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Potential of genetically engineered hybrid poplar for pyrolytic production of bio-based phenolic compounds

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HIGHLIGHTS

- Unique fast and intermediate pyrolysis study of genetically engineered hybrid poplars.
- Steering the pyrolytic production of bio-based phenolics by genetic modification.
- Analysis of phenolic compounds present in the bio-oil using GC–MS.
- PCA combined with K-means for clustering of genetically engineered hybrid poplars.

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ABSTRACT

Wild-type and two genetically engineered hybrid poplar lines were pyrolyzed in a micro-pyrolysis (Py-GC/MS) and a bench scale setup for fast and intermediate pyrolysis studies. Principal component analysis showed that the pyrolysis vapors obtained by micro-pyrolysis from wood of caffeic acid *O*-methyltransferase (*COMT*) and caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*) down-regulated poplar trees differed significantly from the pyrolysis vapors obtained from non-transgenic control trees. Both fast micro-pyrolysis and intermediate pyrolysis of transgenic hybrid poplars showed that down-regulation of *COMT* can enhance the relative yield of guaiacyl lignin-derived products, while the relative yield of syringyl lignin-derived products was up to a factor 3 lower. This study indicates that lignin engineering via genetic modifications of genes involved in the phenylpropanoid and monolignol biosynthetic pathways can help to steer the pyrolytic production of guaiacyl and syringyl lignin-derived phenolic compounds such as guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, syringol, 4-vinylsyringol, and syringaldehyde present in the bio-oil.

1. Introduction

There is an urgent need to increase the use of renewable, abundant, and sustainable resources such as lignocellulosic biomass for the production of commodity/specialty chemicals because of depleting oil resources, growing environmental concerns and increasing energy demands. Specialty chemicals are currently almost entirely obtained from fossil based resources via a series of complex reaction steps (Pyl et al., 2012; Ragauskas et al., 2006). A drastic reduction in the number of reactions steps would be obtained if the specialty chemicals such as bio-based phenolic compounds could be produced directly from biomass instead of classical petrochemical building blocks.

Pyrolysis of lignocellulosic biomass has gained substantial interest specifically for the production of liquid bio-oil. There are three classes of pyrolysis technologies, namely fast pyrolysis primarily for bio-oil production, slow pyrolysis for bio-char production, and intermediate pyrolysis, which is a relatively new type of pyrolysis, for bio-oil and bio-char production. There are numerous published data regarding fast pyrolysis (Carpenter et al., 2014; Venderbosch and Prins, 2010) and a few intermediate pyrolysis study (Torri et al., 2016; Yang et al., 2014). In contrast to fast pyrolysis, so-called intermediate pyrolysis corresponds to considerably lower heating rates than in fast pyrolysis and longer residence times which could lead to secondary reactions in the vapor phase (Yang et al., 2014).

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Researchers are quite convinced that lignocellulosic bio-oils which are complex mixtures of water and hundreds of organic compounds (Djokic et al., 2012) are strong candidates to be used as renewable and sustainable feedstocks for the production of specialty chemicals (Venderbosch and Prins, 2010; Vispute et al., 2010), in particular because they are rich in phenolic compounds originating from lignin (Bu et al., 2014; Choi et al., 2015; Costa et al., 2013; Duman et al., 2011; Kim, 2015). For this reason, there is recently a growing interest to increase the yield of lignin-derived phenolic compounds in bio-oil (Bu et al., 2014; Choi et al., 2015; Kim et al., 2010) in addition to increasing the overall bio-oil yield by optimizing process conditions (Akhtar and Amin, 2012). Furthermore, the development of a robust process necessitates a full understanding of the effect of biomass composition on the overall bio-oil yield and product distribution of the bio-oil (Carpenter et al., 2014). For this purpose, different types of biomasses or isolated biomass fractions have been pyrolyzed using different reactor technologies and process conditions until now (Akhtar and Amin, 2012; Azadi et al., 2013; Carpenter et al., 2014). However, it is difficult to have a clear understanding of the effect of feedstock composition on the bio-oil composition unless well-defined feedstocks which differ only in one property are utilized.

Therefore, in this study the effect of the biomass composition has been investigated using well-defined feedstocks, which were grown under identical and well-controlled conditions. In particular, the bio-oil composition derived from wild-type poplar wood was compared to that of poplar with an engineered lignin composition. Lignin is mainly made from the monolignols coniferyl and sinapyl alcohol and to a lesser amount of *p*-coumaryl alcohol that lead to the formation of guaiacyl, syringyl and *p*-hydroxyphenyl units in lignin structure, respectively. The monolignols are produced from phenylalanine via the phenylpropanoid and monolignol specific pathways (Fig. 1). The lignin of two caffeic acid *O*-methyltransferase (*COMT*) down-regulated lines (ASB2B and ASB10B) presented about a 85% decrease in syringyl/guaiacyl ratio as compared to the wild-type line, whereas their total lignin content was equal to that in the wild-type line (Van Doorsselaere et al., 1995). The lignin of two caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*) down-regulated lines (sccoaomt-16 and sccoaomt-29) had an increase of about 11% in the syringyl/guaiacyl ratio and a 12% reduction in total lignin content, as compared to the wild-type line (Meyermans et al., 2000). The effect of these modifications on the subtle changes of the bio-oil composition has been investigated under both fast micro-pyrolysis and intermediate pyrolysis conditions to understand the role of the operating conditions, the reactor technology and the biomass starting material on the yields of the lignin-derived phenolic compounds. To the authors' knowledge this has not been attempted until now.

Principal component analysis (PCA) combined with the K-means clustering was used to extract and display the systematic variation in the bio-oils produced from genetically engineered and wild-type poplars. This cluster analysis allows not only a simple visualization but it can also be used to determine the statistical independence of the genetically modified lines. The information obtained from this study provides new insights into the possibilities of using genetically modified lignocellulosic biomass derived bio-oils for the production of specialty chemicals instead of fuel.

2. Methods

2.1. Plant material

The genetically engineered *Populus tremula x alba COMT* (ASB2B and ASB10B) and *CCoAOMT* (sccoaomt-16 and sccoaomt-29) down-regulated lines have been described in Van Doorsselaere et al. (1995) and Meyermans et al. (2000), respectively. Six biological replicates of each genetically engineered line and nine replicates of the wild type were grown for 3 months in greenhouse-conditions (16 h:8 h light: dark photoperiod; 23–25 °C). At harvest, the stems were debarked and air-dried. The basal 25 cm stem section was cut with scissors into pieces of 10 mm in length (stems had a diameter of up to 5 mm). The stem pieces of three plants were pooled to obtain two pools per genetically engineered line and three pools of the wild-type line. For fast micro-pyrolysis, the material was further grinded to a powder in a vibratory ball miller (MM400, Retsch, Germany, balls 7 mm i.d.) for 4 min with a speed of 27 Hz. In total 13 samples were prepared for the experiments, as shown in Table 1.

2.2. Fast micro-pyrolysis experiments

The transgenic and wild-type hybrid poplar samples were pyrolyzed using a multi-shot pyrolyzer (EGA/PY-3030D, Frontier Laboratories, Japan). The micro-pyrolysis setup consists of a sampler, a quartz pyrolysis tube that can be preheated to a desired

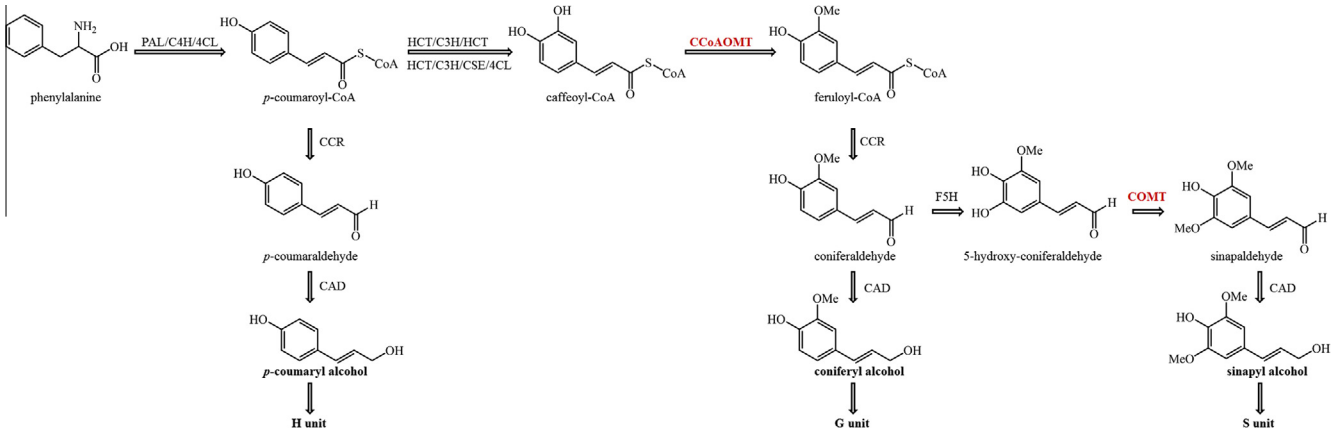

Fig. 1. Simplified phenylpropanoid and monolignol biosynthetic pathway starting from phenylalanine toward the biosynthesis of the monolignols *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol that leads to *p*-hydroxyphenyl, guaiacyl and syringyl units, respectively (Vanholme et al., 2013, 2012). PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate: CoA ligase; HCT, *p*-hydroxycinnamoyl-CoA: quinate shikimate *p*-hydroxycinnamoyltransferase; C3H, *p*-coumarate 3-hydroxylase; CSE, caffeoyl shikimate esterase; *CCoAOMT*, caffeoyl-CoA *O*-methyltransferase; CCR, cinnamoyl-CoA reductase; F5H, ferulate 5-hydroxylase; *COMT*, caffeic acid *O*-methyltransferase; CAD, cinnamyl alcohol dehydrogenase. The steps, which are modified in the pyrolyzed genetically engineered hybrid poplars, are highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)