Stacking \textit{AsFMT} overexpression with a \textit{BdPMT} loss of function enhances monolignol ferulate production in \textit{Brachypodium distachyon}

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SUPPLEMENTAL DATA

\textbf{Figure S1}. Confirming gene expression, and production of an active YFP protein.

\textbf{Figure S2}. Mature \textit{AsFMT:EYFP} expressing transgenic Brachypodium plants grown side-by-side with wild-type and \textit{Bdpmt-1} knockout mutant plants.

\textbf{Figure S3}. Characterization of the \textit{Bdcer1-1} T-DNA mutant.

\textbf{Figure S4}. Characterization of the \textit{Bdcer1-1} T-DNA mutant.
**Figure S1.** Confirming gene expression, and production of an active YFP protein. Images of YFP fluorescence of *AsFMT:EYFP* seedlings (A,B) and mature plants (C,D). The wild-type plants show no YFP fluorescence as either seedlings (A) or 31 days after planting (C), whereas the *AsFMT:EYFP* seedlings strongly fluoresce yellow, indicating expression and production of an active YFP protein. Bottom: Representative Western blot analysis of proteins extracted from independent event T₃-generation plant leaves. (E) Amido black stained membrane showing protein loading. (F) Leaf proteins probed with anti-GFP antibody, 15s exposure. AsFMT:EYFP and EYFP:AsFMT expected band size: 78 kDa; EYFP:GUSPlus expected band size: 99 kDa (arrows). WT stands for wild type. Asterisks indicate lines tested by DFRC for cell-wall-bound ML-FA.
Figure S2. Mature AsFMT:EYFP expressing transgenic Brachypodium plants grown side-by-side with wild-type and Bdpmt-1 knockout mutant plants. From left to right (A) wild type, (B) AsFMT:EYFP, (C) AsFMT:EYFP × Bdpmt-1, and (D) Bdpmt-1.
Figure S3. Characterization of the *Bdcr1-1* T-DNA mutant. (A) Scale diagram of the *BdCCR1* locus, Bradi3g36887. Black and white boxes represent exons and untranslated regions, respectively, and lines indicate introns. The black triangle represents the location of the pJJ2LBA T-DNA insertion (not shown to scale) in seed lot JJ8708 (*Bdcr1-1*) along with relative primer locations (arrows). (B) Agarose gel-electrophoresed PCR products semi-quantitatively amplified from first strand cDNA indicating the amounts of transcript present either upstream (primers F1 - R1, 144 bp) or downstream (primers F2 - R2, 157 bp) of the T-DNA insertion. Note the fainter *Bdcr1-1* bands signifying a partial reduction in *BdCCR1* transcript levels. EF1α (Bradi1g06860) is the loading control (196 bp). Ladder = ThermoScientific 100 bp SM0421. (C) *Bdcr1-1* plants grown in 4”-diameter pots appear indistinguishable from wild type, having a slight yet insignificant delay in growth as determined by daily culm height measurements (n=15, bars are std. dev.).
Figure S4. Characterization of the Bdccr1-1 T-DNA mutant. (A) Klason Lignin quantification of senesced stems plus leaf sheaths of Bdccr1-1 and wild-type Bd21-3. Each biological rep bar represents the mean of three technical reps. (B) Thioacidolysis quantification of Syringyl (S), Guaiacyl (G), and p-Hydroxyphenyl (H) lignin units. Bars indicate SEM for n = 3-5 plants. Significant differences from wild type, as determined by the Student’s t-test, are indicated in plots (A, B) with * for p<0.05 and ** for p<0.01. (C, D) Phloroglucinol staining of transverse stem sections of wild type (C) and Bdccr1-1 (D) taken from plants at similar developmental stages: Scale bar = 100 μm.