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ORIGINAL PAPER

Genetic variations of cell wall digestibility related traits in floral stems of *Arabidopsis thaliana* accessions as a basis for the improvement of the feeding value in maize and forage plants

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Abstract Floral stems of *Arabidopsis thaliana* accessions were used as a model system relative to forage plant stems in genetic variation studies of lignin content and cell wall digestibility related traits. Successive investigations were developed in a core collection of 24 *Arabidopsis* accessions and in a larger collection of 280 accessions. Significant genetic variation for lignin content in the cell wall, and for the two in vitro cell wall digestibility investigated traits, were found both in the core collection and in the large collection. Genotype × environment interactions, investigated in the core collection, were significant with a few genotypes contributing greatly to interactions, based on ecovalence value estimates. In the core collection, genotypes 42AV, 224AV, and 8AV had low cell wall digestibility values, whatever be the environmental conditions. Genotype 157AV, observed only in one environment, also appeared to have a low cell wall digestibility. Conversely, genotypes 236AV, 162AV, 70AV, 101AV, 83AV had high cell wall digestibility values, genotype 83AV having a slightly greater instability across differing environments than others. The well-known accession Col-0 (186AV) appeared with a medium level of cell wall digestibility and a weak to medium level of interaction between environments. The ranges of variation in cell wall digestibility traits were higher in the large collection than in the core collection of 24 accessions, these results needing confirmation due to the lower number of replicates. Accessions 295AV, 148AV, and 309AV could be models for low stem cell wall digestibility values, with variable lignin content. Similarly, accessions 83AV and 162AV, already identified from the study of the core collection, and five accessions (6AV, 20AV, 91AV, 114AV, and 223AV) could be models for high stem cell wall digestibility values. The large variations observed between *Arabidopsis* accessions for both lignin content and cell wall digestibility in floral stems have strengthened the use this species as a powerful tool for discovering genes involved in cell wall biosynthesis and lignification of dicotyledons forage plants. Investigations of this kind might also be applicable to monocotyledons forage plants due to the basic similarity of the genes involved in the lignin pathway of Angiosperms and the partial homology of the cell wall composition and organization of the mature vascular system in grasses and *Arabidopsis*.

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Introduction

It was established a long time ago that lignins are a primary determinant in lessening forage plant cell wall digestibility, even if ''the precise mechanism by which lignification reduces digestibility is unclear'' (Casler and Jung 1999). Lignins indeed have a variable

negative effect on polysaccharide degradation by microorganisms according to their proportions in the cell wall, as well as to their variable structures and the way in which they embed cell wall polysaccharides or cross-link to hemicelluloses via C–C linkages or ferulic acid (FA) bridges (review in Grabber et al. 2004). Beyond phenolic compound characteristics and organization in the cell wall, forage plant intake and digestibility could also be related to variations in contents, structures and ways of deposition of cell wall carbohydrates and possibly cell wall proteins. Lastly, lignified tissue patterning could act upon leaf and stem degradability and friability (Inoué et al. 1994; Jung and Allen 1995; Grabber et al. 2004).

Most perennial and annual forage plants are grasses (Poaceae, monocotyledons) such as ryegrass, fescue and maize, but several protein-rich dicotyledonous forage plants such as alfalfa, clovers or amaranths play an important role in pure or mixed swards. Most studies related to the genetic, genomic and biochemistry of lignins have been conducted on woody dicotyledons or gymnosperm species used for paper pulp production, and a few herbaceous model species dicotyledons (tobacco, zinnia), but with no or very few data related to cell wall digestibility (Anterola and Lewis 2002; Boerjan et al. 2003). Both for the lignins genetic, genomic and biochemistry, and for cell wall digestibility, few data are available in perennial forage grasses, although larger investigations have been devoted to alfalfa among dicotyledons (Guo et al. 2001a, b). In monocotyledons, most of investigations have been done in maize, probably due to its economic importance, the worldwide development of maize genomic investigations, and the easiness in maize plant selfing and RIL progeny production (Ralph et al. 2004). Rice has seemingly not yet been used as a monocotyledon model species for lignification or cell wall biosynthesis or degradability studies, although Abou-el-Enin et al. (1999) have shown large variations of in vitro cell wall digestibility of rice straws, and Zhang et al. (2006) established that the GOLD HULL AND INTERNODE2 (GH2) rice mutant, which exhibits a reddish-brown pigmentation of hull and internodes, was affected in a gene encoding a cinnamyl alcohol dehydrogenase (CAD), a key enzyme of the lignin pathway.

Conversely to rice, many investigations have been devoted to studies of cell wall biosynthesis or the lignin pathway in *Arabidopsis thaliana*, most often based on mutant or down-regulated plants. The efficiency of a dicotyledons model in the investigation of cell wall biosynthesis and lignification in monocotyledons has not as yet been definitively answered. Contradictory to dicotyledons and few exceptional monocotyledons, the vascular system of grasses develops without secondary growth from a bifacial cambium. In both grasses and *Arabidopsis*, the lignified plant cell wall is a composite material made of cellulose microfibrils and an amorphous matrix consisting predominantly of hemicelluloses and phenolics. However, chemical structures of wall components and wall architecture are not similar in typical monocotyledons and dicotyledons. In *Arabidopsis*, the cellulose–xyloglucan framework, with a similar amount of cellulose and xyloglucans, is embedded in a network of abundant pectic polysaccharides, mainly homogalacturonans, usually branched with arabinose or galactose residues. Unlike *Arabidopsis*, grass cell walls have more cellulose than xyloglucan, which are predominantly glucurono-arabinoxylans, with β1,3 and β1,4 mixed glucans, but with very low amounts of pectins (Yokoyama and Nishitani 2004). Phenolics mostly comprise lignins. However, in grasses, they also include cell wall-linked *p*-hydoxycinnamates, *p*-coumaric acid (*p*CA) and FA derivatives, this later one forming extensive cross-linking networks primarily when cells stop expanding. *p*CA and FA contents are both reported to be negatively related with cell wall digestibility (Grabber et al. 1998a,b; Casler and Jung 1999; Fontaine et al. 2003). Correlatively at least for a part, cell wall linked hydroxycinnamic acids are likely contributors to strengthening effects in grass stems (Monties 2003). Despite these differences, Reiter (1998) clearly showed the interest of *Arabidopsis* as a model system for the genetic dissection of cell wall synthesis in higher plants. Several reviews (Costa et al. 2003; Goujon et al. 2003b; Barrière et al. 2004a; Ralph et al. 2004; and Yokoyama and Nishitani 2004) have confirmed the basic similarity of genes involved in the lignin pathway of Angiosperms. Yokoyama and Nishitani (2004) also established that all gene families related to cell wall biogenesis in *Arabidopsis* had orthologs in rice, with an approximately similar number of members, even if genes related to signaling or regulation were not investigated. However, from investigations of Petroni et al. (2002), R2R3MYB genes regulating the biosynthesis of similar phenylpropanoids tended to be structurally very similar within and between species. Similarly, Romero et al. (1998) considered that ''the property to control the biosynthesis of phenylpropanoids was present in the ancestor of the majority of present day R2R3MYB genes''. Therefore, a plant model system should be considered efficacious in the search of genes involved in both cell wall biosynthesis and regulation in forage plants.

As illustrated first by the results of Chapple et al. (1992), Meyer et al. (1996), and Humphreys et al. (1999) for ferulate-5-hydroxylase (F5H), *Arabidopsis* was proven an efficient model system in evidencing genes of the lignin pathway, most often from knocked-out genes (Anterola and Lewis 2002; Boerjan et al. 2003). *Arabidopsis* also appeared as an efficient model system in estimating effects of gene disruption or deregulation in grasses. Significant aldehyde incorporation into lignins, with a higher incorporation of coniferaldehyde than of sinapaldehyde, was found as a signature of CAD deficiency in maize brown-midrib1 (bm1) mutants (Barrière et al. 2004a), which are affected in CAD activity (Halpin et al. 1998). Similarly, null *Arabidopsis* mutants of Atcad-C and Atcad-D have a drastically reduced CAD activity, and incorporated both coniferaldehyde and sinapaldehyde into the lignins (Sibout et al. 2003; 2005), as it was also observed in the lignins of CAD-deficient poplar (Ralph et al. 2001; Kim et al. 2002; Lapierre et al. 2004) and CAD-down-regulated tobacco (Kim et al. 2000; Ralph et al. 2001). Maize bm3 mutants, with a quasi-lack of caffeic acid *O*-methyl transferase (COMT) activity due to an exon 2 alteration in the COMT gene (Grand et al. 1985; Vignols et al. 1995), have a lignin content reduced by 25–40%, a heavily reduced frequency of syringyl (S) units, incorporated 5-hydroxyguaiacyl units and have a highly improved cell wall digestibility (Kuc and Nelson 1964; Lapierre et al. 1988; Ralph et al. 2001, Barrière et al. 2004a, b). Down-regulation of the COMT gene in *Arabidopsis* was proven to have similar consequences on lignin structure and cell wall digestibility (Goujon et al. 2003a), as it has previously been observed in bm3 and also in COMT down-regulated plants of maize (Piquemal et al. 2002).

For some time, the coumarate-3-hydroxylase (C3H) was the unique missing enzyme and gene of the lignin pathway. The ref8 mutant of *Arabidopsis*, whose lignin content is severely decreased and with lignins essentially composed of *p*-hydroxyphenyl (H) units, instead of guaiacyl (G) and syringyl (S) units as found in the wild type, was proven as a C3H (CYP98A3) mutant (Franke et al. 2002a, b). This CYP98A3 P450 3-hydroxylase was also shown to be involved in the 3-hydroxylation on 5-O-shikimate and 5-O-D-quinate esters of *p*-coumarate, instead of the usually considered *p*CA (Schoch et al. 2001). Moreover, Nair et al. (2004) demonstrated that the ref1 mutant of *Arabidopsis*, which has a reduced content in soluble sinapate esters, was affected in a sinapaldehyde dehydrogenase, which exhibited both sinapaldehyde and coniferaldehyde dehydrogenase activities when it was expressed in *E. coli*. In maize and grasses, the biosynthesis pathway of FA remained unknown during a long time. The maize COMT, involved in methylation of 5-hydroxyconiferaldehyde, was established to be uninvolved in the methylation of caffeic acid into FA (Barrière et al. 2004a). The implication here is that the acids in the wall could be produced beyond the monolignol pathway and derived from oxidation of the corresponding aldehydes, rather than acting as precursors of those aldehydes (and hence the monolignols), as it has been considered over the years.

The genetic and genomic basis of forage plant tissue susceptibility to be broken and torn up during eating and ruminating (mechanical qualities), which affects silage ingestibility and digestibility (Barrière et al. 2003a; Fernandez et al. 2004), are largely unknown. However, it should be considered that they are surely not only related to lignin content or structure, but also included carbohydrate organization and cross-linkages in the cell wall. Mutations of genes involved in cell wall polysaccharide biosynthesis were thus shown to affect xylem phenotype and/or lignin content or structure (Turner and Sieburth 2002; Cano-Delgado et al. 2003). Collapsed xylem cells were related to a deficiency in cellulose-synthase genes, and correlatively in secondary cell wall cellulose deposition (Taylor et al. 1999). The *Arabidopsis* FRAGILE FIBER1 (*FRA1*) gene mutation causes a very important reduction in fiber mechanical strength, but without any apparent alteration to the cell wall composition (Zhong et al. 2002). The *FRA1* gene encodes a kinesin-like protein that most likely mediates the activity of cortical microtubules in orienting the cellulose microfibrils during differentiation of xylem cells (Zhong et al. 2002; Nieminen et al. 2004). Conversely, the Arabidopsis FRAGILE FIBER8 gene mutation, which also results in a considerable reduction of stem mechanical strength, corresponds to an alteration of a putative glucuronyltransferase with significant impacts on cell wall composition (Zhong et al. 2005). Relationships between phenolics, cell wall carbohydrates and tissue friability are likely to be new ways of breeding maize and forage for higher ingestibility, for which *Arabidopsis* (and rice) could be convenient model systems.

Based on the study of four maize RIL progenies, 24 genomic locations were found supporting QTL involved in maize cell wall digestibility (Ralph et al. 2004; INRA, ProMaïs, and Génoplante unpublished data). No clear putative candidate genes are publicly available for more than 50% of these cell wall digestibility QTL. Similarly, among the five locations comprising the greater part of the variation explained by QTL, only two had candidate genes (in maize bins 4.05 and 6.06), but none of these genes has been yet

validated. Genetic variation in cell wall digestibility of maize plants appears more likely to be related to more subtle mechanisms or traits than the variation of structural genes of the lignin pathway. The studies of mutants thus appears to be an inescapable tool towards demonstrating new genes involved in cell wall biogenesis and digestibility. However, a mutant phenotype is expressed in a particular genetic background with possible epistatic interactions that might be different from one genotype to another, as observed for the flowering time loci (Koornneef et al. 2004). Moreover, a knock-out gene could have a drastic effect that is not representative of the variation occurring naturally between lines. Correlatively, Nieminen et al. (2004) concluded that ''approaches based on natural variation during secondary development between various *Arabidopsis* accessions may also turn out to be an important avenue to exploit *Arabidopsis* in wood development research''. As a first QTL analysis for lignin content and cell wall digestibility in an *Arabidopsis* RIL progeny strengthened the floral stem of *Arabidopsis* as a model system in studies of forage grass stem digestibility (Barrière et al. 2005), natural variations in *Arabidopsis* should therefore be considered for investigation of genetic and molecular determinants of lignification and cell wall digestibility of forage plants. Numerous accessions of *A. thaliana* are now available, out of which 1,460 are referenced at the ABRC, one of the international stock centers, and more than 500 accessions are available at the INRA Versailles resource center (http://www.dbsgap.versailles.inra.fr/vnat/). Nested core collections maximizing genetic diversity with a limited number of accessions have been established based on single nucleotide polymorphism (SNP) markers and validated by observations of phenotypic traits (McKhann et al. 2004). The objective of this work was to characterize both a 24-accessions core collection and a larger collection for traits related to lignin content and cell wall digestibility in *A. thaliana* floral stems as a tool for further investigations of molecular determinant of lignification and cell wall digestibility in forage plants.

Materials and methods

Accessions were provided by the *A. thaliana* Genomic Resource Center at INRA Versailles (http://www.-ijpb.versailles.inra.fr/en/sgap/equipes/variabilite/crg/index.htm). Each accession is maintained as a Single Seed Descent line and originated from a seed stock obtained from the ABRC, the Nottingham *Arabidopsis* Stock Center or from natural populations (http://www.dbsgap.versailles.inra.fr/vnat/).

In the first experiment, genetic variation for cell wall digestibility related traits was investigated in the core collection of 24 *A. thaliana* accessions defined by McKhann et al. (2004) out of a larger collection of 265 accessions. Floral stems of each accession were produced in four different environmental conditions. Plants were sown at INRA Lusignan in a controlled growth chamber in May, 2003, and harvested at seed maturity in September of the same year (environment E1). Plants were also cropped in a greenhouse at INRA Lusignan, being sown in September, 2003, and harvested at seed maturity during March, 2004, at three successive dates according to their earliness (environment E2). In the growth chamber, the photoperiod was 8 h light/16 h dark, with temperatures equal to 20 and 16°C during day and night, respectively. Light intensity was equal to 160 μmol/m^2/s. In the greenhouse, light was natural lighting and during winter the temperature was controlled so it could not be lower than 5°C. Two and three replicates of each accession were sown in E1 and E2 environments, respectively. Plant samples were also available from two environmental conditions in greenhouses at INRA Versailles in 2002. In environment E3, one replicate of the 24 accessions of the core collection was cropped. In environment E4, one replicate of a selection of 17 accessions out of the 24 accessions of the core collection was also cropped. Cropping conditions ensured a homogeneous material for phenotypic analysis. The temperature was 23°C during the day/15°C at night, with a 16 h light/8 h dark photoperiod. Additional light was added according to natural day length giving 105 μE/m^2/s at the plant level. In both E3 and E4 environments, floral stems were also collected at seed maturity. Based on the true core collection with 24 genotypes as it was established from the larger collection of 265 accessions (McKhann et al. 2004), the genotype 180AV was missing in all experiments, and replaced by the reference accession 186AV (Col-0). Nevertheless, this set of 24 accessions was referred as the 24-accessions core collection. Analyses were not done for genotype 157AV and 200AV in the E1 environment, for genotypes 8AV, 62AV, 83AV 101AV, 157AV, 200AV in the E3 environment, and for genotype 200AV in the E4 environment due to a too low amount of floral stem dry matter (DM). Genotypes 101AV, 157AV, 163AV, 178AV, 252AV, 257AV, 266AV were missing in the E4 environment.

The second experiment was devoted to the study of cell wall digestibility related traits in two large sets of accessions. Plants were cropped at INRA Versailles in 2002 (environment E4, 194 accessions, out of which 17

accessions of the core collection were available) and 2004 (environment E5, 258 accessions), respectively. Only one replicate was cropped in both experiments, with 172 accessions common in each environment. Environmental conditions of cropping were similar as previously described for the E4 environment. Accessions of the core collection were present in the E4 environment as previously listed and only accessions 8AV and 92AV of the core collection were present in the E5 environment. Environments E4 and E5 gathered 280 accessions, out of which 239 accessions belonged to the larger collection of 265 accessions from which the investigated 24-accessions core collection was sampled.

Floral stem samples cleared of siliques were dried in a ventilated oven (65°C). Dry samples were then ground with a hammer mill to pass through a 1-mm screen for later analyses. Crude protein (CP, Dumas nitrogen × 6.25), soluble carbohydrates (SC, Lila 1977), neutral detergent fiber (NDF, which is an estimate of cell wall content, Goering and van Soest 1970), Klason lignin (KL, Dence and Lin 1992) and the in vitro dry matter digestibility (IVDMD, Aufrère and Michalet Doreau 1983) were estimated using near infrared reflectance spectroscopy (NIRS system 6500 spectrophotometer, with wavelengths spaced every 4 nm from 1,100 to 2,500 nm). Calibration equations had been previously developed at INRA Lusignan (Barrière et al. 2005), and calibration regressions were fitted and validated with laboratory analysis of 40 samples chosen on their spectral characteristics as representative of the whole available variation. Coefficients of determination between laboratory analysis and NIRS predictions, and standard errors of prediction, were 0.99 and 1.24 for NDF, 0.91 and 0.76 for KL, 0.99 and 0.90 for CP, 0.99 and 1.43 for IVDMD, respectively. Klason lignin was also expressed as a percentage of NDF (KL/NDF) because this compound is a constituent of the cell wall. Cell wall digestibility was investigated using two complementary estimates. According to Struik (1983) and Dolstra and Medema (1990), the in vitro NDF digestibility (IVNDFD) was computed assuming that the non-NDF part of the plant material was completely digestible [IVNDFD = 100 × (IVDMD–(100–NDF))/NDF]. NDF digestibility is underestimated by this trait, as 10–15% of the non-NDF part is not made soluble during the enzymatic attack (Jung et al. 1994). Cell wall digestibility was also investigated according to the DINAGZ trait of Argillier et al. (1995) and Barrière et al. (2003b), as the in vitro digestibility of the ''non-starch, non-soluble-carbohydrates, and non-crude-protein'' portion, assuming that starch, soluble carbohydrates, and crude protein were completely digestible [DINAGZ = 100 × (IVDMD–ST–SC–CP)/(100–ST–SC–CP)]. After measurements of a random sample, a null content of starch (ST, Ewers method, AFNOR, 1981, EEC ISO 10520.2) was considered for all investigated floral stems of *Arabidopsis* accessions. Because the DINAGZ trait was built for grass investigations, pectin content was not taken into account in the calculation. Using an alfalfa calibration giving NDF contents in *A. thaliana* samples very close to those regularly estimated ($r = 0.94$, with a bias only equal to 1.3%), an average neutral detergent soluble fiber (NDSF, Hall et al. 1997) content was then predicted in all *Arabidopsis* accession stems and used in the DINAGZ estimate in place of ST content. Relationships between cell wall traits and vegetative traits were investigated in E1 and E2 environments with measurements, at harvest stage, of stem number per plot, DM yield per plot, giving DM yield per stem (g/stem). Flowering date was considered at blooming of the majority of stems and recorded in environment E2 as days after sowing.

Variance analysis and mean estimates were performed for each environmental condition of the core collection cropping. Variance analysis was also investigated with data from all environmental conditions, with a model including an environment effect, a replicate nested in environment effect, a genotype effect, and a genotype × environment interaction effect. Means over environments were estimated from a similar model without interaction effects. Genotype stability across environments was investigated for each trait through the estimation of their ecovalence according to Wricke (1962) as $W_i = \sum_j (x_{ij} - x_{i.} - x_{.j} - x_{..})^2$ for genotype i, where $x_{i.}$, $x_{.j}$, and $x_{..}$ are genotype, environment, and general means, respectively. The higher is the ecovalence value, the less stable is the genotype across the environments. Ecovalences were investigated across environments in each subset of the genotypes with available data (22, 18, and 12 genotypes in two, three and four environments, respectively). Variance analysis and mean estimates were also investigated for the two overlapping large collections of accessions with a model including an environment effect and a genotype effect.

Results

24-Accessions core collection

Soluble carbohydrate contents were low in all genotypes with an average value close to 2%, and their variation was also low between genotypes, indicating a

good level of floral stem maturity (Table 1). The crude protein content in mature stems had an average value close to 9%, with higher values in the E2 greenhouse environment. NDF and lignin contents were higher in the E1 environment, with a correlative lower cell digestibility, especially when estimated with the IVNDFD trait.

No significant differences between genotypes were found in NDF content, partly due to the large variations were observed between replicates. Genotype × environment interactions were significant for all traits with significant genotype effects. However, mean squares of interactions were of lower importance than mean squares of genotype for pectin content, KL/NDF and cell wall digestibility traits. Genotype × environment interactions appeared to be of much higher importance than usually observed in forage plant experiments.

Based on genotype mean values, the correlation between IVNDFD and DINAGZ was $r = 0.65$, lower than usually observed in forage maize experiments. Correlations between IVNDFD and KL/NDF and between DINAGZ × KL/NDF were $r = -0.70$ and $r = -0.27$, respectively, the latter being lower than usually observed in forage maize experiments. IVNDFD was weakly correlated with NDF content ($r = -0.39$), and DINAGZ was even more weakly correlated ($r = -0.22$). IVNDFD was correlated more with stem DM enzymatic solubility than DINAGZ ($r = 0.82$ and 0.49, respectively). Stem DM enzymatic solubility was correlated well with NDF content (r = −0.86), just as NDF is a component with variable digestibility, whereas other components are highly or completely digestible.

Genotype mean estimates may be partly unreliable, as genotype × environment interactions were significant for most of the traits. Ecovalences then allowed separating genotypes according to their stability across environments (Table 2). The E3 and E4 environments appeared different from the E1 and E2 environments, and contributed more greatly to the observed genotype × environment interactions. However, no replicates were cropped in the E3 and E4 environments, and genotype values could thus be less accurately estimated. Genotype 257AV was seemingly the most interactive for cell wall digestibility according to the growing conditions, and explained at least 25% of the total ecovalences for IVNDFD and DINAGZ when considering two, three or four environments. Whatever the grouping of environments, four genotypes explained about 50% of IVNDFD ecovalence (257AV, 101AV, 178AV, 25AV) related to high ecovalence for IVDMD and/or NDF content. Similarly, four genotypes explained about 60% of DINAGZ ecovalences (257AV, 178AV, 163AV, 25AV) related to high IVDMD ecovalence (genotype 257AV) or high crude protein and/or soluble carbohydrate ecovalences. About 30% of KL/NDF ecovalence was induced by the genotype 166AV, each of the other genotypes individually inducing less than 5% of the total ecovalence.

Genotype 157AV had the lowest adjusted cell wall digestibility for the two IVNDFD and DINAGZ traits, but it was observed only in the E2 environment. However, this lowest value was observed despite the fact that this environment gave the highest average cell wall digestibility values. Genotypes 42AV, 224AV, and 8AV had low ecovalence values and low cell wall digestibility. Genotypes 224AV had a proportionally lower IVDNDF value than DINAGZ value. Conversely, genotypes 236AV, 162AV, 70AV, and 101AV had low ecovalence values and high cell wall digestibility. Genotype 83AV also had high cell wall digestibility values, with slightly higher ecovalences values. The well-known accession Col-0 (186AV) appeared

Table 1 Variance analysis and environmental means of cell wall traits in floral stems of 23 *A. thaliana* accessions out of a core collection of 24 accessions, plus the reference accession 186AV (Col-0), in four environments

	DM solubility	NDF	Klason lignin	Crude protein	Soluble carbohydrates	Pectins	KL/NDF	IVNDFD	DINAGZ
Mean environment 1	41.2	64.0	14.2	6.4	1.8	10.8	22.2	8.2	21.0
Mean environment 2	60.2	51.0	11.7	14.8	2.2	14.5	22.9	22.0	28.1
Mean environment 3	48.8	58.3	14.3	6.2	2.5	14.6	24.5	12.2	23.1
Mean environment 4	44.3	63.5	12.2	4.0	3.3	13.3	19.2	12.2	21.0
Mean of four environments	48.4	59.4	13.1	7.6	2.5	13.3	22.2	13.6	23.2
σ_r^2	9.3	7.9	0.5	8.1	0.5	0.5	0.8	4.1	2.2
Genotype MS	19.1*	11.4	2.4**	11.9	10.4**	3.7**	6.8**	22.0**	16.1**
Genotype × environment MS	14.0*	9.7	1.3**	10.1	4.5**	1.7**	2.8**	8.0**	7.0**

MS mean squares

*Significant at $P < 0.05$

**Significant at $P < 0.01$

Table 2 Mean value and ecovalences (*W*) across the four environmental E1, E2, E3, E4 conditions of cell wall traits in floral stems of 23 *A. thaliana* accessions out of a 24-accessions core collection, plus the reference accession 186AV (Columbia or Col-0)

Geno type	Accession name	DM solubility	NDF	Klason lignin	Crude protein	Soluble carbohydrates	Pectins	KL/NDF	IVNDFD	DINAGZ	W_{IVNDFD}	W_{DINAGZ}
8AV	Pyl-1	48.1	58.1	13.5	7.6	2.4	14.6	23.2	11.1	21.3	1.3	0.8
25AV	Jea	48.1	59.8	13.0	7.0	4.4	12.8	21.6	13.3	21.7	9.6	16.5
42AV	Bl-1 (N968)	48.0	59.3	13.3	8.0	3.6	13.2	22.6	12.5	21.4	3.6	3.1
62AV	St-0 (N1534)	50.9	56.2	13.0	8.7	2.5	14.4	23.2	13.6	23.7	3.2	0.1
70AV	Kn-0 (N1286)	49.5	59.4	12.4	8.4	1.1	13.4	21.1	15.5	25.1	0.5	1.5
83AV	Edi-0 (N1122)	51.6	57.5	12.4	7.3	2.2	14.2	21.5	16.2	25.4	4.1	3.7
91AV	Tsu-0 (N1564)	48.5	59.4	13.3	6.5	2.2	13.8	22.4	13.9	23.9	9.0	4.3
92AV	Stw-0 (N1538)	47.1	60.6	12.7	7.0	1.5	13.8	21.1	13.5	23.0	6.6	1.6
94AV	Mt-0 (N1380)	47.6	59.1	14.1	8.6	0.8	14.1	24.1	12.0	22.8	5.0	1.4
101AV	Ge-0 (N1186)	50.1	60.1	12.3	9.0	2.8	12.9	20.5	17.4	23.9	17.6	0.9
157AV	Ita-0 (N1244)	42.3	63.9	14.5	2.9	2.7	12.8	22.7	8.8	20.9	–	–
162AV	Ct-1 (N1094)	50.8	58.5	12.2	8.3	1.4	13.4	21.2	16.5	26.3	2.3	1.1
163AV	Can-0 (N1064)	49.1	59.1	13.0	8.9	3.3	13.2	22.1	14.3	22.1	0.8	10.5
166AV	Cvi-0 (N902)	48.7	59.5	13.1	10.9	0.9	11.2	22.3	14.2	25.5	6.3	8.0
172AV	Bur-0 (N1028)	48.8	58.7	13.9	8.8	3.3	12.9	23.8	13.1	22.5	1.6	5.3
178AV	Alc-0 (N1656)	49.5	58.3	12.9	8.9	2.8	12.9	22.1	14.2	23.5	11.8	8.7
200AV	Gre-0 (N1210)	48.8	59.3	12.8	7.3	5.5	13.2	21.8	14.3	20.6	–	–
215AV	Mh-1 (N1368)	49.0	58.6	13.3	8.0	1.4	14.1	22.9	13.3	23.7	8.0	2.6
224AV	Oy-0 (N1436)	46.2	59.4	14.4	7.5	1.3	12.5	24.4	9.8	23.5	7.7	2.6
236AV	Shahdara (N929)	49.4	59.7	12.3	8.1	0.8	13.1	20.8	16.2	26.0	6.2	0.8
252AV	Akita	45.7	61.8	13.6	5.0	1.8	12.8	22.0	12.2	23.7	9.6	0.3
257AV	Sakata	46.6	61.0	13.5	7.1	3.3	12.4	22.3	12.5	22.0	40.5	39.5
266AV	N13 (CS22491)	47.3	60.1	13.2	6.0	4.4	13.1	22.0	12.8	21.2	4.6	6.8
186AV	Col-0 (N1092)	50.3	57.5	12.5	7.4	4.1	13.9	21.7	14.3	22.3	4.3	8.3

Genotype numbers are INRA Versailles identification numbers, and accession names are given with the original stock center number

with a medium cell wall digestibility and a weak to medium level of interaction between environments. Similarly, genotypes 163AV and 166AV had medium cell wall digestibility values, but low ecovalence values only for IVNDFD. Because ecovalences for KL/NDF were not systematically high in genotypes with high cell wall digestibility ecovalences, variation in cell wall digestibility across environments was not likely related to variation in lignin content. However, genotype 166AV, with a very high KL/NDF ecovalence, also had (medium) high cell wall digestibility ecovalences, as it could be expected from the negative effect of lignin on digestibility.

In the E1 and E2 environments, genotype effects were significant for all vegetative traits investigated (Table 3). DM production was lower in growth chamber conditions than in the greenhouse, probably due to lower lighting and temperatures. In the E2 environment, in opposition to values observed in forage plants, correlations between flowering date and DM yield were not positive (negative with yield per plot and null with yield per stem). Correlations between flowering date and NDF or KL/NDF contents were close to zero (– 0.10 and – 0.11, respectively). No significant correlations were found between flowering date and cell wall digestibility. Correlations between yield per stem and cell wall digestibility ranged between – 0.09 and – 0.36, this later value being observed with IVNDFD in environment E1. Observed variation in cell wall digestibility or lignin content did not appear to be related to the investigated vegetative traits. Among accessions with low cell wall digestibility, genotypes 8AV, 157AV, and 224AV were early flowering, while genotype 42AV was late flowering (83, 86, 77, and 128 days after sowing, respectively). Similarly, among accessions of high cell wall digestibility, genotypes 70AV, 162AV, and 236AV were early flowering, while genotype101AV was late flowering (77, 77, 77, and 131 days after sowing, respectively).

280-Accessions collection

Variations for all investigated traits were significant at the $P < 0.01$ level. Ranges of variation for IVNDFD and DINAGZ cell wall digestibility were higher than in the 24-accessions core collection (Table 4). Even if results will need confirmation, due to the low number of replicates, accessions 295AV, 148AV, and 309AV could however be models for low cell wall digestibility. Similarly, accessions 83AV and 162AV, already found in the study of the core collection, and five accessions including 6AV and 20AV, could be models for high

Table 3 Cell wall and agronomic traits in floral stems of 23 *A. thaliana* accessions out of a core collection of 24 accessions, plus the reference accession 186 AV (Col-0) in growth chamber environment E1 and greenhouse environment E2

	KL/NDF	IVNDFD	DINAGZ	Stem number	DM yield	Yield per stem	Flowering date
Mean environment 1	22.2	8.2	21.0	11.7	1.2	0.10	–
Minimum environment 1	19.4	2.0	17.1	5.5	0.5	0.04	–
Maximum environment 1	25.3	15.4	24.6	13.0	2.5	0.21	–
σ_r^2 environment 1	0.4	1.2	1.2	1.4	0.4	0.003	–
Genotype MS environment 1	5.2**	17.4**	7.1**	4.8**	0.07**	0.001**	–
Mean environment 2	22.9	22.0	28.1	15.3	3.8	0.25	93.4
Minimum environment 2	21.1	17.3	25.3	5.3	1.6	0.13	77
Maximum environment 2	25.8	26.4	31.0	20.3	6.7	0.37	133
σ_r^2 environment 2	1.0	5.3	2.7	7.8	0.9	0.005	41.2
Genotype MS environment 2	4.4**	11.9**	7.6**	43.0**	5.0**	0.011*	1247.4**

MS mean squares

*Significant at $P < 0.05$

**Significant at $P < 0.01$

Table 4 Average means and values of genotypes with low and high cell wall digestibility traits, respectively, in a set of 280 *A. thaliana* accessions (environments E4 and E5), including the reference accession 186AV (Col-0)

Genotype	Accession name	DM solubility	NDF	Klason lignin	Crude protein	Soluble carbohydrates	Pectins	KL/NDF	IVNDFD	DINAGZ
280 Accessions	General means	44.6	62.2	13.3	4.7	2.5	13.4	21.4	11.0	21.5
280 Accessions	Minimum values	31.7	52.3	9.7	2.0	0.0	9.4	18.5	4.7	15.1
280 Accessions	Maximum values	58.5	71.7	16.4	10.8	11.7	20.2	24.5	21.2	27.8
295AV	Kil-0 (N1270)	31.7	71.7	16.2	2.4	1.2	10.2	22.4	4.7	16.3
148AV	Mr-0 (N1372)	37.1	66.3	14.2	5.2	1.6	12.2	21.3	5.0	16.6
269AV	N17 (CS22495)	41.1	63.0	14.2	3.6	7.0	10.9	22.5	6.4	17.0
172AV	Bur-0 (N1028)	37.5	67.4	16.4	2.6	4.7	9.7	24.5	7.2	19.2
309AV	Sgd-4	36.4	68.9	13.1	2.9	3.0	11.9	18.8	7.7	16.4
283AV	Ken (N8142)	43.7	61.6	12.7	3.4	8.9	13.0	20.7	8.5	15.1
186AV	Col-0 (N1092)	46.3	62.5	13.2	5.1	1.1	14.8	21.2	14.1	22.6
42AV	Bl-1 (N968)	45.1	62.4	13.8	5.2	5.8	10.9	22.2	12.0	21.5
236AV	Shahdara (N929)	42.5	64.9	12.5	3.1	0.2	12.7	19.4	11.4	24.0
27AV	Dam2	43.6	64.7	12.1	3.8	1.4	11.5	18.5	13.0	24.7
91AV	Tsu-0 (N1564)	47.9	60.3	12.2	2.4	2.6	13.1	20.2	13.8	27.0
114AV	Is-0 (N1240)	47.1	61.8	12.2	3.3	0.9	14.0	19.8	14.5	26.0
223AV	Or-0 (N1432)	51.7	56.9	11.5	5.5	0.8	16.4	20.2	15.0	25.6
162AV	Ct-1 (N1094)	46.7	62.8	11.9	3.7	0.7	14.8	19.1	15.1	24.5
20AV	Cle-3	51.1	58.0	12.0	5.7	1.2	16.1	20.5	15.5	24.7
6AV	Fer-5	50.6	58.4	12.7	7.5	0.8	15.8	22.1	15.9	24.7
83AV	Edi-0 (N1122)	53.6	55.8	11.6	7.7	1.7	13.2	20.5	17.0	27.8
119AV	Li-2 (N1312)	58.1	53.2	9.7	7.3	11.3	16.2	18.5	21.2	19.2

All traits were significant at $P < 0.01$

cell wall digestibility. Seven accessions had a higher IVNDFD than these later (14AV, 76AV, 77AV, 119AV, 260AV, 288AV, 276AV), but could not be considered because they were most likely still immature with high soluble carbohydrate contents (from 6.0 to 11.7%). Digestibility value of accession 82AV, with high IVNDFD and medium DINAGZ, but weaker soluble carbohydrate content (3.8%) than the seven other accessions, had to be confirmed. IVNDFD value of 236AV appeared a lightly lower in this experiment than in the environmental conditions (with replicates) of the core collection studies. Highlighting the fact that lignin content is not the only trait involved in cell wall digestibility, accessions were found with similar cell wall digestibility values, but different in lignin content, or the reverse. Similarly, because IVNDFD and DINAGZ are somewhat different estimates of cell wall digestibility, accessions were found with similar IVNDFD, but different DINAGZ, or the reverse (Table 5).

Based on 273 genotype mean values, excluding accessions with immature stems, the correlation

Table 5 Model accessions of *A. thaliana* with similar values for one out of three traits involved in cell wall lignification and digestibility, and variation in two other trait values in the 280 accessions (lignin content, IVNDFD and DINAGZ digestibility traits, respectively) studied in E4 and E5 environments

Accession names	Acession number	DM solubility	NDF	Klason lignin	Crude protein	Soluble carbohydrates	Pectins	KL/NDF	IVNDFD	DINAGZ
Similar KL/NDF										
RLD1	230AV	45.5	60.7	12.8	4.1	0.8	15.0	21.2	10.2	22.8
M7323S	179AV	39.7	64.0	13.7	3.7	2.1	11.5	21.3	5.6	20.2
Enkheim-T	197AV	49.1	58.1	12.3	4.5	1.4	15.9	21.3	12.3	24.0
Mir-0	216AV	42.3	64.4	13.8	4.5	4.8	11.2	21.4	10.3	19.4
Pyl-1	8AV	46.7	60.1	13.1	6.0	0.4	14.1	21.8	11.3	23.9
Similar IVNDFD										
Le-0	97AV	45.9	60.9	13.1	4.5	3.4	14.5	21.6	11.1	21.1
Gu-0	118AV	46.5	60.2	13.4	4.5	1.8	14.1	22.1	11.2	23.4
Ri-0	160AV	43.0	64.6	13.2	3.4	2.3	13.2	20.6	11.8	21.6
Rak-2	228AV	47.6	59.6	12.6	4.7	1.6	15.3	21.1	12.1	23.2
Hiroshima	254AV	43.7	63.5	13.2	5.1	6.3	11.5	20.7	11.3	18.3
Similar DINAGZ										
Sg-1	112AV	47.6	60.2	12.1	4.9	2.4	15.4	20.2	12.9	21.9
Ep-0	133AV	43.9	62.2	13.6	4.2	2.2	13.5	21.8	9.7	21.7
Cvi-3	302AV	40.8	63.2	13.6	3.3	1.2	12.0	21.5	6.3	22.1
Stw-0	92AV	42.9	64.5	12.8	3.9	1.2	13.0	19.8	11.4	22.3
Gie-0	135AV	43.4	62.3	14.0	4.3	1.4	13.0	22.5	9.1	22.3
References										
Col-0	186AV	46.3	62.5	13.2	5.1	1.1	14.8	21.2	14.1	22.6
WS	244AV	42.6	59.7	13.1	6.0	1.1	15.2	22.0	9.8	21.5
Bay-0	41AV	43.0	63.4	13.8	3.5	0.9	12.8	21.6	10.1	23.1
Shahdara	236AV	42.5	64.9	12.5	3.1	0.2	12.7	19.4	11.4	24.0

between IVNDFD and DINAGZ was low, with $r = 0.51$, a little lower than in the core collection, but probably underestimated due to the lack of replicates. Correlations between IVNDFD and KL/NDF and between DINAGZ and KL/NDF were $r = -0.44$ and $r = -0.27$, respectively, also lower than usually observed in forage maize experiments. Stem DM enzymatic solubility was found to be well correlated with NDF content, as it is the component with variable digestibility ($r = -0.93$). However, IVNDFD and DINAGZ were weakly correlated with NDF content ($r = -0.45$ and -0.39, respectively), as it was observed in the core collection. Also similarly to the core collection, IVNDFD was correlated more with enzymatic solubility than DINAGZ ($r = 0.74$ and 0.50, respectively).

Discussion and conclusion

A great deal of research has proven that there is a large genetic variation in the cell wall digestibility of both inbred lines and maize hybrids (Dolstra et al. 1993; Lundvall et al. 1994; Argillier et al. 2000; Méchin et al. 2000; Fontaine et al. 2003; Barrière et al. 2004b). In early maize lines, cell wall digestibility ranged between 24 and 40% when estimated with IVNDFD, and between 43 and 61% when estimated with DINAGZ. Genetic variation in in sacco IVNDFD cell wall digestibility of rice straw ranged from 21.2 to 31.1% (Abou-el-Enin et al. 1999). Compared to a dicotyledonous plant, average *A. thaliana* stem IVNDFD was a little lower than the average alfalfa IVNDFD (about 15%) observed within or between cultivars (Julier et al. 2000). Even if mean cell wall digestibility was much lower in *Arabidopsis* accessions than in grasses, a greater or an at least similar range of variation was observed for both IVNDFD and DINAGZ traits, encouraging its use as a model system. In maize, genotype × environment interactions for cell wall digestibility were very often small compared to principal effects (Méchin et al. 2000; Fontaine et al. 2003; Barrière et al. 2004b). For maize cell wall traits, the mean square of the genotype effect was thus often ten times higher than the interaction mean square, whereas it was found to be only three times higher in *Arabidopsis*. Stability across environment of plant yield, earliness and standability are traits taken into account by maize breeders, that could contribute to the stability of cell wall digestibility. Genotype × environment interactions for lignin content in the cell wall were also considerably higher in *Arabidopsis* than in maize.

As it was already investigated in the *Arabidopsis* Bay-0 × Shahdara RIL progeny (Barrière et al. 2005), QTL analysis for cell wall digestibility traits should be

performed in different other RIL progenies, primarily those currently developed at INRA Versailles. Out of the Versailles 24-accessions core collection of *Arabidopsis*, genotypes 157AV and 224AV, both with low cell wall digestibility, and genotypes 162AV and 236AV, both with high cell wall digestibility, are involved in RIL progeny creation (http://www.dbsgap.versailles.inra.fr/vnat/). RIL progeny 20RV, corresponding to the cross of 186AV (Col-0) as male parent with 172AV and composed of about 350 lines, is mapped and available. The progenies of crosses of Col-0 with the accessions 162AV, 166AV, 180AV, and 236AV will certainly be available in 2006. These four RIL progenies may be especially put forward for consideration as models for further investigation of the genetic and molecular determinants of plant cell wall digestibility. Moreover, RIL populations with 25AV, 157AV, and 224AV, each comprising about 400 lines, will soon be mapped. However, it could be suggested to create RIL progenies in crosses with accessions with extreme values for cell wall digestibility related traits (Table 4), such as 295AV, 283AV, 269AV for low cell wall digestibility, and 83AV for high cell wall digestibility, but also 236AV and 162AV, with high cell wall digestibility and which are already involved in RIL production with crosses to Col-0. Systematic QTL analysis of lignification and cell wall digestibility traits in floral stems of several available or developed Arabidopsis RIL progenies should give a large overview of genes involved in these pathways that could not be so easily and efficiently obtained elsewhere than in such a model system. The understanding of traits involved in cell wall digestibility can also be based on comparisons of accessions with similar values in one trait (i.e., DINAGZ), but with different values in other traits (i.e., LK/NDF, IVNDFD), and reciprocally (Table 5). The comparison of 302AV and 112AV, with similar DINAGZ and lignin content, but double IVNDFD, and the comparison of 118AV and 254AV with similar IVNDFD, but different DINAGZ, could give information on the non-NDF portion possibly involved in cell wall digestibility variation. The comparison of 179AV and 197AV, with equal lignin content, but greatly different IVNDFD or DINAGZ cell wall digestibility could give information on traits involved in cell wall digestibility at a given lignin content.

A. thaliana is currently used successfully as a model system for studying quantitative traits of interest in widely grown crops. Genetic dissection of crop earliness is largely based on studies of the regulation of the plant time to flower in *A. thaliana* (Komeda 2004; Kramer and Hall 2005; Parcy 2005; Quesada et al. 2005). Genetic dissection of grain yield is also successfully investigated in *A. thaliana* by targeting analysis of its physiological and developmental constituents (Van Camp 2005). *A. thaliana* is an essential tool in investigations fatty acid biosynthesis (Murphy 1999; Ohlrogge et al. 2000) and storage in seeds, including QTL analyses (Hobbs et al. 2004). QTL for nitrogen use efficiency, which is one of the most important traits limiting plant growth, were shown by Loudet et al. (2003) in the Bay-0 × Shadara RIL progeny. Correlatively, QTL controlling root growth, which is often a limiting factor of water and nitrogen uptakes, were investigated in the same RIL progeny (Loudet et al. 2005), allowing further fine-mapping and cloning of genes of kinds yet unknown. Heterosis is the foundation of improvements in maize and allogamous plants, but its underlying determinants are still unknown. According to the results of Barth et al. (2003), *A. thaliana* also expressed a great heterosis for biomass yield, a trait of major interest in forage crop breeding, and could thus be a model species for investigating molecular basis of heterosis. Similarly, gene expression was compared in *A. thaliana* lines and hybrids allowing genetic analyses of dominance and heterosis (Vuylsteke et al. 2005). The extent of epistasis for quantitative traits was also investigated in *A. thaliana* RIL progenies (Malmberg et al. 2005). Based on tagged mutant or deregulated gene investigations, many studies have undoubtedly proven that *A. thaliana* was a powerful tool in finding genes involved in cell wall biosynthesis and lignification of both dicotyledons and monocotyledon plants. Because large variation in cell wall digestibility was demonstrated between *A. thaliana* accessions, natural variation between accessions should now be considered as an available complementary way to understand the mechanisms involved in forage plant cell wall digestibility. The range of cell wall digestibility between *A. thaliana* accessions is of a similar or greater magnitude than what was observed between maize lines of high and low cell wall digestibility. QTL analysis in progenies of *A. thaliana* accessions with divergent cell wall digestibility related traits will therefore allow the discovery of unsuspected mechanisms and genes involved in grass feeding value. Moreover, allelic sequencing of candidate genes and SNP studies could also be investigated as phenotypic values of accessions are available (or attainable) for large collections with significant variations in cell wall digestibility related traits. Another powerful approach would be based on comparative kinetics of gene expression in floral stems of accessions with contrasted cell wall digestibility or lignification that could be based on the CATMA microarray (Complete Arabidopsis Transcriptome MicroArray, http://www.catma.org).

Ehlting et al. (2005) thus identified a set of candidate genes for lignin biosynthesis and fiber differentiation according to profile global changes in gene expression along the developmental gradient of stem maturation. Significant original knowledge on still unknown genes involved in cell wall traits related to cell wall feeding value will therefore arise from *A. thaliana* studies with valuable applications in forage maize and grass plants, even if specific works will have to be developed on grasses, primarily maize and rice.

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