary metabolism, causing growth and developmental abnormalities in Sorghum. The transcriptome and metabolite data sets from this study will be useful in future to increase our understanding of the shikimate pathway and its link to primary and secondary metabolism in Sorghum.

**Keywords:** Biomass and yield, DAHPS development, metabolism, shikimate pathway.

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