

# Complete depletion of monolignol *p*-coumarate conjugates in CRISPR/Cas9-generated maize *pmt* mutants towards a lignocellulose biorefinery



Dyoni M. Oliveira<sup>1,2</sup>, Marina de L. S. Saleme<sup>1,2</sup>, Marcelo de F. Lima<sup>1,2,3</sup>, Klaas Vandepoele<sup>1,2</sup>, Thijs Vangeel<sup>4</sup>, Korneel Van Aelst<sup>4</sup>, Rebecca Smith<sup>5</sup>, Alexandra A. Chanoca<sup>1,2</sup>, Thatiane R. Mota<sup>1,2</sup>, Yasmine Vanhevel<sup>1,2</sup>, José N. Junior<sup>1,2</sup>, Jordi Geerts<sup>6</sup>, Iris Cornet<sup>6</sup>, Kris Morreel<sup>1,2</sup>, Bert Sels<sup>4</sup>, John Ralph<sup>5</sup>, Ruben Vanholme<sup>1,2</sup>, Wout Boerjan<sup>1,2</sup>

<sup>1</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium; <sup>2</sup>VIB Center for Plant Systems Biology, Ghent, Belgium; <sup>3</sup>Department of Biochemistry, Rural Federal University of Rio de Janeiro, Seropédica, Brazil; <sup>4</sup>Department of Microbial and Molecular Systems, Center for Sustainable Catalysis and Engineering, KU Leuven–University of Leuven, Leuven, Belgium; <sup>5</sup>Department of Biochemistry, and U.S. Department of Energy Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, USA; <sup>6</sup>Biochemical Wastewater Valorization and Engineering Group, University of Antwerp, Antwerp, Belgium

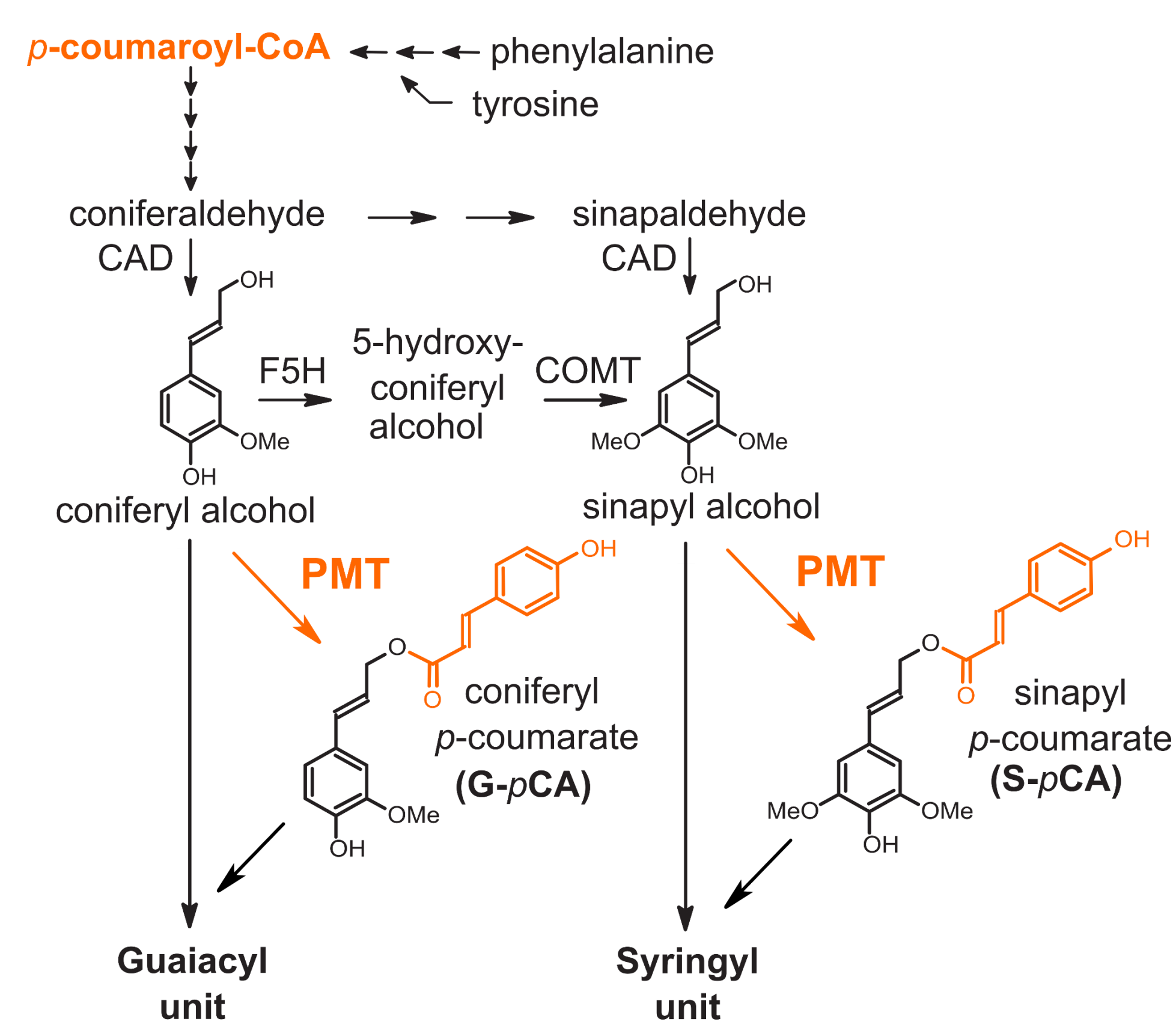
## BACKGROUND

Maize is an important crop for food and animal feed, with potential as **sustainable feedstock for biorefining**.

Lignin poses a major challenge for the **efficient conversion of lignocellulose** into renewable fuels and chemicals.

**Engineering of lignin structure** is pivotal for the success of the lignocellulose biorefinery.

***p*-Coumaroyl-CoA monolignol transferase (PMT)** acylates monolignols with *p*-coumarates (*p*CA) producing monolignol *p*CA conjugates, which are oxidatively incorporated into lignin.

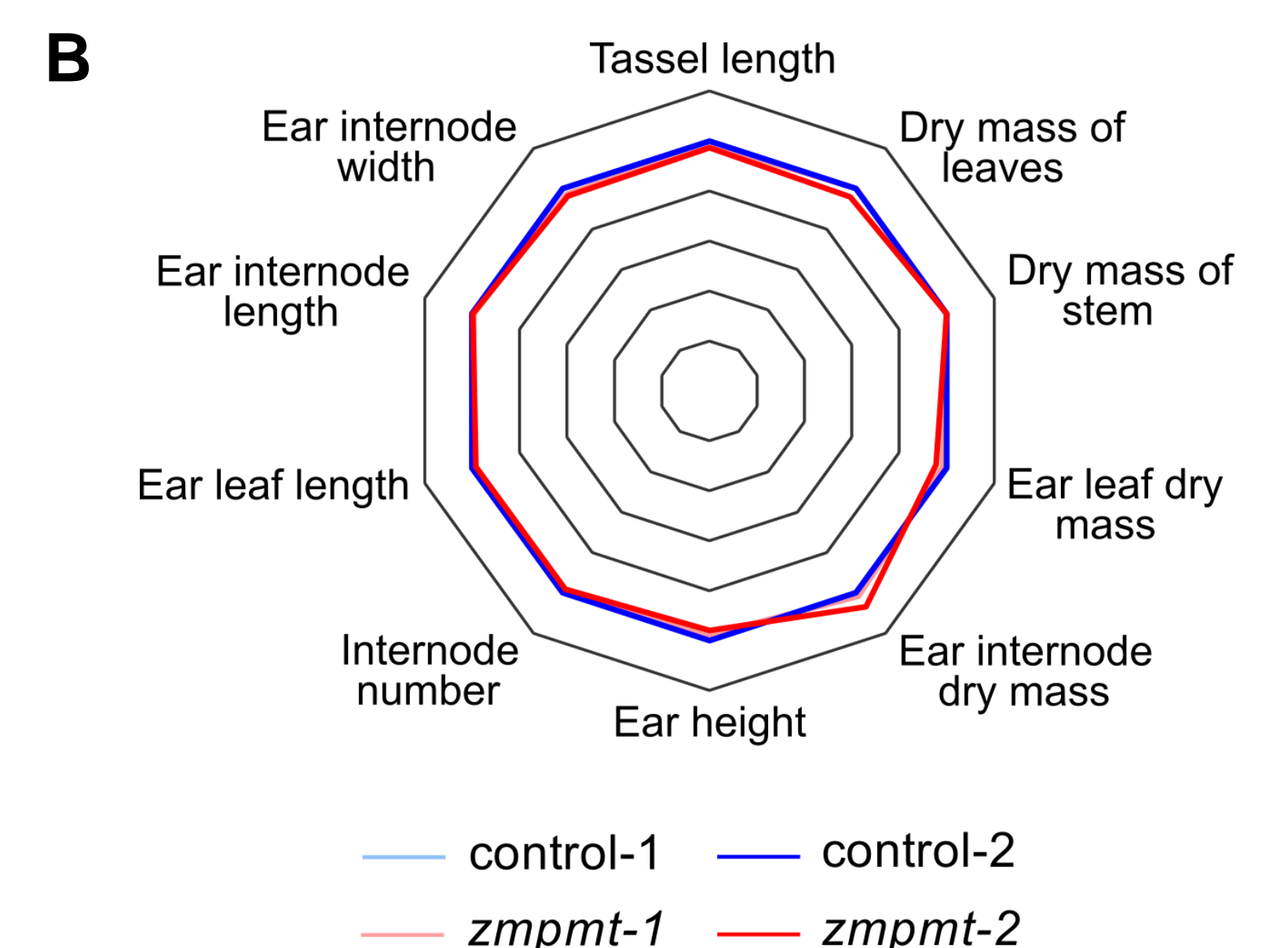
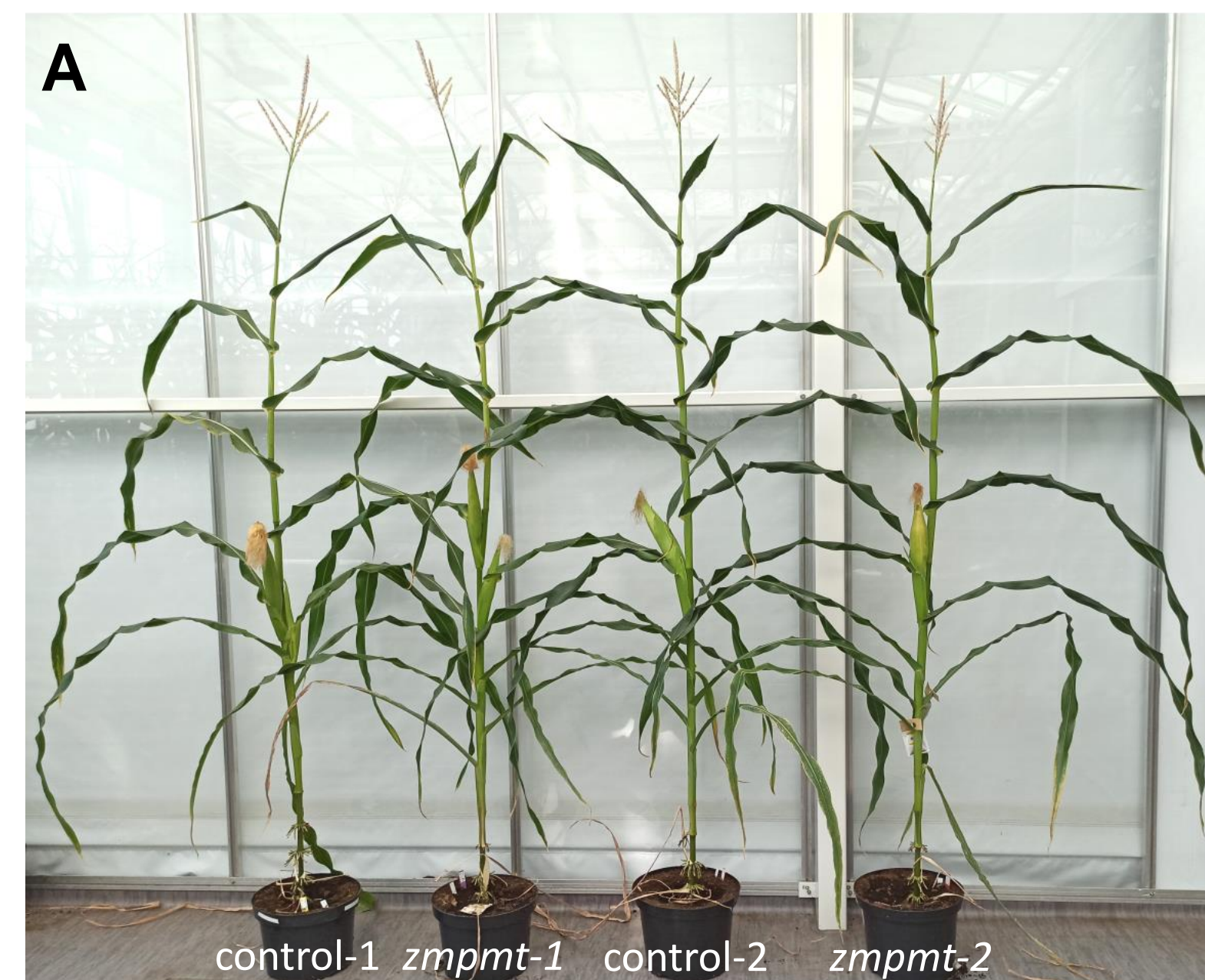


Simplified representation of the lignin biosynthesis in grasses.

We aim to evaluate the potential of CRISPR/Cas9-generated ***zmpmt* knockout mutants towards lignocellulose biorefinery**.

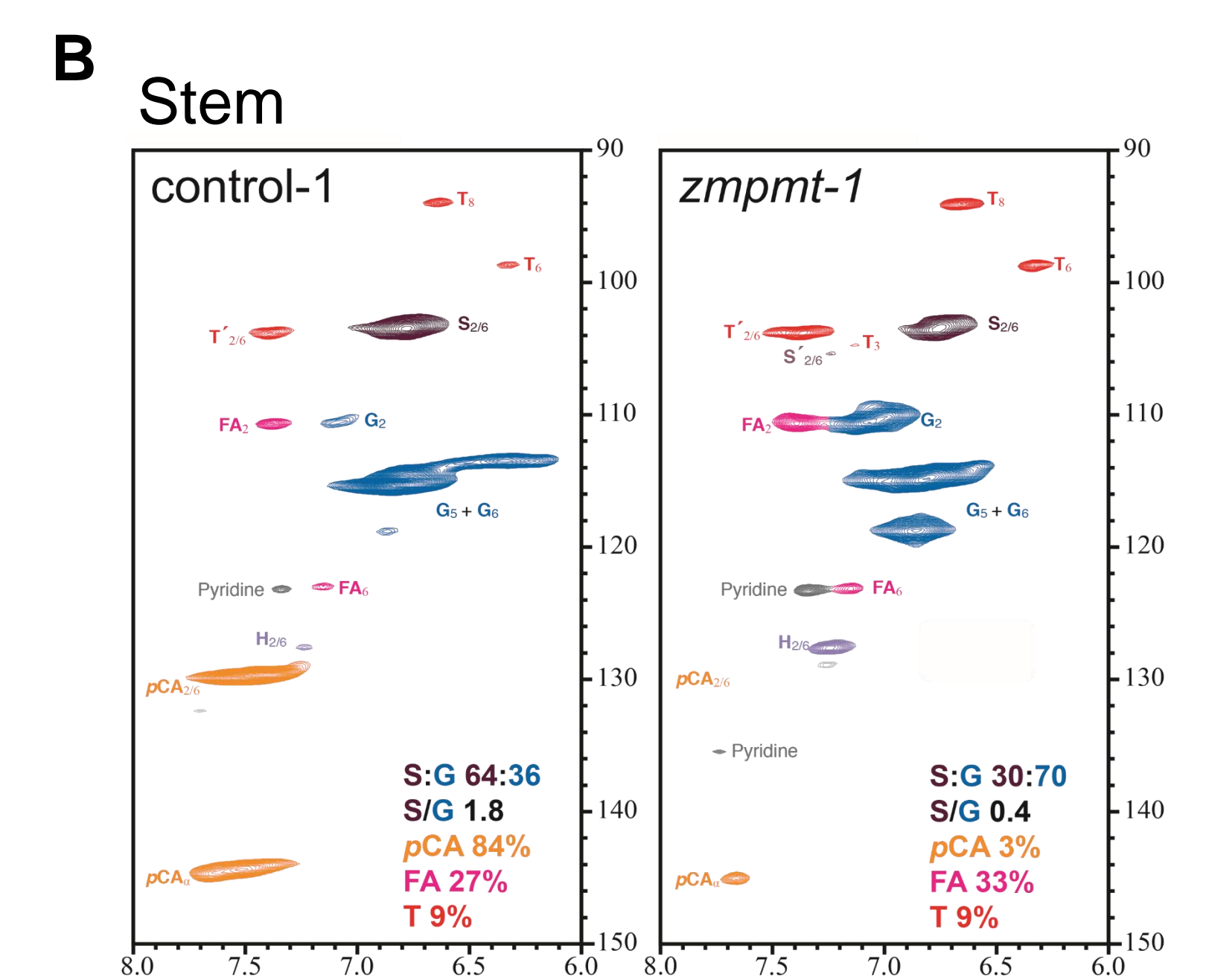
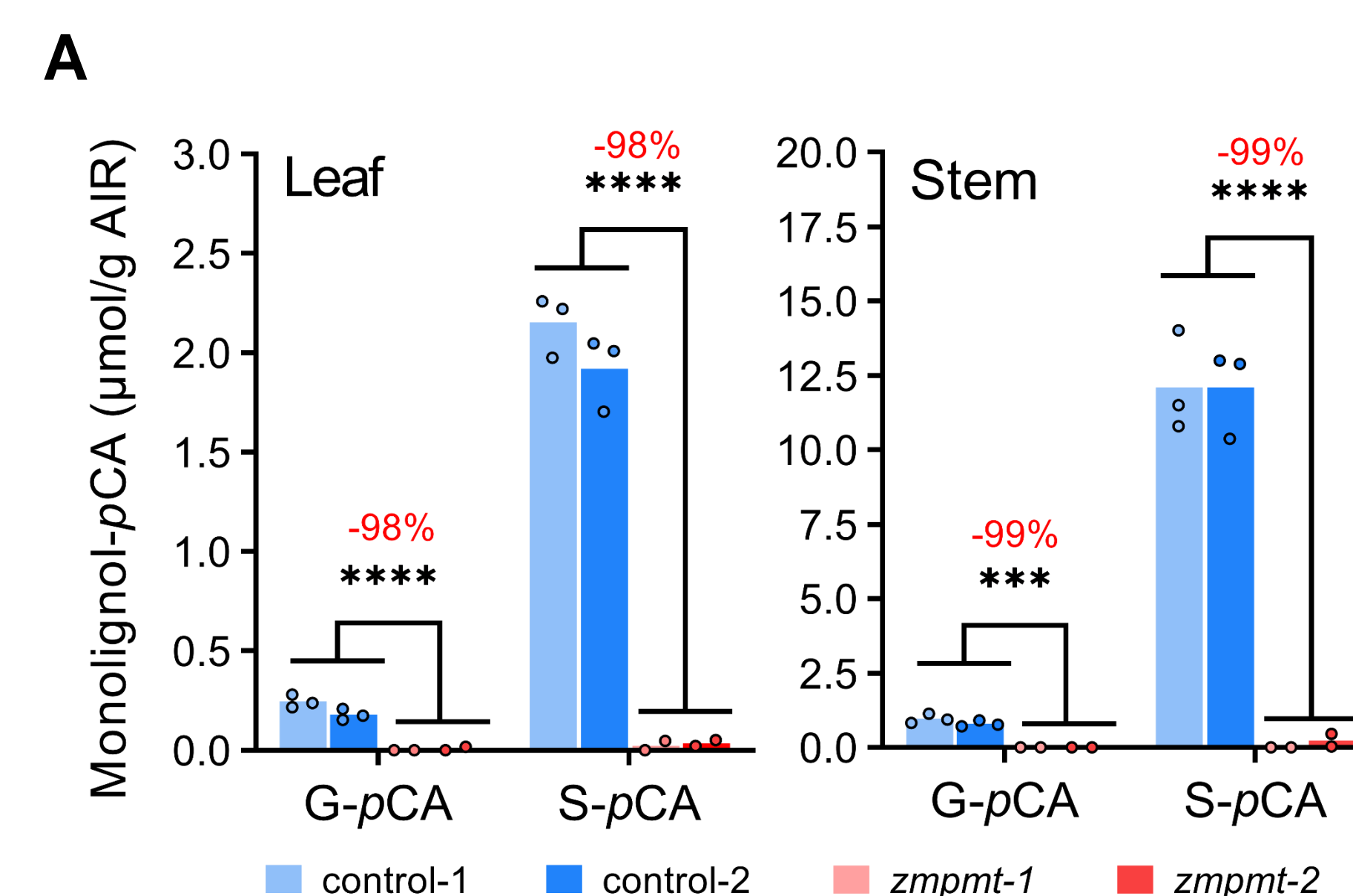
## RESULTS

### *zmpmt* mutants grow similarly to control lines



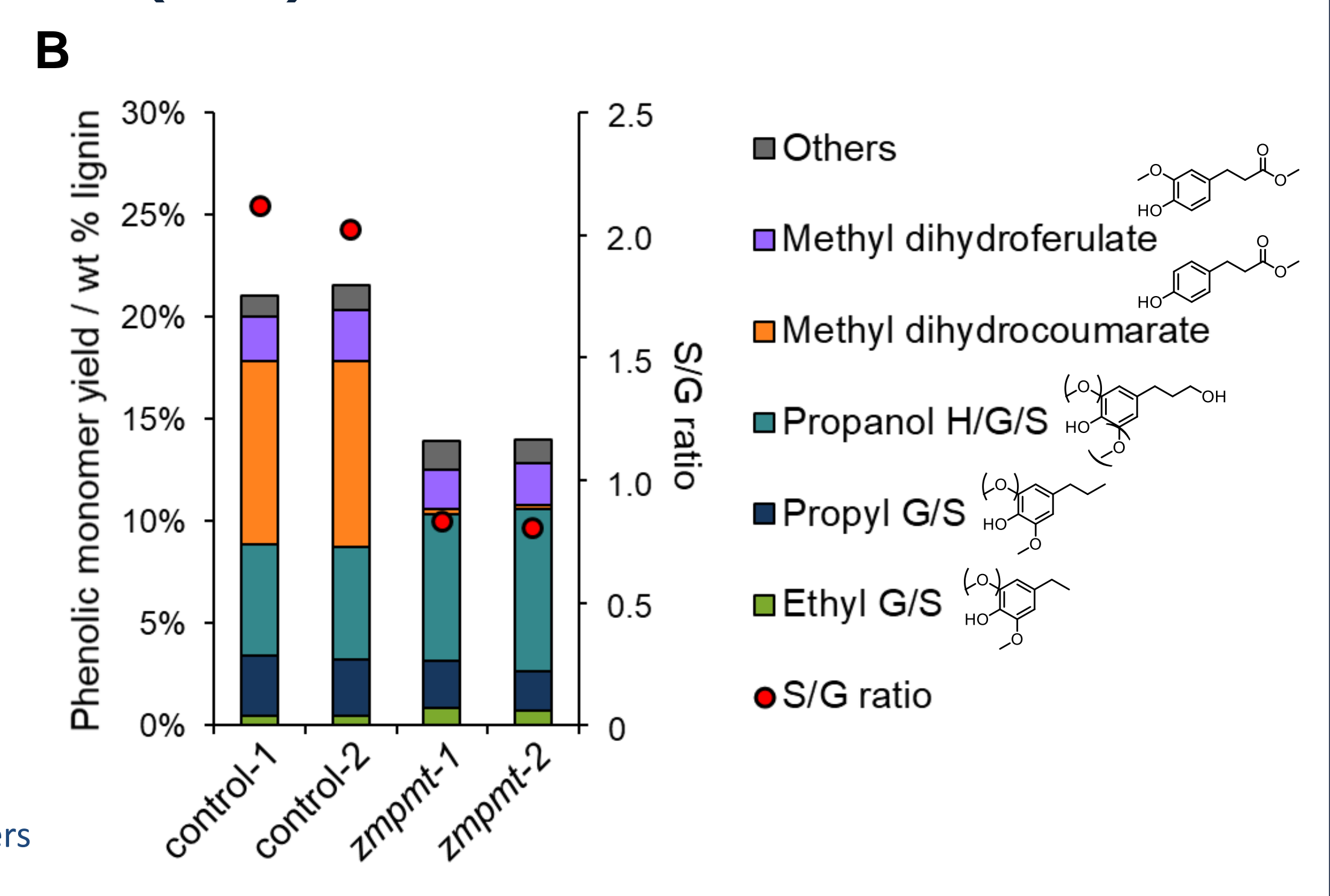
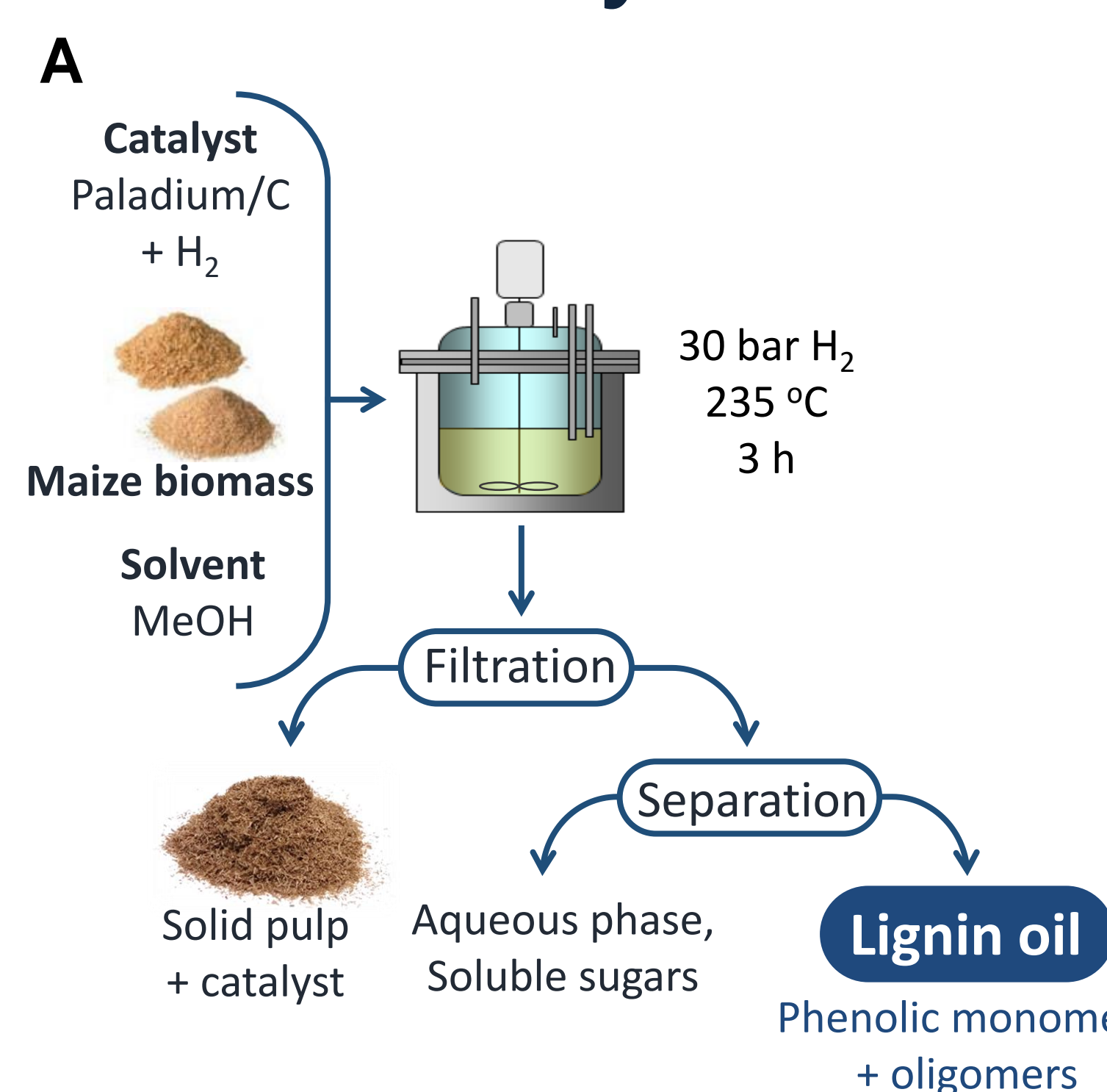
Representative photo of controls and *zmpmt* mutants (A). The spider web presents phenotypic measurements of mutants relative to the respective controls (B).

### Complete depletion of lignin *p*-coumaroylation in *zmpmt* mutants



*zmpmt* mutants do not incorporate *p*CA into lignin (A). Representative 2D-NMR spectra of maize stem showing strong reductions in S/G ratio and *p*CA levels in stem (B).

### Reductive catalytic fractionation (RCF) of maize stem biomass



General scheme of the RCF process of maize stem biomass and downstream separation (A). Phenolic monomers and S/G ratio in lignin oil of controls and *zmpmt* mutants (B).

## CONCLUSION

- The complete disruption of lignin *p*-coumaroylation does not affect the phenotype and growth of maize in greenhouse conditions.
- Differences in levels of monolignol *p*-coumarates may predict RCF yields.

## CONTACT

✉ [dyoni.oliveira@psb.vib-ugent.be](mailto:dyoni.oliveira@psb.vib-ugent.be)  
 🐦 @BioenergyVIB  
 🌐 Dyoni M. Oliveira