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Lignin manipulation for fibre improvement

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1. Introduction

For centuries plant fibres have been used in a number of commercial areas including textiles, construction, paper and pulp, reinforced composites, and as biomass for energy production. These fibres come from a whole host of crops ranging from cotton, jute and flax for textiles; wood crops such as poplar, eucalyptus and conifers for paper and pulp; and cereal crops such as maize, sorghum and barley to provide straw, bedding and animal fodder. In more recent years the popularity of fibre crops in some of these areas has been superseded by synthetic fibres such as those made from plastic or glass. Environmentally, these synthetic fibres are non-renewable and continue to accumulate as sources of pollution. The impact of this pollution has led to a renewed interest in the use of plant fibres as a sustainable commodity for the future.

With the exception of cotton fibres, which are almost pure cellulose, most plant fibres also contain hemicelluloses, pectins and lignins connected together in a complex network. Although each of these components is likely to have an effect on the properties of the plant fibre that is produced, little is known of the basic biological processes underlying the production of most of these polymers. It has been estimated that 15% of the transcribed genome of *Arabidopsis* comprises genes directly involved in cell wall metabolism but few of these genes have been studied in any detail.

P. Ranalli (ed.), Improvement of Crop Plants for Industrial End Uses, 129–153.

Of the different cell wall polymers, the most intensively studied to date has been lignin. Interest in lignin biosynthesis has been driven mainly by the perceived value that improvements in the extractability of lignin would have to the pulp and paper industry. Manipulating genes in the lignin biosynthesis pathway has also been shown to have an impact on the digestibility of certain forage crops. Most of the genes in the lignin biosynthesis pathway (Fig. 5.1) have now been cloned, characterized and manipulated in a range of species including *Arabidopsis,* herbaceous woody angiosperms (tobacco) and trees (poplar and eucalyptus). This available data offers a starting point for further research into fibre crop improvement by focusing on manipulating lignin genes. Although genetic modification (GM) of crop plants offers the greatest potential for rapidly improving fibre traits, research in this area is currently limited due to public concerns over the safety of GM. As an alternative approach, conventional breeding can still benefit from the latest biotechnological tools which are being used to identify candidate genes for marker-assisted breeding.

2. State-of-the-Art and the latest advances of the sector

2.1 Lignin

Lignin is an important component of plant secondary cell walls. It is a complex polymer of phenylpropanoid units that is cross-linked to other components, including cellulose, within the wall. The integration of lignin into the cell wall strengthens and maintains the structure of the cell. Lignin also offers the plant some protection against external threats such as herbivores and pathogens. During papermaking, lignin is difficult to degrade, demanding the use of highly toxic chemicals during its extraction, which go on to pollute the environment. As a component in forage crops, lignin makes it difficult for animals to break down the plant fibre to release the available energy. Much research has been done on manipulating the genes that are involved in lignin biosynthesis in an attempt to make lignin that is more amenable to extraction during pulping (Baucher et al. 2003), and to improve the digestibility of forage crops (Barriere et al. 2003).

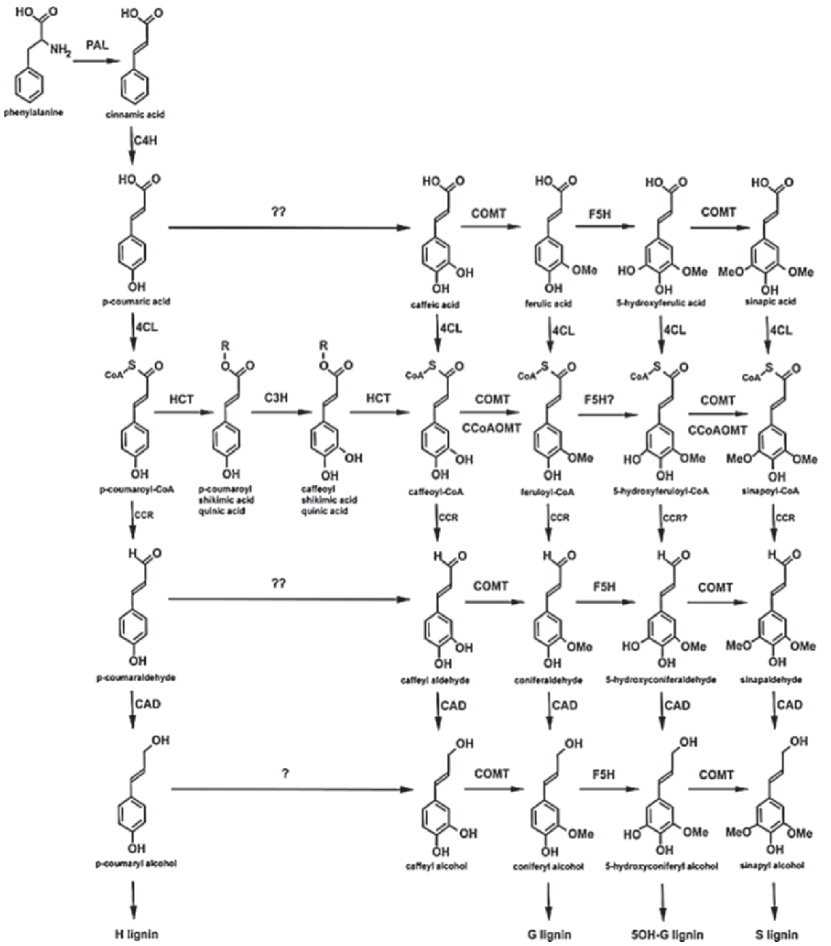

Fig. 5.1. The lignin biosynthesis pathway (adapted from Raes et al. 2003). PAL, Phenylalanine ammonia lyase; C4H, Cinnamate-4-hydroxylase; 4CL, 4-Coumarate:CoA ligase; HCT, Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyltransferase; C3H, Coumarate-3-hydroxylase; COMT, Caffeate 3-*O*-methyltransferase; CCoAOMT, Caffeoyl CoA 3-*O*-methyltransferase; CCR, Cinnamoyl-CoA reductase; F5H, Ferulate-5-hydroxylase; CAD, Cinnamyl alcohol dehydrogenase.

There are three monomers (monolignols) produced by the lignin pathway that become major components of the lignin polymer - *para*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monomers (Fig. 5.2). These monomers differ only in their degree of methoxylation. The percentage of each monomer that is incorporated into lignin varies from species to species, and also within plants from cell type to cell type (Campbell and Sederoff 1996). Generally, lignin in gymnosperms is predominantly composed of G units while angiosperm lignin is a mixture of G and S units, with lignin in the grasses additionally containing H units.

The content, composition and structure of lignin within cell walls, as well as the type and frequency of linkages between lignin and other wall polymers, influence the mechanical and industrial properties of plant fibres. A considerable body of research, summarized in the next section, illustrates how the physical and chemical properties of fibres can be modified by manipulating lignin. Most of this work focuses on improving wood for applications in pulping or improving forages as animal feedstocks. However the results of this research are equally relevant to the improvement of other types of fibre crop, irrespective of whether or not they have applications in pulping or animal feed production, since they demonstrate the types and extent of manipulation that is possible without adverse effects on plant health and viability.

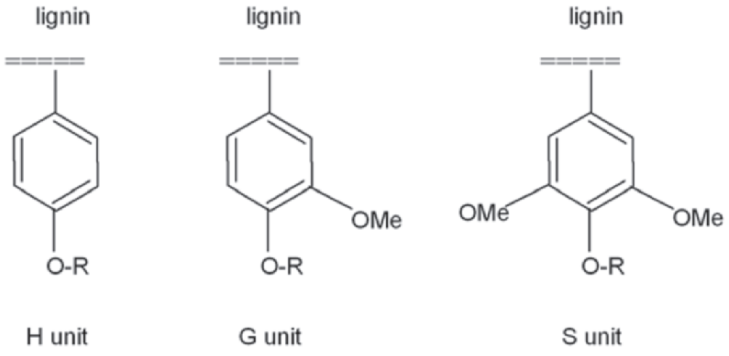

Fig. 5.2. The major monomers incorporated into lignin: *para*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units.

2.2 Manipulation of genes involved in lignin biosynthesis

All of the genes known to be involved in lignin biosynthesis have been targeted for manipulation or mutation in model plants such as *Arabidopsis*, tobacco, or poplar. There are currently ten genes known to be involved in monolignol production. The roles of each of these genes have been characterized following up- or down-regulation of gene activity in transgenic plants, either in a single construct or in combination with other lignin genes. Mutants have also been isolated for some of the lignin genes. A brief explanation of their role in the pathway follows along with results from studies of gene manipulation.

2.2.1. Phenylalanine ammonia lyase (PAL)

PAL is the first enzyme in the pathway and catalyses the deamination of phenylalanine to trans-cinnamic acid (Wanner et al. 1995). This reaction is regulated by a feedback loop that inhibits PAL activity in the presence of high levels of trans-cinnamic acid (Blount et al. 2000). Down-regulating PAL activity in tobacco (Sewalt et al. 1997a) leads to a decrease in the content of G lignin, but does not alter the content of S lignin. Although down-regulating PAL can have a big impact on lignin content, it frequently leads to the production of plants with pleiotrophic detrimental phenotypes making PAL unsuitable as a target for improving fibre qualities while maintaining normal plant growth.

2.2.2. Cinnamic acid 4-hydroxylase (C4H)

Trans-cinnamic acid is hydroxylated by C4H to produce p-coumaric acid. C4H belongs to the family of cytochrome P450-dependent monooxygenases. It has been shown that C4H activity is tightly co-ordinated with PAL activity (Koopmann et al. 1999). Down-regulating activity of C4H in tobacco (Sewalt et al. 1997a) and alfalfa (Reddy et al. 2005) plants results in a reduction in lignin content and a decrease in the S/G ratio. Although the lignin composition in the C4H down-regulated alfalfa plants was not hugely altered, there was a large improvement in digestibility, suggesting that lignin content rather than lignin composition may be a key determinant in improving digestibility.

2.2.3. Coumarate:CoA ligase (4CL)

There are multiple isoforms of 4CL in *Arabidopsis* which catalyze the formation of CoA esters of p-coumaric acid, caffeic acid, ferulic acid, 5-hydroxyferulic acid and sinapic acid (Raes et al. 2003). Down-regulating activity of 4CL in tobacco plants results in reduced lignin content and the plants with the greatest lignin reductions were stunted and had collapsed xylem vessels (Kajita et al. 1997). The S/G ratio in the plants was decreased, although studies in plants other than tobacco report an increased S/G ratio (as reviewed by Boerjan et al. 2003). In poplar, reduced lignin in 4CL-down-regulated plants is apparently compensated for by an increase in cellulose in healthy plants that grow taller than normal (Hu et al. 1999), although the growth effects depend on the promoter used to drive the transgene (Li et al. 2003a).

2.2.4. HydroxycinnamoyI-CoenzymeA Shikimate/Quinate Hydroxycinnamoyl Transferase (HCT)

HCT has recently been identified as acting upstream and downstream of C3H. It catalyzes the conversion of *p*-coumaroyl-CoA to the corresponding shikimic acid or quinic acid esters. It also catalyzes the reverse reaction leading to caffeoyl-CoA. Down-regulation of HCT results in plants with a reduced lignin content, containing less S units, and more H units. *Arabidopsis* plants severely down-regulated in HCT (with ~1% of wild type activity) have almost no S units in the interfascicular fibre cells. These plants have a dwarf phenotype and are sterile (Hoffmann et al. 2004).

2.2.5. Coumarate-3-hydroxylase (C3H)

C3H catalyzes the hydroxylation of shikimic acid and quinic acid esters to caffeoyl units (Schoch et al. 2001). C3H is a P450-dependent monooxygenase. The *Arabidopsis* C3H-defective mutant (*ref*8) has a dwarf phenotype, yielding significantly less lignin than the wild type. *Ref*8 lignin is composed almost exclusively of H units which are only found in trace amounts in the wild type (Franke et al. 2002). A reduction in hemicellulose and lignin was found in C3H down-regulated alfalfa plants (Reddy et al. 2005) which showed evidence of compensatory cellulose accumulation, as previously reported in 4CL down-regulated poplar plants. Although the most severely down-regulated alfalfa plants showed delayed growth, the majority of the plants were phenotypically similar to wild type alfalfa despite having dramatically altered lignin. These alfalfa plants had a massive increase in H units with the biggest increase in digestibility yet found in forage crops. The dramatic changes in lignin structure due to the altered

composition of the polymer are thought to be responsible for the significant digestibility improvement (Ralph et al. 2006).

2.2.6. Caffeic acid 3-O-methyltransferase (COMT)

COMT has been implicated to act at different levels in the pathway (Inoue et al. 1998; Maury et al. 1999). It is currently thought to be predominantly active at the level of the aldehydes and alcohols, leading to S lignin biosynthesis. Down-regulation of COMT in tobacco produces plants with a phenotype indistinguishable from wild type plants. The amount of lignin is not significantly reduced, but the composition of the lignin is altered, containing less S units, and abnormally high amounts of 5-hydroxyguaiacyl (5-OH-G) units (Atanassova et al. 1995; Pinçon et al. 2001a). The incorporation of 5-OH-G units following down-regulation of COMT has also been reported in poplar (Jouanin et al. 2000) and in maize (Lapierre et al. 1988). COMT mutants have been reported in maize (Vignols et al. 1995) and sorghum (Porter et al. 1978). These mutants have a brown midrib phenotype and show reductions in lignin content along with improvements in digestibility (Cherney et al. 1991; Bout and Vermerris 2003). COMT is one of the main candidates for improving digestibility.

2.2.7. Caffeoyl CoA 3-O-methyltransferase (CCoAOMT)

CCoAOMT methylates caffeoyl-CoA to give feruloyl-CoA. Down-regulating activity of CCoAOMT leads to a reduction in lignin content. It has no effect on S units, but shows a reduction in the amount of G units in tobacco (Pinçon et al. 2001a) and alfalfa (Guo et al. 2001a) plants. The alfalfa CCoAOMT down-regulated plants reportedly had a much higher digestibility than wild type plants (Guo et al. 2001b). In poplar, plants suppressed in CCoAOMT made a lignin that was less cross-linked than normal and increased levels of free and bound *p*-hydroxybenzoic acid were detected in the cell walls (Zhong et al. 2000).

2.2.8. Cinnamoyl-CoA reductase (CCR)

CCR is a key enzyme in the pathway converting cinnamoyl-CoA esters to their respective cinnamaldehydes. This is the first committed step in monolignol biosynthesis leading to the production of coniferaldehyde, 5-OH coniferaldehyde and sinapaldehyde. Down-regulating CCR in a variety of species has revealed its important role in regulating lignin content. CCR-suppressed tobacco plants have large reductions in lignin content and changes to lignin structure (Ralph et al. 1998; Piquemal et al. 1998; O'Connell et al. 2002). Similar changes to lignin content and structure

have been identified in transgenic or mutant *Arabidopsis* plants with reduced CCR activity (Jones et al. 2001; Goujon et al. 2003). Many of these CCR-deficient plants do not develop completely normally and have stunted growth, altered leaf morphology and collapsed or irregular xylem vessels. The changes in lignin seem to result in a disorganization and loosening of the secondary walls of fibres and vessels, leading to mechanical weakness (Chabannes et al. 2001a,b; Pinçon et al. 2001b; Goujon et al. 2003).

2.2.9. Ferulate-5-hydroxylase (F5H)

F5H preferentially catalyzes the 5-hydroxylation of coniferaldehyde (Humphreys et al. 1999) leading to the production of the S lignin monomer. F5H is a member of the CYP84 family of P450-dependent monooxygenases. The *Arabidopsis* F5H-deficient mutant (*fah1*; Meyer et al. 1996) exhibits only trace amounts of S units, while over-expression of F5H under the control of the C4H promoter results in a lignin that is almost entirely composed of S units (Marita et al. 1999). Despite the absence of S units, the *fah1* mutant shows no change in cell wall degradability by rumen micro-organisms (Jung et al. 1999). Down-regulation of F5H in alfalfa (Reddy et al. 2005) shows a reduction in S units with no effect on digestibility.

2.2.10. Cinnamyl alcohol dehydrogenase (CAD)

CAD is the final enzyme in the monolignol biosynthesis pathway and reduces *para*-coumaraldehyde, coniferaldehyde and sinapaldehyde into their corresponding alcohols. Down-regulating CAD in various species including tobacco and poplar produces plants with a red-brown colour in the stem wood. This is thought to be due to an increase in the amount of aldehydes incorporated into the lignin in these plants (Higuchi et al. 1994; Baucher et al. 1996). Due to this colour, a number of potential CAD mutants have been identified in maize and sorghum (see Cherney et al. 1991), and recently in rice (Zhang et al. 2006). Experiments on CAD-antisense tobacco plants indicated that the structural and compositional changes of the tobacco lignin changed the mechanical properties of the stem wood, although the possible impact of these changes on plant health and wood processing properties were not directly addressed (Hepworth and Vincent 1998, 1999). However, long-term field trials of poplar trees with reduced CAD activity have shown that tree growth and fitness is normal as are ecological interactions with insects and soil microbes (Pilate et al. 2002) although the trees have significant pulping benefits (discussed below).

2.2.11. Manipulation of multiple monolignol biosynthesis genes

Although some of the genes in the lignin biosynthesis pathway appear to be good candidates for crop improvement, the greatest results may come through manipulating more than one gene at a time. There are a few reports where two or more lignin genes have been targeted simultaneously. Both COMT and CCoAOMT have been simultaneously suppressed in tobacco (Zhong et al. 1998; Pinçon et al. 2001a) and alfalfa (Guo et al. 2001a). In tobacco, the double gene suppression promoted a greater reduction in lignin content than could be achieved by targeting either gene alone. Similarly, tobacco suppressed in both CAD and CCR showed synergistic effects of the two genes in reducing lignin quantity (Chabannes et al. 2001a). Plants suppressed in both genes had lignin decreases of approximately 50% compared to 32% and 12% reductions in plants suppressed in CCR or CAD alone. Simultaneous suppression of COMT and CCR has been achieved in tobacco by crossing plants down regulated in the single genes (Pinçon et al. 2001b). In this case, the effects of CCR suppression on lignin predominated - plants had reduced lignin content and increased S/G ratio but lignin changes typical of COMT suppression were not detected, possibly due to insufficient levels of COMT down-regulation (Pinçon et al. 2001b). Down-regulating CAD and COMT together in tobacco produces plants with a normal phenotype and reduced lignin content, while plants down-regulated in CAD, COMT and CCR together reduces lignin content but produces a stunted phenotype (Abbott et al. 2002). In poplar trees where F5H was up-regulated in tandem with 4CL down-regulation, additive effects were observed compared to transformants where only one of the genes had been manipulated. Lignin composition was altered and lignin content was reduced while cellulose content was increased (Li et al. 2003a). This work illustrates the potential for manipulating different complex traits affecting fibre quality by targeting relatively few genes (Halpin and Boerjan 2003).

2.2.12. Manipulation of monolignol transport and polymerisation

Considerable scope exists for manipulating lignin by targeting processes other than monolignol biosynthesis, such as monolignol transport or polymerization. Indeed these late steps in lignin deposition might be expected to be particularly suitable targets as unintentional effects on connected biochemical pathways could be avoided. Unfortunately, the details of how monolignols are transported and how they become polymerized within plant cell walls is still unclear and several possible mechanisms have been proposed (see Halpin 2004 for review). Monolignols are assumed to be exported from the cell as glucosides, although direct evidence for this is still lacking. In *Arabidopsis*, a number of UDP-glucosyltransferases have been

identified that are capable of glucosylating lignin monomers or their direct precursors (Lim et al. 2001, 2005). Similarly, a gene encoding a coniferin β-glucosidase that is capable of de-glucosylating monolignols has been found in pine (Dharmawardhana et al. 1999). Consistent with a potential role in deglucosylating exported monolignols prior to polymerization, the encoded protein is found in secondary cell walls of developing xylem (Samuels et al. 2002). Manipulating the expression of either the UDP-glucosyltransferases or the β-glucosidases that act on monolignols might be a useful strategy for manipulating lignin content or composition in crops, but, as yet, there are no reports of such work in the literature.

The identity of the enzymes involved in polymerizing lignin monomers is still under debate with peroxidases, laccases, and other phenoloxidases all proposed to play a role. Controversy also exists as to whether lignin polymerization is a random or highly ordered process, perhaps mediated by 'dirigent' proteins (Davin et al. 1997). Several reports describe the effects of manipulating the expression of various peroxidase genes *in planta*. In most cases, modification of anionic peroxidase expression had little effect on lignin deposition in tobacco (Lagrimini et al. 1997a,b) although aspen with reduced activity of a stem-specific anionic peroxidase (prxA3a) had a moderate reduction in lignin content (Li et al. 2003b). Similarly, suppression of laccase activity in poplar had no effect on lignin amount or composition (Ranocha et al. 2002). By contrast, suppression of a cationic peroxidase (TP60) in tobacco apparently reduced lignin content by 40-50% (Blee et al. 2003). However the exact roles of the multiplicity of different peroxidases and laccases present in different plant species, and the possibility of significant functional redundancy between them, makes effective modification of lignin via manipulation of monolignol polymerization an uncertain prospect.

2.2.13. Fibre improvement for pulp and paper making

During pulp production from plant fibres, lignin has to be removed from cellulose in order to produce good-quality paper. However, due to its complex structure, lignin is very difficult to extract, demanding the use of highly toxic chemicals which can pollute the environment. In order to make pulping easier and more environmentally benign, either (a) the lignin content of fibres could be reduced or (b) the extractability of lignin could be improved by modifying its chemical structure. Pulping studies have already been performed on some of the transgenic lignin-modified plants described in the last section and the results demonstrate the potential that exists for manipulating fibres to improve pulping properties.

Transgenic poplar suppressed in CAD activity has been evaluated by chemical pulping analyses after growth in the greenhouse (Baucher et al. 1996; Lapierre et al. 1999) and after two four-year field trials in France and the UK (Pilate et al. 2002). In all cases, the changes to lignin structure in CAD-suppressed plants made delignification easier using the chemical Kraft pulping process. In pulps made from the transgenic lines, the kappa number, a measure of residual lignin in the pulp after cooking, was reduced compared to that of pulp made from wild type plants. Subsequent bleaching of the pulps was also easier and other pulp properties were not adversely affected by the lignin modification. In a pine *cad* mutant, no enhanced delignification was evident after Kraft pulping (MacKay et al. 1999), probably reflecting the differences in lignin structure between gymnosperms and angiosperms. However, pulp produced from the mutant by the soda pulping process had a lower kappa number than wild type. Poplars overexpressing F5H also show significant improvements of Kraft pulping efficiency. Pulps from the F5H-expressing plants had lower kappa numbers and increased brightness compared to wild type (Huntley et al. 2003). The authors estimate that pulp throughput could be increased by 60% and consumption of pulping chemicals decreased by this genetic improvement. Not all lignin modifications are beneficial for pulping, however. Wood from field-grown COMT-down regulated poplars has proved to be more difficult to pulp than wood from wild type trees. The modified lignin was more difficult to extract and the kappa number was higher while pulp brightness after bleaching was lower than that of wild type trees (Pilate et al. 2002).

Pulping performance has also been assessed for transgenic tobacco with various modifications to lignin biosynthesis (O'Connell et al. 2002; Kajita et al. 2002). The Kraft pulp produced from CAD- or CCR-suppressed tobacco had a lower kappa number than pulp made from wild type plants (O'Connell et al. 2002). However, after bleaching, the pulp from the low-CCR plants had reduced brightness. This appeared to be due to a higher content of unextracted chlorophyll (O'Connell et al. 2002). Tobacco suppressed in 4CL has also been shown to be improved for Kraft pulping, having a higher efficiency of delignification, higher pulp yield, and improved subsequent bleaching, than pulp from wild type plants (Kajita et al. 2002).

As well as indicating how trees grown for pulping might be improved, this work also illustrates the potential for using and manipulating less traditional crops for paper production. The tobacco plants analyzed made high quality pulps that could be bleached to high levels of brightness and made into strong papers (O'Connell et al. 2002). This suggests a new opportunity for tobacco to be grown as a pulp crop and illustrates the potential for manipulating other annual crops for improved fibre production for pulp and paper making.

2.2.14. Fibre improvement for the forage industry

Forage crops are the main source of nutrition for ruminating animals. In 2005, over one billion metric tons of forage crops were produced worldwide. The main bulk of this forage material comes from maize and alfalfa production (Fig. 5.3).

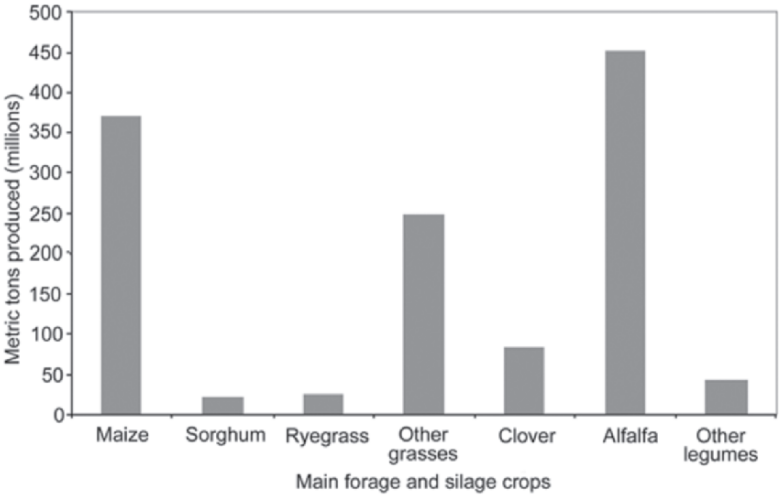

Fig. 5.3. Worldwide production of crop plants grown for forage and silage in 2005 in millions of metric tons (from FAOSTAT data).

The presence of lignin in these crops has a detrimental effect on their digestibility by ruminants (Cherney et al. 1991) due to the resistance of lignin to degradation by micro-organisms. Altering the content and/or composition of lignin by genetic manipulation of genes in the lignin biosynthetic pathway can result in changes to the digestibility of crop plants (see Barriere et al. 2003 for review). Numerous studies have shown that by down-regulating expression of certain genes in the lignin pathway the ratio of S/G monomers can be manipulated. Along with changes in lignin content, even small changes in the S/G ratio can have a significant impact on fibre digestibility as can changes to lignin structure. As already mentioned above, decreases in lignin content in C4H down-regulated alfalfa resulted in a large improvement in digestibility, but the massive structural changes resulting from the incorporation of increased levels of H lignin in C3H-deficient alfalfa had an even greater effect on digestibility. CAD- and COMT-deficient transgenic tobacco and alfalfa have also been shown to have slightly improved digestibility (Bernard-Vailhe et al. 1996, 1998). PAL-suppressed transgenic tobacco or CCR-deficient transgenic *Arabidopsis*

indicated similar improvements to enzymatic digestibility (Sewalt et al. 1997b; Goujon et al. 2003).

Although modifications to digestibility have been demonstrated in GM plants, many of the species concerned are not normally used for forage. A better illustration of how lignin might be manipulated to improve digestibility comes from data on a family of naturally-occurring or chemically-induced lignin mutants, the brown midrib mutants. These mutants have been much used in digestibility studies and have been instrumental in furthering understanding of the basic processes involved during lignification of grass cell walls.

In maize, four naturally occurring brown midrib (*bm*) mutants exist (named *bm*1, *bm*2, *bm*3 and *bm*4), the first of which was discovered in 1924. Brown midrib mutants have now been found in a number of other crop species including sorghum (Bout and Vermerris 2003), millet (Cherney et al. 1988), sudangrass (Fritz et al. 1981), and recently rice (Zhang et al. 2006). These mutants all exhibit a reddish or golden brown pigment in the midrib and stem. Most brown midrib mutants are susceptible to lodging, possibly due to brittleness of the stem caused by a reduction in lignin content. *Bm*1 is the result of a mutation in or near the CAD gene (Halpin et al. 1998) and *bm*3 is the result of a mutation in the COMT gene (Vignols et al. 1995). The genes for *bm*2 and *bm*4 have not yet been identified but it is thought that, if not directly involved in lignin biosynthesis, they could play a related role as transcription factors (Vermerris and Boon 2001) or be involved in transportation of monolignols to the cell wall (Barriere et al. 2004).

Studies of the *bm*1 mutant shows that both the total lignin content and the structure of the lignin polymer are altered (Halpin et al. 1998). These plants have improved lignin extractability, perhaps making them good candidates as novel pulping materials, and have modest improvements in digestibility. The lignin content in *bm*3 plants is reduced by 25-40%, with a reduction in S units. *Bm*3 maize mutants have been grown commercially in the USA for a number of years. Fig. 5.4 shows the combined results from 18 agronomic and dairy cattle feeding trials comparing *bm*3 and regular corn. On average the yield from *bm*3 corn was reduced by 6%, while acid detergent fibre (ADF) and neutral detergent fibre (NDF) were reduced by 3% and 2% (Lauer and Coors 1997). ADF is a measure of the lignin and cellulose components and is used to predict energy content, while NDF is a measure of the total cell wall, used to predict dry matter (DM) intake potential. From this data, calculations were made to predict milk/ton and milk/acre. *Bm*3 corn showed a 4% increase in milk/ton, but a 2% decrease in milk/acre (Lauer and Coors 1997). Clearly, digestibility in *bm*3 is greatly improved compared to wild type maize, although this is at the expense of lower yields. Digestibility in maize decreases after flower-

ing, while yield increases due to grain production. A trade-off therefore exists between digestibility and yield when deciding the optimum harvest time.

Brown midrib mutants have been created in sorghum by chemical mutagenesis. *Bmr*12, *bmr*18 and *bmr*26 are the result of point mutations generating premature stop codons in a COMT gene (Bout and Vermerris 2003). The *bmr* mutants are allelic and show similar characteristics to maize *bm*3 but are distinct in some respects, e.g. *bmr*12 flowers 7 days early (Pedersen et al. 2005).

Further work is needed to engineer or select plants with the increased digestibility found in *bm*3 mutants, while maintaining yield and resistance to lodging. The identification of COMT alleles showing a less dramatic effect than *bm*3 may efficiently improve digestibility without effecting these changes (Barriere et al. 2003).

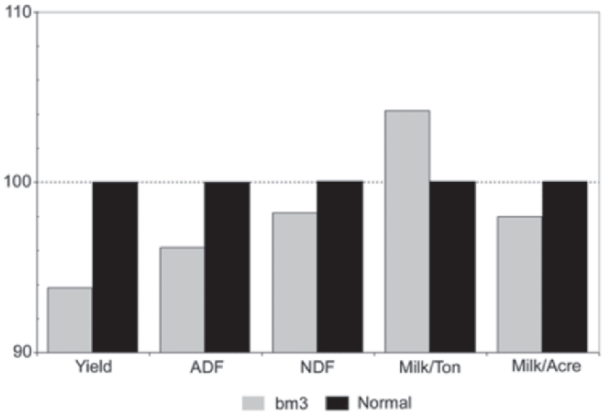

Fig. 5.4. Average yield, ADF, NDF, Milk/Ton and Milk/Acre of *bm3* corn mutants relative to normal corn (100%) compiled from 18 trials (Lauer and Coors 1997).

2.2.15. Crop improvement for textiles and other markets

Besides their use in papermaking and as components of animal forage, one of the biggest markets for natural fibres is still the textile industry. However, an increasing range of new markets are becoming more important, including potential for use of plant fibres in biocomposites for car manufacturing, construction and other industries, and as raw materials for energy production either as biomass for burning or for conversion into biofuels. These emerging commercial opportunities are fuelling new research

initiatives into the 'tailoring' of plant cell walls for different applications but, as yet, few results of such work have been published.

Cotton production dominates the textile market; in 2005, 66 million tons of cotton seed and 23 million tons of cotton fibre were produced worldwide. Cotton fibres, being mostly cellulose, are not targets for lignin manipulation. Instead, major targets in cotton are to improve the yield and produce longer, more uniform fibres to improve the quality of materials for clothing and furnishings. Nevertheless cotton is an example of a genetically modified fibre crop that is already widely deployed commercially, offering hope for growing acceptance of the production of transgenic fibre crops in the future. It is estimated that 28% of the global cotton planted in 2005 was genetically modified (James 2005). Most GM cotton contained an insecticidal *Bt* gene from *Bacillus thuringiensis* which protects the plants from bollworm, an important pest on cotton. However, a significant proportion (37%) of the global GM cotton additionally contained a herbicide-tolerance gene, along with the *Bt* gene, illustrating the state-of-the-art in engineering 'stacked' GM traits into crops and the potential for future manipulation of complex traits, such as fibre quality, with multiple transgenes.

Besides cotton, there are other fibre crops which are of interest to the textile industry (Table 5.1). Of these crops, jute is the most widely grown and has a greater yield per hectare than cotton. The biggest producer of jute is India where 2.8 million tons were produced last year. Jute is used to make lower quality products such as heavy duty clothing, home furnishings and carpets. Jute is 100% biodegradable and has a high tensile strength and a strong resistance to heat and fire. Improvements in the quality of jute fibres would include making the fibres finer so that they would be suitable for use in higher quality products. A jute mutant, *d*eficient *l*ignified *p*hloem *f*ibre (*dlpf*), was recently reported. It contains 50% less lignin, and has a greater amount of cellulose than normal (Sengupta and Palit 2004). This mutant has low levels of PAL activity and has a wavy, undulated, stem phenotype. Although shorter than wild type jute, most of the tissues other than the phloem fibres develop normally. The phloem fibres in the mutant are reduced and are present as single fibres rather than in bundles as they are found in the wild type. The tensile strength of the fibres was not significantly affected even though lignin was greatly reduced. The separateness of the fibres without loss of tensile strength is a trait that could be of interest to textile producers since the fibres have to be mechanically separated from bundles during their preparation for textile production. This is the first lignin mutant to be found in jute, and it shows that there is potential for improving jute by manipulating genes on the lignin pathway.

Table 5.1. Worldwide production, area harvested and yield of crops suitable for use as textiles in 2005 (from FAOSTAT data).

Crop	Production Metric tons $x10^5$	Area Harvested Hectares $x10^3$	Yield Hectograms per hectare $x10^3$
Kapok fruit	3.93	208.00	18.89
Seed cotton	666.66	35217.38	18.93
Cotton lint	232.64	66665.98	3.49
Flax fibre and tow	7.72	501.25	15.40
Hemp fibre and tow	0.67	52.44	12.87
Jute	28.62	1351.91	21.17
Jute-like fibres	3.88	296.20	13.11
Ramie	2.50	131.66	18.95
Sisal	3.28	384.82	8.51
Agave fibres nes	0.58	53.20	10.89
Abaca (Manila hemp)	1.01	147.92	6.83
Fibre crops nes	2.79	89.93	31.02

Production, area harvested and yield of the main crops grown worldwide in 2005 which are suitable for the use in textile making (FAOSTAT data, 2005)

Other crops, such as hemp, are in much lower production although they have the advantages that they can be grown in more moderate climates than cotton, and need reduced inputs in order to achieve high yields (Ebskamp 2002). Along with flax, hemp is one of the most widely grown natural fibre crops in Europe. While its predominant use is in the clothing and home textile industries, hemp has a very wide range of potential and novel applications and is also used for paper, building materials, toiletries, composts, fuel and foods. To make textiles, lignin and pectins have to be removed from the fibre (Wang et al. 2003) and decreasing the lignin content could therefore reduce processing costs and improve the softness of the fibre. It has been reported that improving the fineness of fibre would widen its application in clothing and industrial textiles and that this could be done by breeding hemp varieties containing smaller fibre diameters (Ranalli and Venturi 2004). This reduction in diameters might also potentially be achieved through reducing lignin by manipulating lignin biosynthetic genes.

3. Future and expected developments

Future directed improvement of fibre crops, either by genetic manipulation or conventional breeding, requires increased research effort into identifying the genes involved in all aspects of cell wall biosynthesis. The ongoing development of a variety of genomics tools, following the sequencing of the *Arabidopsis*, rice and poplar genomes, is enabling increasingly rapid identification and functional characterisation of previously 'unknown' genes. Technologies such as DNA microarrays which allow global and parallel analysis of the gene expression profile of an organism or tissue under different conditions are providing vast amounts of information. Transcript profiling in plants is already being used to investigate a wide range of developmental processes, including secondary cell wall deposition. Even microarrays that cover only a small portion of a genome can yield useful data. For example, xylem microarrays have been used to investigate gene expression in distinct development zones within the wood-forming tissues of hybrid aspen (Hertzberg et al. 2001). This analysis revealed the strict transcriptional regulation that must underlie the observed stage-specific expression of different cell wall biosynthesis genes. Similar approaches are currently being used to investigate secondary cell wall formation in fibre crops such as flax and hemp (Ebskamp 2002). Identification of the genes underlying fibre development and cell wall biosynthesis will enable increasingly sophisticated GM strategies to manipulate the expression of combinations of those genes in order to develop 'designer' fibres, improved for conventional and novel applications in the future. However, even without resorting to GM strategies, the identification of increasing numbers of genes affecting fibre quality will enable more rapid breeding improvement through marker assisted selection.

One of the greatest potential benefits of gene expression studies will be the possibility of identifying candidate genes by linking gene expression data to fibre quality QTLs (quantitative trait loci). The development of QTLs, along with comprehensive molecular markers and linkage maps, has enormous potential for marker-assisted breeding (see Collard et al. 2005 for review), enabling breeders to increase the precision of selection and more quickly advance the rate of improvements in crop plants. QTLs have been mapped for a range of agronomically important traits in many plant species. Several QTLs influencing wood properties have already been mapped in tree species used for pulp production, and forage quality QTLs have been identified in several crop species. However, few studies have yet identified the specific genes that underlie these QTLs, although progress is beginning to be made by simply mapping the few genes (such as lignin biosynthetic genes) that have already been shown to affect wood properties in order to see whether they co-locate with mapped QTLs. For

instance, in eucalyptus, specific single nucleotide polymorphisms (SNP) that are significantly associated with microfibril angle, have recently been found in a CCR gene (Thumma et al. 2005). Similarly, in maize, a CCoAOMT gene has been shown to co-localize with a QTL for cell wall digestibility and lignin content (Guillet-Claude et al. 2004). The identification of candidate genes that co-localize with QTLs will enable the development of improved early-selection markers since the linkage between conventional markers and genes can be lost during recombination. Future research will increasingly combine data from QTL analysis of phenotype, genotype and expression levels from microarrays (eQTLs) in genetical genomics strategies (Kirst et al. 2004).

4. Conclusion

Modern, marker-assisted plant breeding, and genetic modification technologies have the potential to improve plant fibres for a variety of conventional and novel end uses. To exploit this opportunity to the full, much research is still needed to identify and characterise the genes involved in fibre cell wall biosynthesis. Using genomic and post-genomic technologies, including transcript profiling, the identification of these genes should proceed at an increasing pace. Verification of the functional role of those genes by isolating the corresponding mutants or by genetic manipulation will also yield the critical information on how the activity of specific genes affects fibre properties. This information can then be applied for fibre improvement. This strategy is already exemplified by the huge body of work over the past decade into the lignin biosynthesis pathway. To date, most, perhaps all, of the genes involved in lignin biosynthesis have been identified and functionally characterized. Mutants and transgenics where expression of those genes has been altered have indicated how fibres can be improved for applications such as pulping and forage use. Modified lignin trees, genetically manipulated for improved pulping, have already been released in controlled field trials, and similar maize mutants with improved digestibility have been marketed in the USA and elsewhere for several years. This work indicates the real opportunities for manipulating lignin and other cell wall polymers for fibre improvement. Continuous innovation in fibre crop breeding either via GM approaches or marker assisted selection, is not only desirable, but is necessary if we are to meet the predicted increased future demand for sustainable, environmentally-friendly, production of natural fibres.

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