**Leaf: Photosynthesis**

Among 40 pathways scored for leaf-specific expression, the most significant are biosynthetic pathways for chlorophyll, beta-carotene, xanthophylls, methylerythritol, antheraxanthin, violaxanthin, rhamnose, geranylgeranyldiphosphate and flavin/flavonol and catabolic/degradation pathways of chlorophyll and xylose. The well-known and well-characterized genes *lesion22* (*les22, GRMZM2G044074*), a uroporphyrinogen decarboxylase (EC:4.1.1.37) that catalyzes the decarboxylation of uroporphyrinogen-III to coproporphyrinogen-III (Hu et al., 1998) and is engaged in both heme and chlorophyll synthesis, and the *oil yellow1* (*oy1, GRMZM2G419806*), a protoporphyrin IX magnesium chelatase (EC: 6.6.1.1) that catalyzes the committed step in chlorophyll synthesis (Sawers et al., 2006), were specifically expressed in leaf. This is not unanticipated given that the visual phenotypes reported for *les22* are epidermal lesions and *oy1* shows yellow tissues apparent in leaf. However, homologs of *les22* were expressed in both leaf and anther, and *oy1* homologs were expressed in leaf, anther, and embryo tissues (Figure 4iii). This may indicate a change in organ-specific expression subsequent to duplication (sub-functionalization) of members of this gene family. In addition, the expression of genes involved in chlorophyllide a biosynthesis (where chlorophyllide a is a metabolite produced in reactions leading up to chlorophyll biosynthesis) is observed primarily in leaf as expected, but also at reduced levels in embryo and anther (Figure 4iii). The porphyrin synthesis reaction catalyzed by protoporphyrinogen oxidase (EC:1.3.3.4) in the chlorophyllide a biosynthetic
pathway has been shown in rice to improve response to drought stress (Phung et al., 2011), indicating that its activity may be of broad impact beyond chlorophyllide a biogenesis. The maize protoporphyrinogen oxidase encoding gene GRMZM2G039396 is over expressed in leaf, while GRMZM2G078033 is over-expressed in both, anther and endosperm, and may be primarily engaged in heme synthesis.

**Root: Phenylpropanoid (Lignin) Biosynthesis**

A number of biosynthetic pathways of secondary plant products including the initial reactions of phenylpropanoid biosynthesis (i.e., steps leading up to 4-coumarate synthesis and eventually lignin (Figure 4v) and suberin biosynthesis (Supplementary Figure S2), monolignol, ferulate and sinapate biosynthetic pathways) show over expression in the root. For example, two well-characterized genes *cinnamoyl CoA reductase2 (cnr2, GRMZM2G131836)* (Pichon et al., 1998; Tamasloukht et al., 2011) and *brown midrib3 (bm3, AC196475.3_FG004)* (Vignols et al., 1995; Piquemal et al., 2002) are involved in lignin biosynthesis (Figure 4v). The *cnr2* gene product *cinnamoyl CoA reductase2* (EC:1.2.1.44) catalyzes the reduction of 4-coumaroyl-CoA to coumaraldehyde — the first committed step of the lignin-specific branch of monolignol biosynthesis, and also catalyzes feruloyl-CoA reduction to coniferaldehyde. The *bm3* gene encodes the enzyme caffeate O-methyltransferase (EC:2.1.1.68) that catalyzes the synthesis of sinapaldehyde and S-adenosyl-L-homocysteine from 5-hydroxy-coniferaldehyde and S-
adenosyl-L-methionine (SAM). Our analysis shows that cncr2 is preferentially expressed in roots (Figure 4v), which is consistent with previous studies (Pichon et al., 1998; Fan et al., 2006). In addition, we found that of the several known cncr2 homologs (Guillaumie et al., 2007), two genes, GRMZM2G146031 and GRMZM2G017285, are expressed preferentially in the root and at a higher level than cncr2. In contrast, the homologous cncr1 (GRMZM2G131205) (Tamasloukht et al., 2011), known to be widely and constitutively expressed in all lignified tissues (Pichon et al., 1998), was not present in the differentially expressed gene list based on our tissue-specific filtering and cutoff values. The down-regulation of cncr1 gene in one maize mutant has been associated with improved cell wall digestability, altered lignin structure in cell walls of sclerenchyma cells surrounding the vascular bundles, and deregulation of kinesin and katanin genes that are involved in cellulose microfibril deposition (Tamasloukht et al., 2011). Initially the fact that these genes appear to be uniquely expressed in roots came as a surprise given that lignin is deposited in specific cell types throughout the plant. For example, the bm3 mutant (Vignols et al., 1995) exhibits a reddish brown pigmentation of the leaf midrib beginning at the four to six leaf stage. Mutations in bm3 are known to alter lignin composition and plant digestibility for ruminants, and therefore constitute prime candidates in the breeding of silage maize for cattle feed and bioenergy production. Even though these roles indicate an effect of bm3 throughout the plant, it has been reported that expression is strong in roots and weak in other tissues (Collazo et
al., 1992; Vignols et al., 1995) and this is also consistent with our observations, indicating strong expression in root.

The lignin biosynthetic pathway gene gpm105, (GRMZM2G035584) (Falque et al., 2005), a putative shikimate O-hydroxycinnamoyltransferase (EC: 2.3.1.133) (Fernandes et al., 2002; Gardiner et al., 2004; Falque et al., 2005), shows root-specific upregulation (Figure 4v). Shikimate-O-hydroxy-cinnamoyltransferase catalyzes the transfer of small chemical groups (other than the aminoacyl groups), leading to synthesis of 4-coumaroyl-shikimate from 4-coumaroyl-CoA and shikimate. It is also involved in the synthesis of trans-5-O-(4-coumaroyl)-D-quininate in a reaction similar to the shikimate reaction where shikimate is replaced by L-quinate as a reactant. Another gene, pal1 (GRMZM2G074604) that encodes phenylalanine ammonia lyase1 (EC:4.3.1.24), is involved in the initial reactions of lignin biosynthesis (Andersen et al., 2007) (MaizeCyc pathway ID PWY1F-467) and was found to be highly expressed in root, as well as in anther. Expression of pal1 gene in more than one tissue may be anticipated, given that phenylalanine ammonia lyases (PALs; EC: 4.3.1.24) catalyze the entry point to the general phenylpropanoid pathway, which participates in tyrosine metabolism, phenylalanine metabolism, nitrogen metabolism, alkaloid biosynthesis, flavonoid biosynthesis and monolignol biosynthesis. Its expression is also known to be impacted by age, light, phytochrome, wounding, infection and growth modifiers (Camm and Towers, 1972; Chen et al., 2009; Wada and Takeno, 2010).

Additional genes in the initial steps of the lignin biosynthesis pathway that show preferential expression in root include GRMZM2G140817 encoding for the
enzyme 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase (EC: 1.14.13.36), and two homologs: GRMZM2G033952 and GRMZM2G332522 likely to encode caffeoyl-CoA O-methyltransferase (EC:2.1.1.104). For EC:2.1.1.104, there are two additional homologs of which GRMZM2G099363 over-expresses in root and anther, and GRMZM2G077486 that preferentially expresses in the leaf sample.

REFERENCES

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