ABSTRACTS (MASTER THESIS)

Generation and characterization of rice *CAD2 CAldOMT1* double mutants with altered lignin content and structure

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Lignin, an abundant aromatic polymer derived from oxidative couplings of monolignols and related phenylpropanoids, is one of the major components of lignocellulosic biomass. Bioengineering of lignin has long been a major research focus, especially because lignin negatively impacts numerous biomass utilization processes such as chemical pulping and bioethanol production. More recently, engineering lignin to facilitate its conversion into value-added aromatic commodities has also received considerable attention. In this context, our laboratory has been studying lignin engineering using rice, a grass model plant, to explore molecular breeding approaches for improving the properties of grass biomass.

Recent studies in our laboratory have demonstrated that regulations of rice lignin biosynthetic genes encoding cinnamyl alcohol dehydrogenase (CAD) and 5-hydroxyconiferaldehyde *O*-methyltransferase (CAldOMT) differently impact structures and properties of rice lignocellulose. Downregulation of *OsCAD2*, one of the major rice CAD genes, leads to unusual incorporation of *p*-hydroxycinnamaldehydes into lignin polymers along with a slight reduction in lignin content [1]. On the other hand, downregulation of *OsCAldOMT1*, a major rice *CAldOMT*, resulted in altered lignins largely depleted in syringyl and tricin units [2,3]. Both *OsCAD2*- and *OsCAldOMT1*-deficient rice displayed significantly increased biomass saccharification efficiency compared to wild-type rice [1,2]. Interestingly, however, they show distinctively different time-dependent saccharification profiles, suggesting that differences in lignin content/structure of the two lignin-modified rice differently affect the properties of lignocellulose. In this study, to further investigate the relationship between altered lignin content/structure and lignocellulose properties of *OsCAD2*- and *OsCAldOMT1*-deficient rice, new double-knockout mutants for *OsCAD2* and *OsCAldOMT1* (*cad2 caldomt1*) were generated, and their modified lignocellulose structure as well as enzymatic saccharification efficiency were assessed comparatively with the previously developed rice transgenics in which either of *OsCAD2* or *OsCAldOMT1* alone is downregulated.

Anticipated *cad2 caldomt1* double knockout mutants were successfully generated via targeted mutagenesis in *OsCAD2* single knockout background [1] using CRISPR/Cas9 expression vector designed to target *OsCAldOMT1*. Genotyping for selected T_0 lines suggested that they have bi-allelic mutations that should lead loss of *OsCAldOMT1* function. Chemical and NMR analyses on cell walls isolated from *cad2 caldomt1* clearly demonstrated that they produce altered lignins affected by loss-of-functions of both *OsCAD2* and *OsCAldOMT1*. Our preliminary saccharification experiments suggested that *cad2 caldomt1* T_0 lines also displayed synergistic increase in saccharification efficiency via loss-of-functions of both *OsCAD2* and *OsCAldOMT1*, although a deeper investigation should be conducted with T_1/T_2 progeny lines. Overall, we contemplate that further analysis of the developed rice transgenic lines would provide new insights into molecular breeding approaches to improve grass biomass for future biorefinery.

References

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