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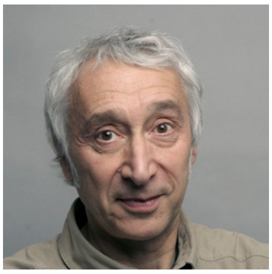
Maydica special issue - Exploitation of grass cell wall related traits for improving biofuel productions: an overview

Yves Barrière[1], **Mario Motto**[2]

[1]INRA, Unité de Génétique et d'Amélioration des Plantes Fourragères, 86600 Lusignan, France
[2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Maize Research Unit, via Stezzano 24, 24126 Bergamo, Italy



The exploitation of petroleum reserves has enabled modern industrialization, consumer product diversification, and global mobility (van Maris et al, 2007). The consequences of this utilization of fossil carbon-derived fuels – the release of carbon dioxide into the atmosphere – are no longer deniable. It is also clear that the increasingly high demand for fossil carbon will eventually deplete the existing stocks, with consequences not only in the area of energy but also in the wider chemical industry (van Maris et al, 2007; Lin and Tanaka, 2006).

Alternative energy initiatives worldwide have now rekindled large interest in the development of new and cost-effective processes for converting plant-derived biomass to fuels. For instance, in the US, the Energy Independence and Security Act (EISA) of 2007 and in Europe, the EU funded 7th Research Framework Program had an increased focus on biofuels support R&D needed to enable the achievement of the respective volumetric and cost targets set forth to bring us closer to independence from fossil transportation fuels. In the EU, it is expected by 2030 that one-fourth of the transportation fuels will be derived from biofuels.

Currently, biofuels are largely derived from maize grains, sugarcane or soybean oil, but researchers and investors are increasingly upbeat about another biofuel feedstock, lignocellulose (Somerville, 2007), with a great reduction of competition with food supplies.

In particular, lignocellulosic biomass represents an abundant, inexpensive, and locally available feedstock continually replenished by photosynthetic reduction of carbon dioxide using sunlight energy for the conversion to carbonaceous fuels (Ragauskas et al, 2006; Gray et al, 2006). Despite their great abundance, only 2% of this resource is currently used by humans. Therefore, research into the feasibility of using plant cell walls in the production of cost-effective biofuels is desirable. The main bottleneck for using cell wall materials is the recalcitrance of walls to efficient degradation into fermentable sugars (cf. Li et al, 2008). Manipulation of the wall polysaccharide biosynthetic machinery or addition of wall structure-altering agents should make it possible to tailor cell wall composition and architecture to enhance sugar yields upon cell wall digestion for biofuel fermentation. Study of the biosynthetic machinery and its regulation is still in its infancy and represents a major scientific and technical research challenge (Farrokhi et al, 2006 for a review). Of course, any change in cell wall structure to accommodate cost-efficient biofuel production may have detrimental effects on plant growth and development due to the diverse roles of walls in the life of a plant. However, the diversity and abundance of cell wall structures present in the plant kingdom gives hope that this challenge can be met.

Genetic variation for cell wall related traits

In maize, for instance, investigations carried out for cattle feeding and silage breeding indicated that a large variation in lignin content and cell wall digestibility occurred among inbred lines and hybrids (review in Barrière et al, 2004). A greater digestion of cell walls in animal rumen or during enzymatic solubility essays was considered to be related to a better degradability during industrial processes. Inbred lines with higher cell wall degradability are currently available for breeding: lines with low cell wall degradability can be used as model system towards the discovery of cell wall degradability determinants. However, the maize germplasm used in current breeding programs represents only a small fraction of the available genetic resources, mostly corresponding to germplasm used for grain breeding programs. Consequently, it is interesting to carry out investigations of cell wall related-traits in other accessions, old lines, and exotic resources, to discover new regulatory mechanisms or alleles beneficial for enhancing cell wall degradability. Additionally, new selection criteria to improve biofuel genotypes should be developed by considering the notion that susceptibility to chemical pre-treatments is probably different from susceptibility to enzyme hydrolysis without pretreatment.

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Genes involved in cell wall biosynthesis

The knowledge of pathways leading to the synthesis of the different bricks forming cell walls is a crucial clue towards the discovery of the corresponding genes involved in these pathways and affecting their regulation (for a review see Zhong et al, 2010). The different steps of the three monolignol biosynthesis are fairly well known, and the corresponding genes mostly identified. One outstanding issues concern the role of the different members in each gene family in the different metabolic pathways towards either the synthesis of each monolignol, or the synthesis of monolignols in the different tissues. Another unanswered question is centered on the identification of the O-methyltransferase allowing the methoxylation of caffeoyl-aldehyde into coniferaldehyde. An unknown, but promising, field of investigation should be based on the capacity of lignin polymers to incorporate various phenolics in place of the three regular constitutive monolignols (Grabber et al, 2012; Vanholme et al, 2012). Such unusual monomers can be incorporated in lignins of wild-type plants with no or lower impacts on growth than those observed for reduced lignin contents. However, the modified physico-chemical and stereo-chemical properties of the modified lignin polymer could be a basis for original plant improvement for biofuel production.

A list of candidate genes involved in secondary wall biosynthesis and assembly

Most investigations directed to deciphering the cell wall traits were based on dicotyledonous and gymnosperm plants, with the objectives of reduced costs and pollution during paper pulping production. Plant cell walls were also investigated in the Arabidopsis model system, especially for the understanding of monolignol gene regulation by upstream (master) transcription factors (Zhong and Ye, 2009). Additionally, several investigations have also described genes involved in cell wall carbohydrate biosynthesis, including especially mutants with irregular xylem or brittle stalks (e.g. Courtial et al, this issue). A list of maize ortholog genes, including those involved in cell wall carbohydrate and phenolic compound biosynthesis, have been singled out, comprising between 400 and 800 genes, according to investigations. The most important bias of such investigations is related to the specificities the grass cell wall, without secondary cambium, and conversely with high ferulate cross-linkages.

In search of QTLs for cell wall related traits

Quantitative Trait Loci (QTLs) sustaining cell wall related-traits are available from several maize RIL progenies, A recent survey, has indicated that approximately 125 QTLs affecting cell wall degradability, which correspond to 35 non-overlapping positions (Courtial, 2012). Co-localizations of cell wall degradability and lignin QTLs occurred in nearly 2/3 of the estimated non-overlapping positions. After lignin content, the two most frequent co-localizations of QTLs involved in cell wall degradability were those with cross-linking ferulate bridges and p-coumaric acid content QTLs. Progress is now expected from meta-QTL analyses, taking also advantages of the marker physical positions. RIL phenotyping should also be carried out using specific biofuel traits, such as saccharification yield, before and after pretreatments.

Expression studies and the cell wall gene networks

Assuming that differences in cell wall degradability between inbreds could be related to differences in gene expression, transcription profiling studies, during successive stages of internode growth and development, can give a large picture of genes switching on and off secondary cell wall biosynthesis and deposition, and their co-regulation. Observed transcriptomic variation should give clues in the search for either the gene function of the candidate to be discovered or probably, less frequently, the candidate itself. The lower expression of several phenylpropanoid genes in lines of higher cell wall degradability is very likely related to the degree of efficiency or expression of a transcription factor rather than to an allelic variation of one or more lignin genes, with pleiotropic consequences (such as the co-regulation observed in the comparisons of normal and brown-midrib mutants). Furthermore, variations in cell wall degradability may be related to variation in extent of lignified tissue assembly, rather than differences in intensity of secondary wall deposition in each tissue. Such variation might correspond to variable expression of other regulatory genes, which might be highlighted via differential expression studies of specific families of genes including genes related to auxin-regulated patterning mechanisms. Finally, bio-analysis approaches from large set of expression data may be a tool towards the discovery of specific grass genes, which have no orthologs in model plants such as Arabidopsis.

Genetic engineering

Genetic engineering is most often an unavoidable tool for candidate gene validation. For instance, altering the expression of an appropriate transcription factor regulating secondary wall biosynthesis or tissue patterning regulation might be a relevant strategy in plant breeding programs directed to improve cell wall degradability. This strategy allows affecting a group of target genes in a metabolic pathway in a coordinated manner. Moreover, when their metabolism and polymerization rules will be better understood, the modification of lignin monomer ratio, of monomer acylation, and the incorporation of unusual monomers could lead to a lignin polymer structure with reduced masking effects on cell wall carbohydrates and/or more susceptible to industrial pre-treatments.

Transposon tagging mutants and candidate gene validation

Transposon tagging is a powerful strategy for gene discovery, which is however limited by the large number of plants to be observed for such cell wall related traits, especially in forward genetics approaches when no candidate genes are a priori considered (EMS mutants could likely be similarly considered). A favorable situation is certainly for highly heritable traits that could be estimated on plantlets, as it has successfully been done for the maize low ferulate mutant (Jung and Phillips, 2010). Transposon tagging is probably a more efficient strategy when it is used as a functional validation tool. Such mutants could thus be searched for genes underlying a support interval of QTLs, reduced after fine mapping to a set of nearly 100 genes.

Association mapping for cell wall related gene identification

During the last decade, association mapping emerged as a complementary approach to identify polymorphisms driving phenotypic variation, and alleles of interest for agronomic purposes. A crucial aspect of association genetics is the knowledge of linkage disequilibrium (LD) In maize, LD was shown to decrease fast with physical distance within genes, precluding of a high resolution of the method (Rafalski, 2002). Moreover, several studies have suggested that recombination may strongly differ within and between maize genes, since LD nearby candidate genes may persist much longer than within genes. Additionally, polymorphisms shown to be responsible for phenotypic variation were found to be located in regulatory rather than coding regions (Salvi et al, 2007; Ducrocq et al, 2008). Finally, genome-wide LD that results from population stratification can generate non-functional, spurious associations. In maize, intense stratification results from genetic drift during geographical expansion and increased relatedness among elite inbreds derived by plant breeders. A technical bias could also arise from the fact that arrays were developed from SNP discovered on the B73 line, or late US related germplasm, which are not representative of the germplasm used in early and medium-early maize breeding programs. Significant further methodological investigations are likely requested before a routine use of association mapping in cell wall gene discovery.

Model species

Due to its considerable genetic and genomic backgrounds, the easiness in self-pollination and hybrid production, and large seed yield per plant, maize is indeed a relevant model species for identifying traits underlying cell wall degradability variations in grass species. It is then questioning whether another grass model plant could be of interest for the improvement of cell wall traits towards biofuel production. Results are still expected from investigations of cell related-traits (degradability and saccharification texts) in EMS mutant collection of *Brachypodium* (C3 grass). Similar results could also be expected from investigations in Setaria (C4 grass). In addition to investigations in maize, these two species are likely efficient tools for faster candidate genes validation, after their discovery as underlying support interval of cell wall related QTLs.

In the current issue

To contribute to the ongoing discussion in this area, we present in this special issue a series of papers reporting original information. This will increase our knowledge of the grass cell wall assembly and highlight new strategies for breeding maize and grass fitting better, in more environmentally friendly conditions, to the production of biofuels and bioproducts.

In this issue, a first set of papers focus on the composition and organization of the maize secondary cell wall and the consequence on cell wall degradability, and the search for the corresponding genetic determinants.

The paper by Wong Quai Lam and colleagues (2013) is dealing with maize cell wall degradability, from whole plant to tissue level, and illustrates different scales of complexity.

The effects of different mutations in the *ZmCAD2* gene that underlie the maize brown-midrib1 (bm1) phenotype with similar effects on lignin characteristics and have potential interest for bioenergy production, has been investigated by Barrière and coauthors (2013).

The article by Courtial and co-workers (2013) highlights breeding grasses efforts for silage feeding value or capacity to biofuel production. In addition, the paper gives an updated list of genes involved in maize secondary cell wall biosynthesis and assembly.

A second set of papers focuses on genetic resources for the corresponding traits in maize.

Specifically, the paper by Muttoni and colleagues (2013) gives results on cell wall composition and biomass digestibility diversity in Mexican maize (*Zea mays* L) landraces and inbred lines developed at CIMMYT.

The contribution of Tothne Zsubori and coauthors (2013) reports information on yield and chemical composition of plant parts of silage maize (*Zea mays* L) hybrids and their interest for biogas production.

Information from the previous cited studies was enlarged to maize allied species.

Eubanks and coworkers (2013) present data concerning high sugar Eastern gamagrass (*Tripsacum dactyloides* L) cultivars as potential biofuel feedstock.

An agronomic and digital phenotyping evaluation regarding sweet sorghum public varieties and F_1 hybrids, with potential for ethanol production in Spain, was provided by the contribution of Codesido and colleagues (2013).

The final article by Pilù and coworkers (2013) emphasizes the importance of *Arundo donax* as an energy crop. The paper makes evident the pros and cons of the utilization of this perennial plant.

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