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LIGNOCELLULOSIC BIOFUELS – CHALLENGES AND POTENTIALS

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ABSTRACT

Lignocellulosic biomass consist of inedible parts of woody grass plants, stalk of sweet sorghum and agricultural residues, are the sources for the 2nd generation biofuels. Lignocellulosic biomass comprises of many different polysaccharides cellulose, hemicelluloses, phenolic polymer lignin and proteins. The problem of the 2nd generation feed stock is the extraction of the sugars located inside the lignin and cellulose structure. To convert lignocellulosic biomass to biofuels the complex polysaccharides and lignin must be broken down or hydrolysed into simple sugars. This process of bioconversion of cellulose to ethanol involves pre-treatment, saccharification and fermentation. The other challenges include types of the biomass and their availability round the year, logistics, and production technologies. These challenges take in identification and improvement of energy crops like sweet sorghum, switch grass, miscanthus, alfa alfa, etc., by biotechnological approaches by generating feed stocks with low lignin content and modified traits which can tolerate biotic and abiotic stresses with improved cellulose content. Down regulation of key enzymes involved in lignin biosynthetic pathway may be a promising approach to decrease or alter the hard lignin content in lignocellulosic feed stock materials. With the advent of genetic engineering and crop improvement strategies in energy crops may provide continuous supply of feed stock for the production of biofuels as an alternative source of energy. To benefit the environment and also to meet the global demand of fossil fuels, the biofuels produced from lignocellulosic biomaterial by biotechnological route may help in decreasing the emission of green house gasses and also save our food crops.

KEYWORDS: **Agricultural waste, Bioconversion, Bio-ethanol, Down regulation, Lignin, RNAi**

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INTRODUCTION

In developing countries growing population, increased fossil fuel use and competition for limited resources for fertile land, water and demand for vehicle fuels is increasing and excess use of petrol and diesel leads to increasing atmospheric CO_2 concentration, and the potential for significant greenhouse gas-mediated climate change.[1] The International Energy Agency (IEA) expects that biofuels will contribute 7% of total fuel use by 2030.[2] First generation biofuels are produced directly from food crops like sugarcane, maize and oilseed crops like peanut and soybean. Bio- ethanol is eventually derived from starch, sugar and vegetable oils. Important to note that the properties and structure of the biofuel itself does not change between generations, but rather the source from which the fuel is derived changes. Maize and sugarcane are the most commonly used first generation biofuel feed stock. Second generation biofuels widely known as lignocellulosic fuels and these are most promising renewable resource for biofuel production. What separates them from first generation biofuels is the fact that feedstock used in producing second generation biofuels are generally not food crops and these lignocellulosic generation biofuels are derived from different feed stocks which include mostly inedible parts of woody grass plants, stalk of sweet sorghum plants and agricultural residues are the major sources for the 2nd generation biofuel production. Second generation bioethanol production fulfils the impractical gap of first generation since it employs non-edible feedstock sourced from agriculture and forestry wastes[4]. Lignocellulosic and starchy materials in their biomass is convertible to fermentable sugars that are able to be further processed, resulting in bioethanol as the end product. Ligncellulosic biomass is an abundant renewable feedstock source that can be converted biofuels by using chemicals and fermentation process. Plant cell wall mainly composed of three organic polymers: cellulose, hemicellulose and lignin. These polymers are also major components of lignocellulosic biomass. 40-45% of Cellulose monomer molecules D-Pyran glucose units arrange regularly by beta 1-4-Glycosidic bonds, gather into bundles, and determine the framework of the cell wall. Fibers are filled with 25-30% of hemicellulose monomers D-Xylose, mannose, L-arabinose, galactose, glucuronic acid with β-1-4 Glycosidic bonds in main chains and β- 1-2, β-1-3, and β- 1-6-glycosidic bonds in side chains[3] and 20-25 % of lignin made up with three monomers Guaiacylpropane (G), syringylpropane (S), phydroxyphenylpropane by various ether bonds and carbon-carbon bond, mainly β-O-4 ether bond[3]. The structure of the plant cell wall is compact and different bonding among cellulose, hemicellulose, and lignin exists. Cellulose and hemicellulose or lignin molecules are mainly coupled by a hydrogen bond. In addition to the hydrogen bond, there is the chemical bonding between hemicellulose and lignin.

CHARACTERISTICS OF LIGNOCELLUOSIC BIOMASS

Lignocellulosic materials including agricultural wastes, forestry residues, grasses and woody materials have great potential for bio-fuel production. Their biomass is made up of complex structural polysaccharides, cellulose, hemicelluloses and lignin [5,6] Several second generation biofuel crops like sweet sorghum feed stock consists of cellulose (45%), hemicelluloses (27%) and lignin (21%) are constituents of plant cell walls.[7] Typically, most of the agricultural lignocellulosic biomass is comprised of about 10% - 25% lignin, 20% - 30% hemicellulose, and 40% - 50% cellulose.[8,9] Cellulose is the most common and abundant component of all plant matter and is responsible for mechanical strength and chemical stability to plants. While, hemi-cellulose consist of repeated polymers of pentose, and hexose sugars and depending upon the source their structural composition varies.[10] Whereas the lignin contains three aromatic alcohols (coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol) produced through a biosynthetic process and forms a protective seal around the other two components *i.e.*, cellulose and hemicelluloses. Polysaccharides are cross linked in the plant cell walls with the hydrophobic network of lignin. Lignin physically obstructs enzymatic bioconversion as it plays negative role in converting biomass to biofuels[11]. Delocalization of lignin is crucial and essential to enable complete digestion of secondary cell walls of plants by cellulase and hemicellulases. Different crops and their biomass composition and their biofuel yield mentioned in table 1.

CHALLENGES OF LIGNOCELLULOSIC BIOFUELS

The most promising technology for the conversion of the lignocellulosic biomass to fuel bio-ethanol is based on the enzymatic breakdown of cellulose using enzymes. The complex materials present in plant cell wall are highly recalcitrant towards bioconversion of carbohydrates into bio-ethanol, bio-butanol compared to simple polysaccharide starch. The conversion of lignocellulosic biomass to bio-ethanol involves 3 major process *viz*., pre-treatment with strong acid or alkali followed by polysaccharide hydrolysis to simple sugars followed by bioconversion of simple sugar to bio-ethanol through fermentation process.[12] The presence of lignin in plant cell walls negatively impacts these conversion steps and interferes with the fermentation to produce biofuels.[13,14] Plant cell walls binds together with lignin with three well known monolignol precursors : *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These result, respectively, in the hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monomer units of the lignin polymer. The relative proportion of monolignols determines the ease of cell wall deconstruction by enzymatic or biocatalyst-mediated mechanisms.[15] The polymerization of three monolignols and their cross-linking with phenolic acids to hemicellulosic sugars are keys to the recalcitrance of cell walls to enzymatic hydrolysis that is required to release sugars for biofuel production. High S/G ratios are considered favorable for deconstruction in angiosperms[16] but the reverse is true for alfalfa, and switchgrass. [15,17] To increase the surface accessibility of carbohydrate polymers to the hydrolytic enzymes, which is a key step toward efficient utilization of biomass for bio-ethanol or other advanced biofuels production through pretreatment process. Lignocellulose conversions carried out at ⩽50 °C have several limitations and recent research efforts focuses on the importance of thermophilic bacteria and thermostable enzymes to overcome the limitations of existing lignocellulosic biomass conversion processes.[18]This treatment has been considered as the most expensive processing step in cellulosic ethanol processes, representing about 18% of the total cost.[19] Therefore, developing a cost-effective and efficient biomass pretreatment technology is the most critical need for lignocellulosic biofuels. Attempts to exploit Lignocellulosic biomass into commercial ethanol production, recent research efforts have been devoted to the techno-economic improvements of the overall conversion process, in addition to screen out promising feedstock materials[20].

MODIFICATION OF LIGNIN CONTENT IN FEEDSTOCK MATERIALS

Lignin tightly binds to hemicellulose and cellulose, thereby blocking the access of hydrolytic enzymes, and also inhibiting the activities of hydrolytic enzymes and the process of fermentation during the bioconversion stages.[21] Through advent of Genetic engineering we can directly manipulate the genes involved in the lignin biosynthetic pathway by RNAi silencing, blocking the expression of the genes by artificial zinc finger chimeras.[22] or by manipulating transcription factors that regulate the expression of single to multiple lignin synthesis gene(s).[23,24,25] Silencing the gene(s) involved in the monolignol biosynthetic pathway is currently the most straight forward way to reduce lignin content. Down-regulation of any of enzymes involved in lignin biosynthetic pathway *viz.,* the Caffeic acid 3-*O*-methyltransferase EC 2.1.1.68 (COMT), Cinnamyl alcohol dehydrogenase EC 1.1.1.195 (CAD), 4-Coumarate: coenzyme A ligase EC 6.2.2.12 (4CL) etc in the feed stock materials to produce transgenic plants with a normal growth phenotype, but with reduced lignin content, altered lignin composition, we can increase saccharification efficiency, and in return increase bio-ethanol and other biofuels production. Most of the lignin biosynthetic enzymes have been down regulated in different crops by RNAi and Antisense technology for theproduction of lignocellulosic biofuels. (Table 2)

POTENTIAL OF LIGNOCELLULOSIC BIOFUELS

Lignocellulosic biofuels are gaining attention as a possible solution to decrease our dependency on fossil fuels imported from oil reserve rich nations and produce a cleaner burning fuel while not significantly affecting the price of agricultural commodities. Production of biofuels from lignocellulosic biomass could alleviate the dependence on fossil fuels, and this notion has led to developing biofuel feedstock from crops like switchgrass, sweet sorghum, miscanthus, alfalfa and also focus on new biofuel conversion technologies.[26] Lignocellulosic biomass has considerable environmental and resource advantages over non-renewable fossil fuels. Second-generation biofuels are made by turning crops into liquid fuels using more sophisticated chemical processes and include biohydrogen *i.e.,* hydrogen gas made from crops and mixed alcohols like bioethanol, biobutanol etc. They are generally more efficient than first-generation biofuels because they release more energy per volume, and some crops like sweet sorghum are beneficial in making grain for food and also enable to make biofuels from the stalk materials. To reduce greenhouse gas emissions cellulosic ethanol is a promising technology in near future.[27]

FUTURE REQUIREMENT OF BIOFUELS

The Government of India has planned to boost biofuels market over the next few years and 5 % blending of biodiesel with regular diesel and 10% ethanol with gasoline could boost the market from 65 billion rupees to 500 billion rupees by 2022.[28] The country would require 6.75 billion litres of biodiesel and 4.5 billion litres of ethanol for blending over the next six years. The Government of Brazil enhanced blending of ethanol for gasoline from 25 to 27 per cent in the year 2015. Brazilian ethanol production is projected at 30.68 billion litres of ethanol from sugarcane for the year 2016. The ethanol-use mandate has been mandatory in Brazil since 1977 blend of ethanol to gasoline and now rise of 25 per cent. In case of biodiesel, a blend of 10 per cent recommended by the end of 2020. The continual growth in commercial aviation fuels and more stringent environmental legislations have led to immense interest in developing green aviation fuels from renewable lignocellulosic biomass. The biofuels derived from sawdust basically met the major specifications of jet fuels by a novel transformation of biomass into bio-jet and diesel fuels involving catalytic pyrolosis, alkylation and hydrogenation of saw dust. This transformation potentially provides a valuable path for the development of green aviation biofuels utilizing lignocellulose biomass [29].To meet the global demand and decrease the utilization of fossil fuels, generation of biofuels from lignocellulosic biomass is the major route. Without diverting the food crops like maize and sugarcane and also without disturbing the cultivable lands for the production of bio-ethanol and higher alcohols, lignocellulosic biomass plays an vital role with the aid of genetic engineering and development of energy crops and better utilization of lignocellulosic biomass. Further, generation and utilization of biofuels benefit the environment by decreasing the emission of green house gasses and further economically benefit the farmers in adaptation of cultivating energy crops for the production of biofuels.

Table 1
Biomass composition of various energy crops and their biofuel yield

Item	**Sweet sorghum**	**Switch Grass**	**Sugar cane**	**Maize**	**Miscanthus**	**Alfa Alfa**
Photosynthetic type	C4, perennial	C4, perennial	C4, perennial	C4, perennial	C4, perennial	C4, perennial
Crop duration	3-4 months	Once or twice per year	12-18 months	4-6 months	3-4 times from second year onwards	Three to four times a year
Lignin(%)	25	12	10	7	12	20
Cellulose(%)	45	45	40	35	45	50
Hemicellulose(%)	30	31	27	16.8	27	30
Fermentable sugars (%)	Sucrose - 70%, Glucose- 20%, Fructose - 10%	Sucrose Glucose Fructose (18-27%)	Sucrose (99%)	4-12% oligomeric sugars	Glucose-36.96% Xylose-22.12% Mannose Arabinose	Glucose – 306g/Kg biomass Xylose- 99g/Kg biomass Arabinose- 21g/Kg biomass
Biofuel yield (Ethanol)	220g /Kg Dry stem biomass	421 gal/acre	19.5 gal/ton	756 gal/acre	1198 gal/acre	182g of ethanol from 92g of glucose

Table 2
Down regulation of lignin biosynthetic enzymes in different crops for lignocellulosic biofuel production

Energy Crop	**Switch Grass**	**Sugar cane**	**Maize**	**Alfa Alfa**
Enzymes down regulated	4CL [31] COMT[32,33] CAD[34,35]	COMT[36]	COMT[37] CAD[38]	C4H[39] HCT[39] C3H[39] CCoAOMT[39] COMT[39,40] F5H [39] CAD[39]
Targeted approach	RNAi	RNAi	Antisense	Antisense

C4H: Cinnamate 4-Hydroxylase (EC 1.14.13.11)
C3H: Coumarate 3-Hydroxylase (EC 1.10.3.1)
COMT: Caffeate O-Methyltransferase (EC 2.1.1.68)
CCoAMT: Caffeoyl-Coenzyme A O- Methyltransferase (EC 2.1.1.104)
F5H: Ferulate 5-Hydroxylase (No EC number Assigned)
4CL: 4-Coumarate: Coenzyme A Ligase (EC 6.2.1.12)
CAD: Cinnamyl Alcohol Dehydrogenase (EC 1.1.1.195)

CONCLUSION

Reduced lignin content in lignocellulosic biomass to improve saccharification efficiency through enzyme hydrolysis, reduce the cost of biofuel production. Reducing lignin content has increased cellulose hydrolysis efficiency in model plants such as alfa alfa and Arabidopsis[30]. Further improvements of feedstock quality in other plant species by genetic engineering along with efficient bioprocessing and conversion technologies will lead to economical biofuel production in near future. By using biotechnological approaches in generating feed stocks with low lignin content and modified traits which can tolerate to biotic and abiotic stresses the generated lignocellulosic biofuels may bring benefits to developing countries by reducing their dependency for oil from mid-East countries and also increase in economic growth of marginal farmers. Further, lignocellulosic fuels may replace use of fossil fuels to produce significantly lower green house gases and also save our food crops in diverting them for bioconversion to biofuels.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Parry ML, editor. Climate change 2007-impacts, adaptation and vulnerability: Working group II contribution to the fourth assessment report of the IPCC. Cambridge University Press; 2007.
2. IEA, Jan: http://www.iea.org/publications/freepublications/publication/essentials2.pdf. 2007.
3. Chen H. Research Methods for the Biotechnology of Lignocellulose. InBiotechnology of Lignocellulose 2014 (pp. 403-510). Springer Netherlands.
4. Aditiya HB, Mahlia TM, Chong WT, Nur H, Sebayang AH. Second generation bioethanol production: A critical review. Renewable and Sustainable Energy Reviews. 2016 Dec 31;66:631-53.
5. Abramson M, Shoseyov O, Shani Z. Plant cell wall reconstruction toward improved lignocellulosic production and processability. Plant Science. 2010 Feb 28;178(2):61-72
6. Somerville C, Youngs H, Taylor C, Davis SC, Long SP. Feedstocks for lignocellulosic biofuels. science. 2010 Aug 13;329(5993):790-2.
7. Kim M, Day DF. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. Journal of industrial microbiology & biotechnology. 2011 Jul 1;38(7):803-7.
8. Hafiz Muhammad Nasir I, Ishtiaq A, Muhammad Anjum Z, Muhammad I. Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by Trichoderma viride under SSF and its detergent compatibility. Advances in Bioscience and Biotechnology. 2011 May 23;2011.
9. Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial & engineering chemistry research. 2009 Mar 20;48(8):3713-29.
10. Iqbal HM, Kyazze G, Keshavarz T. Advances in the valorization of lignocellulosic materials by biotechnology: an overview. BioResources. 2013 Apr 26;8(2):3157-76.
11. Zeng Y, Zhao S, Yang S, Ding SY. Lignin plays a negative role in the biochemical process for producing lignocellulosic biofuels. Current opinion in biotechnology. 2014 Jun 30;27:38-45
12. Mielenz JR. Ethanol production from biomass: technology and commercialization status. Current opinion in microbiology. 2001 Jun 1;4(3):324-9.
13. Keating JD, Panganiban C, Mansfield SD. Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. Biotechnology and bioengineering. 2006 Apr 20;93(6):1196-206.
14. Li X, Weng JK, Chapple C. Improvement of biomass through lignin modification. The Plant Journal. 2008 May 1;54(4):569-81.
15. Chen F, Dixon RA. Lignin modification improves fermentable sugar yields for biofuel production. Nature biotechnology. 2007 Jul 1;25(7):759-61.
16. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. Annual review of plant biology. 2003 Jun;54(1):519-46.
17. Fu C, Mielenz JR, Xiao X, Ge Y, Hamilton CY, Rodriguez M, Chen F, Foston M, Ragauskas A, Bouton J, Dixon RA. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. Proceedings of the National Academy of Sciences. 2011 Mar 1;108(9):3803-8.
18. Bhalla A, Bansal N, Kumar S, Bischoff KM, Sani RK. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes. Bioresource technology. 2013 Jan 31;128:751-9
19. Yang B, Wyman CE. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioproducts and Biorefining. 2008 Jan 1;2(1):26-40.
20. Zabed H, Sahu JN, Boyce AN, Faruq G. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. Renewable and Sustainable Energy Reviews. 2016 Dec 31;66:751-74.
21. Abramson M, Shoseyov O, Shani Z. Plant cell wall reconstruction toward improved lignocellulosic production and processability. Plant Science. 2010 Feb 28;178(2):61-72.
22. Sánchez JP, Ullman C, Moore M, Choo Y, Chua NH. Regulation of Arabidopsis thaliana 4-coumarate: coenzyme-A ligase-1 expression by artificial zinc finger chimeras. Plant biotechnology journal. 2006 Jan 1;4(1):103-14.
23. Zhou J, Lee C, Zhong R, Ye ZH. MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in Arabidopsis. The Plant Cell. 2009 Jan 1;21(1):248-66.
24. Zhao Q, Gallego-Giraldo L, Wang H, Zeng Y, Ding SY, Chen F, Dixon RA. An NAC transcription factor orchestrates multiple features of cell wall development in Medicago truncatula. The Plant Journal. 2010 Jul 1;63(1):100-14.
25. McCarthy RL, Zhong R, Fowler S, Lyskowski D, Piyasena H, Carleton K, Spicer C, Ye ZH. The poplar MYB transcription factors, PtrMYB3 and PtrMYB20, are involved in the regulation of secondary wall biosynthesis. Plant and Cell Physiology. 2010 Jun 1;51(6):1084-90.
26. Carroll A, Somerville C. Cellulosic biofuels. Annual review of plant biology. 2009 Jun 2;60:165-82.
27. Lynd LR. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. Annual review of energy and the environment. 1996 Nov;21(1):403-65.
28. Availablefrom:http://biofuelsnews.com/display_news/10886/india_sets_out_2022_plan_to_beef_up_biofuels_production/
29. Zhang Y, Bi P, Wang J, Jiang P, Wu X, Xue H, Liu J, Zhou X, Li Q. Production of jet and diesel

biofuels from renewable lignocellulosic biomass. Applied Energy. 2015 Jul 15;150:128-37.

30. Weng JK, Akiyama T, Bonawitz ND, Li X, Ralph J, Chapple C. Convergent evolution of syringyl lignin biosynthesis via distinct pathways in the lycophyte Selaginella and flowering plants. The Plant Cell. 2010 Apr 1;22(4):1033-45.
31. Xu B, Escamilla-Treviño LL, Sathitsuksanoh N, Shen Z, Shen H, Percival Zhang YH, Dixon RA, Zhao B. Silencing of 4-coumarate: coenzyme A ligase in switchgrass leads to reduced lignin content and improved fermentable sugar yields for biofuel production. New phytologist. 2011 Nov 1;192(3):611-25.
32. Baxter HL, Mazarei M, Fu C, Cheng Q, Turner GB, Sykes RW, Windham MT, Davis MF, Dixon RA, Wang ZY, Stewart Jr CN. Time Course Field Analysis of COMT-Downregulated Switchgrass: Lignification, Recalcitrance, and Rust Susceptibility. BioEnergy Research. 2016:1-4.
33. Tschaplinski TJ, Standaert RF, Engle NL, Martin MZ, Sangha AK, Parks JM, Smith JC, Samuel R, Jiang N, Pu Y, Ragauskas AJ. Down-regulation of the caffeic acid O-methyltransferase gene in switchgrass reveals a novel monolignol analog. Biotechnology for biofuels. 2012 Sep 21;5(1):1.
34. Fu C, Xiao X, Xi Y, Ge Y, Chen F, Bouton J, Dixon RA, Wang ZY. Downregulation of cinnamyl alcohol dehydrogenase (CAD) leads to improved saccharification efficiency in switchgrass. BioEnergy Research. 2011 Sep 1;4(3):153-64.
35. Saathoff AJ, Sarath G, Chow EK, Dien BS, Tobias CM. Downregulation of cinnamyl-alcohol dehydrogenase in switchgrass by RNA silencing results in enhanced glucose release after cellulase treatment. PloS one. 2011 Jan 27;6(1):e16416.
36. Jung JH, Fouad WM, Vermerris W, Gallo M, Altpeter F. RNAi suppression of lignin biosynthesis in sugarcane reduces recalcitrance for biofuel production from lignocellulosic biomass. Plant Biotechnology Journal. 2012 Dec 1;10(9):1067-76.
37. Piquemal J, Chamayou S, Nadaud I, Beckert M, Barriere Y, Mila I, Lapierre C, Rigau J, Puigdomenech P, Jauneau A, Digonnet C. Down-regulation of caffeic acid O-methyltransferase in maize revisited using a transgenic approach. Plant Physiology. 2002 Dec 1;130(4):1675-85.
38. Fornalé S, Capellades M, Encina A, Wang K, Irar S, Lapierre C, Ruel K, Joseleau JP, Berenguer J, Puigdomènech P, Rigau J. Altered lignin biosynthesis improves cellulosic bioethanol production in transgenic maize plants down-regulated for cinnamyl alcohol dehydrogenase. Molecular Plant. 2012 Jul 31;5(4):817-30.
39. Chen F, Dixon RA. Lignin modification improves fermentable sugar yields for biofuel production. Nature biotechnology. 2007 Jul 1;25(7):759-61.
40. Guo D, Chen F, Inoue K, Blount JW, Dixon RA. Downregulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-methyltransferase in transgenic alfalfa: impacts on lignin structure and implications for the biosynthesis of G and S lignin. The plant cell. 2001 Jan 1;13(1):73-88.