Genetic analysis in recombinant inbred lines of early dent forage maize. II-QTL mapping for cell wall constituents and cell wall digestibility from per se value and top cross experiments

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Maydica 47 (2002): 9-20

GENETIC ANALYSIS IN RECOMBINANT INBRED LINES OF EARLY DENT FORAGE MAIZE. II – QTL MAPPING FOR CELL WALL CONSTITUENTS AND CELL WALL DIGESTIBILITY FROM *PER SE* VALUE AND TOP CROSS EXPERIMENTS

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Received October 3, 2001

ABSTRACT - Quantitative trait loci (QTL) affecting feeding value traits in forage maize were evidenced and mapped in a RIL (Recombinant Inbred Lines) progeny from the cross between the two early dent lines of contrasted cell wall digestibility F288 and F271. Traits studied were cell wall content and composition, whole plant digestibility and cell wall digestibility. Field evaluation was performed among a set of 131 RILs studied *per se* (3 years, 3 locations in year 1, 2 locations for the other two years, 3 replicates per location), and after top crossing with the early flint line F286 (2 years, 3 locations per year, 3 replicates per location). Heterosis was considered as the difference between hybrid (RIL x F286) value and average value of the parents (RIL and F286 *per se*). The genotypic effects were highly significant and always greater than genotype x environment interactions. Transgressive lines were observed for digestibility traits only towards higher values in RILs *per se*, and only towards lower values in top cross. A map was achieved with 108 SSR markers, chosen to cover the entire genome. However, large monomorphic areas were found because the two parental lines were both related to line Co125. Putative QTL were identified through composite interval mapping, and are given for LOD values higher than 2.0. Additive x additive epistatic effects were investigated. QTL observed in RILs *per se* were logically more numerous than QTL found in top cross, but some QTL found in top cross were not found in RILs *per se*. QTL involved in heterosis for cell wall content or digestibility were also observed. Five major clusters of QTL were found for digestibility and lignification traits. In descending order for their LOD values or percentage of explained phenotypic variation, the clustered QTLs were located. in bins 6.06, 3.05, 2.08, 6.01 and 9.02. QTL for NDF, Hcell/NDF and Cell/NDF were also found in bins 2.08 and 6.06. QTL greatly involved in heterotic effects, both for digestibility traits and lignin content, were observed in bins 5.05 and 8.05. Significant simultaneous improvement of top cross yield, earliness, protein content and digestibility was proven to be possible by MAS based on the high yielding RIL n° 143 and five other RILs, sources of favorable alleles for the respective traits under breeding.

KEY WORDS: *Zea mays* L.; Silage maize; QTL analysis; Yield; Digestibility, Cell wall; Lignin; Cellulose; Hemicellulose.

ABBREVIATIONS

NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
KL	Klason Lignin
Hcell	Hemicellulose
Cell	Cellulose
CP	Crude Protein
SC	Soluble Carbohydrates
ST	Starch
IVDMD	*In Vitro* Dry Matter Digestibility
IVNDFD	*In Vitro* NDF Digestibility
DINAG	(IVDNSC) *In Vitro* Digestibility of the "Non-(Starch and soluble Carbohydrates)" part
DINAGZ	*In Vitro* Digestibility of the "Non-(Starch, soluble Carbohydrates and crude Protein)" part
SNDFD	*In vitro* (Sheep) estimated NDF Digestibility

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INTRODUCTION

The extensive use of the whole maize plant for dairy and meat cattle feeding, especially as silage, is relatively recent, despite the fact that as early as 1791, A.A. PARMENTIER had observed that "cows eat maize forage greedily, and it makes them yield a lot of milk". The first maize silage was made in France in 1852 by A. GOFFART (C. DEMARQUILLY, com pers), but the surface area of maize cropped for silage making began to increase significantly only one century later. This development was especially extensive between 1973 and 1983, when the surface area increased from 1 to 3 million hectares in the European Union. Silage maize is now the most important annual forage crop in the European Union,

predominantly in northern Europe, with an area close to 3.5 million hectares. Thanks to its high, regular yield, good, consistent energy content, digestibility and ingestibility, the maize plant is essential to maintain the economic and technical viability of dairy cattle breeding.

Maize bred for grain produce was first used as forage maize, but it was later clearly established that a good grain maize was not best suited for forage (BARRIÈRE *et al.*, 1997a, b). Genetic variation for *in vivo* whole plant and cell wall digestibility in silage maize has been reported in different studies (review by BARRIÈRE *et al.*, 1997a), showing both the feasibility and the necessity of improving this trait in forage maize. Forage maize energy value and digestibility were proven in these animal tests to be almost entirely dependent on cell wall digestibility and starch concentration, two traits that are not genetically linked. From experiments with cattle (BARRIÈRE *et al.*, 1997a), the optimum starch content in maize silage was later established to be close to 27 to 30%, depending on the quantity of concentrates in the diet. As a consequence, the essential target for maize breeders should be the improvement of cell wall digestibility, without forgetting important agronomic traits such as yield, earliness with respect to cropping areas, lodging tolerance, and tolerance to dry and cold conditions.

Genetic variation for *in vitro* digestibility of the whole plant has been reported in numerous studies over the past 25 years using rumen fluid or enzymatic solutions. More recently, it was largely established that enzymatic solubility, but also quality traits such as starch, cell wall or lignin content, could be accurately predicted by near infra red spectroscopy (NIRS) for routine analysis of breeding materials (review by BARRIÈRE *et al.*, 1997a). Even if the question is still debatable, it seemed of interest for the purpose of breeding and molecular approaches to take the starch content of the plant directly into account, and to use an *in vitro* criterion of cell wall digestibility, rather than a criterion of the resulting whole plant digestibility. STRUIK (1983), and DOLSTRA and MEDEMA (1990), proposed computing a cell wall digestibility index, assuming that the whole non cell wall part of the plant was completely digestible. ARGILLIER *et al.* (1995) proposed taking into consideration the non starch, non soluble carbohydrate part of the plant, assuming that these two constituents were completely digestible.

With the development of molecular markers, especially SSR, allowing the achievement of dense linkage maps, it is now possible to more accurately investigate the genetic and molecular determinants of complex traits, dissected into their underlying QTL, and then to investigate their colocalisation with genes or EST. Until now, QTL analysis for agronomic and quality traits in forage maize have been reported in few studies. LÜBBERSTEDT *et al.* (1997a, b and 1998) published the first QTL analysis devoted both to whole plant agronomic and quality traits, investigating QTL affecting whole plant digestibility. MÉCHIN *et al.* (2001) gave data on QTL related to cell wall digestibility, in a RIL family whose parents had not been chosen for their contrasted cell wall digestibility.

A recombinant inbred line (RIL) family was developed at Inra Lusignan (France) from the cross between two inbred lines differing greatly in cell wall digestibility. In a companion paper, results were given for agronomic traits (BARRIÈRE *et al.*, 2002). Genetic variation and QTL analysis were investigated in the present study, after *per se* and top-crossed experiments, in order to investigate the genetic basis of traits related to digestibility and lignification in forage maize.

MATERIAL AND METHODS

Plant material

RILs were developed at INRA Lusignan (France) by single seed descent from the cross between the elite inbreed early dent line F271, originating from a pedigree breeding in the cross Co125 x W103, and the early dent line F288, originating from a pedigree breeding in the cross F244 x F252. Because F252 is a progeny of a single cross hybrid with Co125, some consanguinity is expected between F271 and F288, more or less exacerbated by the selection for grain yield and earliness (BARRIÈRE *et al.*, 2002). F271 is more susceptible to lodging than F288, but gives earlier and higher yield hybrids than F288. Above all, F271 and F288 were proven to have contrasting values for cell wall digestibility, both for *per se* value and combining ability value (ARGILLIER *et al.*, 1995, 2000). Furthermore, this RIL family was specifically developed for studies of quality traits in forage maize. A set of 135 RILs was evaluated on a *per se* value basis, and top crossed with F286, which is an early flint elite line with a high cell wall digestibility (at least 2 percent points more than F288 in *per se* value), a low lignin content in the cell wall (1.5 percent points less than F288 in *per se* value), and a good combining ability value with early dent lines.

Field experiments, and forage quality evaluation

Field experiments were carried out in 2 locations over 3 years (1998, 1999 and 2000) for RIL *per se*, at Lusignan (Vienne), Le Pin (Orne), with an extra location at Rennes (Ile et Vilaine) in 1998. Field experiments were carried out in 3 locations over 2 years (1999 and 2000) for top cross, at Lusignan (Vienne), Le Pin (Orne) and Mons (Somme). Each year, top cross and RIL *per se*

(135 RIL), and both parents, were evaluated in generalized alpha-lattice designs with, in each location, three replicates for the tested RIL and nine replicates for the parents. Each experimental plot was a 5.2 m long single row of 37 plants. Row spacing was 0.75 m, and the resulting density was 95,000 plants/ha. Irrigation was applied in Lusignan during summer to prevent water stress. At the silage harvest stage [about 30 to 35% of dry matter (DM)], the plots were machine-harvested with a forage chopper. A representative sample of 1 kg chopped material per plot was collected.

Whole plant samples were dried in an 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) (Kjeldahl nitrogen x 6.25), starch (ST) (AFNOR, 1981, Ewers method, EEC ISO 10520.2), soluble carbohydrates (SC) (LILA, 1977), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) (GOERING and VAN SOEST, 1970), Klason lignin (KL) (Dence et 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 1100 to 2500 nm). Calibration equations were provided by SHB Libramont (Belgium), and calibration regressions were validated with laboratory analysis of 40 samples. Coefficients of determination between laboratory analysis and NIRS predictions and standard errors of prediction were respectively 0.92 and 0.35 for crude protein content, and 0.97 and 1.67 for starch content, 0.93 and 1.19 for soluble carbohydrate content, 0.93 and 1.77 for NDF, 0.95 and 1.13 for ADF, 0.83 and 0.36 for ADL, 0.79 and 0.77 for Klason lignin, and 0.88 and 1.89 for IVDMD. Hemicellulose and cellulose were then estimated respectively as NDF – ADF and ADF – ADL. Because these compounds are constituent of the cell wall, hemicellulose, cellulose, ADL and Klason lignin were expressed as percentage of NDF (respectively Hcell/NDF, Cell/NDF, ADL/NDF, KL/NDF).

Cell wall digestibility was investigated using three different 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 plant material was completely digestible. According to ARGILLIER *et al.* (1995a), estimates of the *in vitro* digestibility of the "non starch and non soluble carbohydrates" part (DINAG, or English acronym IVDNSC) could be computed assuming that starch and soluble carbohydrates were completely digestible. A modified DINAG criterion, namely DINAGZ, was estimated in a similar way as DINAG and used here, after adding crude protein to the completely digestible constituents (BARRIÈRE and EMILE, 2001). The formula were

IVNDFD = 100 x (IVDMD – (100 – NDF)) / NDF

DINAG = 100 x (IVDMD – ST – SC) / (100 – ST – SC)

DINAGZ = 100 x (IVDMD – ST – SC – CP) / (100 – ST – SC – CP)

In vivo NDF digestibility (SNDFD) was estimated according to the best multi-linear regression obtained by the INRA – Pro-Maïs network (unpublished, 2000), on the average value of 136 hybrids studied both for the *in vivo* digestibility with sheep in digestibility crates and the *in vitro* characteristics of green forage (406 mini-silos and elementary measurements, for 3 years).

SNDFD = 1.615 + 1.985 CP + 0.471 DINAGZ + 0.372 IVNDFD
(r^2 = 0.63, rsc = 3.1).

This regression was only carried out on hybrids, as no inbred lines were used in the *in vivo* measurements.

Data analysis

Analyses of variance were first carried out following the standard procedure of a fixed model with genotype, environment (= year-location), block, sub-block and interaction effects, as

$$Y_{ijkl} = \mu + E_j + B_k.E_j + SB_l.B_k.E_j + G_i + G_i.E_j + e_{ijkl}$$

with Y_{ijkl} = observed value for a given trait, μ = grand mean, E_j = environment effect, $B_k.E_j$ = block within environment effect, $SB_l.B_k.E_j$ = sub-block within block and environment effect, G_i = genotype effect, $G_i.E_j$ = genotype * environment interaction, and ε_{ijkl} = residual error. The variance of genetic effects σ_g^2, genotype x year-location interactions σ_{gxe}^2, and random error σ_r^2, were then estimated in accordance with the standard procedure of a mixed model with random genotype and genotype x year-location interaction effects, using the SAS statistical package (SAS, 1989, with "varcomp" procedure and a restricted maximum likelihood method). As genotype x year-location interactions were always low, broad-sense heritabilities (plot basis, n plot per genotype) were estimated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_r^2/n)$ after pooling the variance of genotype x year-location interactions with the variance of random error. Phenotypic correlation between RIL *per se* and top cross were computed on the mean basis over all available years and locations. Phenotypic correlation between RILs *per se* and top cross were computed on the mean basis over all available environments. Heterosis, considered as the difference between hybrid value and average value of the parents, on the mean basis over all environments, was computed for the i^{th} RIL as $H_i = [TC_i - (RIL_i + F286)/2]$ for each of the investigated traits, where TC_i is the average top cross value of the i^{th} RIL, RIL_i is its *per se* value, and F286 is the *per se* value of the tester line F286. However, for a given trait, positions, effects, and percentages of phenotypic variance of QTL observed for heterosis did not depend on the value observed in F286, but only on the difference $[TC_i - (RIL_i)/2]$, as [– (F286)/2] was a constant.

Molecular marker data and development of the linkage map

In accordance with their bin location, 341 simple sequence repeat (SSR) markers were chosen in the maize database (Maize DB, www.agron-missouri.edu) throughout the genome. Out of these markers, 107 giving different banding patterns in the two parental lines F271 and F288 were successfully used on 131 RILs. One additional SSR marker on chromosome 9 was made in Inra Lusignan (lus1). Some chromosomal areas remained unmarked, most probably due to the consanguinity between F271 and F288 (BARRIÈRE *et al.*, 2002). DNA extraction was performed in accordance with the procedure of SAGHAI-MAROOF *et al.* (1984), on leaf tissue sampled from a 10 day old, 3 visible leaf plant. Primer pairs were synthesised by Isoprim (Toulouse, France). PCR was performed, according to the SSR protocol of the "INRA maize microsatellites users" network (BARRIÈRE *et al.*, 2002). The linkage map was developed using Mapmaker (version 3.0b, LINCOLN *et al.*, 1992).

QTL identification

As previously described in BARRIÈRE *et al.* (2002), QTL mapping was based on means of 131 RILs *per se* and top cross values over years and locations, using the method of Composite Interval Mapping [CIM, ZENG (1994)] implemented in the PLABQTL computer package (UTZ and MELCHINGER, 1995 and 1996). PLABQTL uses the regression method (HALEY and KNOTT, 1992) in combination with selected markers as cofactors. Cofactors were selected by stepwise regression (option cov SELECT) with an "F-to-enter" and an "F-to-delete" value of 7. This F value was chosen to keep markers significant at the 1% level. For all analyses, a LOD

TABLE 1 - *Estimated genetic and environmental components of variance, mean, maximum, and minimum performances of F288 x F271 RIL progenies in per se and top cross experiments (** = significant at P < 0.001).*

RIL *per se*	σ_g^2	σ_{gxe}^2	σ_r^2	var(σ_g^2)	mean	mini	maxi	F271	F288
NDF	3.24**	1.41	3.30	0.1988	45.9	41.4	51.5	47.6	46.3
Hcell/NDF	1.11**	0.32	0.73	0.0215	42.4	39.0	44.7	40.7	44.2
Cell/NDF	0.73**	0.25	0.54	0.0097	51.3	49.6	54.2	51.7	50.4
ADL/NDF	0.25**	0.06	0.11	0.0011	6.2	4.8	7.8	7.6	5.4
KL/NDF	0.96**	0.18	0.49	0.0154	14.3	12.2	17.5	16.1	13.0
IVDMD	2.92**	1.16	1.49	0.1520	69.3	63.9	73.4	63.5	70.5
DINAGZ	4.52**	1.29	1.43	0.3443	51.3	45.7	55.7	43.6	53.8
IVNDFD	9.50**	1.83	2.80	1.4797	33.1	25.4	39.0	23.3	36.9
RIL x F286	σ_g^2	σ_{gxe}^2	σ_r^2	var(σ_g^2)	mean	mini	maxi	F271 x F286	F288 x F286
NDF	0.95**	0.48	3.83	0.0246	41.9	39.1	44.0	40.4	43.0
Hcell/NDF	0.21**	0.03	0.90	0.0011	42.2	40.8	43.3	41.6	42.5
Cell/NDF	0.15**	0.03	0.65	0.00059	51.4	50.5	52.7	51.4	51.5
ADL/NDF	0.04**	0.01	0.09	0.00003	6.4	5.0	6.9	6.9	6.0
KL/NDF	0.13**	0.02	0.60	0.00045	15.1	14.1	16.8	15.7	14.9
IVDMD	0.58**	0.27	2.02	0.0086	71.2	69.4	73.1	71.3	71.4
DINAGZ	0.57**	0.17	1.57	0.0072	50.3	48.0	52.0	48.4	52.0
IVNDFD	1.17**	0.29	2.60	0.0286	31.3	27.9	33.4	29.1	33.4
SNDFD	0.63**	0.15	1.47	0.0085	51.7	48.9	53.2	49.8	53.6

threshold of 2.0 was used, yielding an individual type I error rate close to 0.25% and an experimentwise error rate of 30% [result obtained by the permutation test method of CHURCHILL and DOERGE (1994)] suitable for the biological interpretation of QTLs and linkage patterns. LOD thresholds equal to respectively 2.7, 3.0 and 4.0 allowed an experimentwise error rate respectively close to 10.5 and 1%. QTL positions were estimated where the LOD score reached its maximum in the region under consideration. A LOD support interval was constructed for each QTL (LANDER and BOTSTEIN, 1989). As the question of the interval support is not fully resolved in the case of CIM, the LOD support intervals must be considered as underestimates. QTL with more than 20 cM separating support intervals were considered to be different. The percentage of phenotypic variance ascribed to an individual QTL is estimated in PLABQTL with the approximate standard error of KENDALL and STUART (1961). The additive effects of QTL were estimated as half the difference between the phenotypic values of the respective homozygotes.

RESULTS

Map and consanguinity

Detailed results were previously given by BARRIÈRE *et al.* (2002). The map agrees with other maps previously published and with results available in the maize database (MaizeDB, www.agron.missouri.edu). However, some large areas were monomorphic, because F271 and F288 both had line Co125 in their pedigree. Chromosome 10 thus appeared very similar between F271 and F288, and polymorphous markers were found only in bins 10.06 and 10.07. Large monomorphic areas, more than 60 cM long, were also found in chromosomes 1 (one area) 2 (one area), 5 (one area), 7 (three areas) and 9 (two areas). Consanguinity between F271 and F288 lines was thus found close to 2.5/8, more than twice the expected 1/8 value. This showed that the breeding effort probably favored Co125 alleles. As a consequence, QTL mapping in the F288 x F271 RIL progeny could only apply to 70 to 75% of the maize genome. However, these areas are strongly involved in the feeding value of forage maize because large differences were observed for cell wall digestibility between these two early dent lines.

Mean and genetic variation analysis

Genotype effects were highly significant for all investigated traits ($P < 0.001$), and much higher than genotype x environment interaction effects in RILs *per se*, but only higher in top cross. Genetic variances were highly significant for all studied traits in RILs *per se* and top cross experiments (Table 1). F288 and F271 effectively provided RIL progenies differing largely in digestibility related traits. However, in top cross experiments, genetic variation was low for the two lignin content traits. Transgressive segregations were observed for all traits related to NDF and NDF composition both in RILs *per se* and top cross experiments, and both for lower and higher values. But for each of the investigated digestibil-

TABLE 2 - *Putative QTLs identified for NDF content, hemicellulose content and cellulose content from 131 RILs in F288 x F271, experimented in 1998, 99, 00 (7 locations) for their per se value, and experimented in 1999, 00 (6 locations) for their top cross value (QTLs were italicized when LOD were lower than 2.7, add is the estimated additive effect of the QTL, and favorable parental line (fav line) increased the value of the trait).*

Evaluation type	trait	chr-pos	bin	support interval	lod	R^2	add	fav line
top-cross	*NDF*	*1-248*	*1.09/10*	*234-270*	*2.5*	*8.5*	*0.39*	*F271*
	NDF	*6-198*	*6.08*	*180-212*	*2.2*	*7.4*	*0.43*	*F271*
	Hcell/NDF	*2-76*	*2.04*	*66-84*	*2.0*	*6.7*	*0.15*	*F288*
	Hcell/NDF	2-134	2.08	114-146	2.8	9.7	0.18	F288
	Hcell/NDF	6-168	6.06	154-194	7.9	24.2	0.36	F288
	Cell/NDF	2-76	2.04	66-84	3.4	11.2	0.17	F271
	Cell/NDF	6-166	6.06	150-194	4.7	15.3	0.26	F271
RIL *per se*	*NDF*	*1-96*	*1.02*	*86-118*	*2.6*	*8.9*	*0.57*	*F288*
	NDF	3-56	3.03	42-70	5.1	16.5	1.02	F271
	NDF	9-146	9.05/06	138-186	4.6	14.9	0.81	F271
	Hcell/NDF	2-74	2.04	64-82	3.4	11.3	0.34	F288
	Hcell/NDF	4-38	4.05	28-48	3.8	12.9	0.37	F288
	Hcell/NDF	6-168	6.06	154-192	9.2	27.7	0.75	F288
	Hcell/NDF	9-114	9.02	104-126	4.9	15.8	0.45	F271
	Cell/NDF	*1-134*	*1.04*	*126-148*	*2.4*	*7.9*	*0.23*	*F271*
	Cell/NDF	2-68	2.03	64-82	3.6	12.0	0.30	F271
	Cell/NDF	2-100	2.06	88-116	3.1	10.4	0.34	F271
	Cell/NDF	4-42	4.05	32-48	3.7	12.7	0.27	F271
	Cell/NDF	6-166	6.06	150-190	5.5	17.5	0.70	F271
	Cell/NDF	*9-156*	*9.05/06*	*144-190*	*2.3*	*7.8*	*0.22*	*F288*
heterosis	*NDF*	*3-142*	*3.05*	*132-152*	*2.6*	*8.6*	*0.33*	*F288*
	NDF	*8-24*	*8.04*	*14-40*	*2.0*	*6.8*	*0.28*	*F271*
	Hcell/NDF	*3-144*	*3.06*	*134-170*	*2.1*	*7.0*	*0.11*	*F288*
	Hcell/NDF	*8-20*	*8.04*	*14-32*	*2.5*	*8.5*	*0.11*	*F271*
	Cell/NDF	*3-172*	*3.08*	*158-178*	*2.1*	*7.0*	*0.08*	*F288*

ity traits, transgressive segregations were observed in RILs *per se* only towards higher values and no line was lower than F271, whereas in top cross, no line was higher than F288, and transgressive segregations were then observed only for lower values. F271 was 7 percent points lower than F288 in lines *per se* value for IVDMD, but only 0.1 percent lower (non significant) in top cross with F286, which is of good digestibility. However, for DINAGZ, the difference between F271 and F288 reached 10.2 percent points in lines *per se*, and was still equal to 3.6 percent points in top cross (and respectively 13.6 and 3.8 percent points for IVNDFD). Possibly, a flint line with a lower cell wall digestibility than in F286, such as F2 which is 2 percent points lower than F288, would have allowed a greater genetic variation between top crossed RILs.

Broad-sense heritabilities, estimated on a plot basis, were very high for each investigated trait (h^2>0.77). In both RILs *per se* and top cross, the genetic correlation between the two cell wall digestibility estimates (DINAGZ and IVNDFD) was high and of similar value (r_g = 0.88). In both RILs *per se* and top cross experiment, the genetic correlations between cell wall digestibilities and ADL/NDF were very high and ranged between –0.88 and –0.93. But correlations were lower when KL/NDF was considered instead of ADL/NDF, and, in this case, correlations were also lower for DINAGZ (close to 0.6) than for IVNDFD (close to 0.8). Correlation between the two lignification traits (ADL/NDF and KL/NDF) was lower in top cross (r_g = 0.65) than in RILs (r_g = 0.71). ADL/NDF and KL/NDF did not measure the same part of the lignin content in

TABLE 3 - *Putative QTLs identified for lignification traits from 131 RILs in F288 x F271, experimented in 1998, 99, 00 (7 locations) for their per se value, and experimented in 1999, 00 (6 locations) for their top cross value (QTLs were italicized when LOD were lower than 2.7, add is the estimated additive effect of the QTL, and favorable parental line (fav line) increased the feeding value of the trait, ie decreased lignin content).*

Evaluation type	trait	chr-pos	bin	support interval	lod	R^2	add	fav line
top-cross	ADL/NDF	2-126	2.08	120-142	3.1	10.3	0.06	F288
	ADL/NDF	*3-144*	*3.06*	*134-166*	*2.6*	*8.6*	*0.07*	*F288*
	ADL/NDF	*3-238*	*3.09*	*220-256*	*2.0*	*6.6*	*0.06*	*F288*
	ADL/NDF	6-188	6.06	172-198	5.8	18.5	0.12	F288
	ADL/NDF	*7-214*	*7.04*	*208-244*	*2.6*	*8.7*	*0.06*	*F288*
top-cross	KL/NDF	9-130	9.02	122-144	3.8	12.3	0.17	F271
RIL *per se*	ADL/NDF	3-140	3.05	132-150	3.2	10.5	0.17	F288
	ADL/NDF	*6-20*	*6.01*	*10-24*	*2.1*	*7.2*	*0.12*	*F288*
	ADL/NDF	6-184	6.06	162-194	6.5	20.4	0.29	F288
	ADL/NDF	9-110	9.02	98-122	3.3	11.0	0.19	F271
RIL *per se*	KL/NDF	1-108	1.02	86-120	3.6	11.8	0.39	F288
	KL/NDF	6-184	6.06	158-192	8.6	26.1	0.65	F288
	KL/NDF	9-100	9.02	66-102	3.6	12.0	0.31	F271
heterosis	ADL/NDF	1-290	1.11	280-302	3.1	10.8	0.06	F288
	ADL/NDF	*4-212*	*4.09*	*208-224*	*2.1*	*7.7*	*0.04*	*F271*
	ADL/NDF	5-162	5.05	146-172	4.1	13.5	0.07	F288
	ADL/NDF	6-22	6.01	18-40	3.2	10.5	0.05	F271
	ADL/NDF	8-40	8.05	26-44	2.6	8.8	0.04	F288
heterosis	KL/NDF	1-134	1.04	128-146	5.0	16.1	0.17	F271
	KL/NDF	6-164	6.06	146-200	3.9	12.7	0.22	F271
	KL/NDF	*8-20*	*8.04*	*12-28*	*2.4*	*7.9*	*0.10*	*F271*

the cell wall. Genetic correlations between Hcell/NDF and cell wall digestibilities were positive in both top cross and RILs *per se* and ranged from 0.60 to 0.78). Conversely, genetic correlations between Cell/NDF and cell wall digestibilities were negative, lower, and ranged from –0.25 to –0.43. Genetic correlations between cell wall digestibilities and starch content were very low, as expected, and ranged from –0.22 to 0.09. In top cross experiments, genetic correlations between yield and DINAGZ or IVNDFD were close to zero (r_g = 0.09 and 0.14), allowing simultaneous improvement in yield and feeding value. Similar low values were observed between cell wall digestibility and earliness (dry matter content at harvest or silking date) and between cell wall digestibility and crude protein content.

Phenotypic correlations between cell wall digestibility estimates in RILs *per se* and topcross were, as expected, high (r = 0.71 and 0.79 for DINAGZ and IVNDFD respectively), and, also as expected, higher than for IVDMD (r = 0.63). Phenotypic correlation between lignification in RILs *per se* and top cross was higher for ADL/NDF (r = 0.75) than for KL/NDF (r = 0.62). Phenotypic correlations between RILs *per se* and top cross were also high for other components of NDF, Hcell/NDF (r = 0.78) and Cell/NDF (r =0.81), but lower for NDF content (r = 0.58).

QTL Analysis

Eighty-eight putative QTL were found for the investigated traits (Tables 2, 3, and 4), often clustered in the same chromosome areas, and only distributed over 20 non overlapping positions. Only five QTL were observed in isolated locations, two of which had a LOD value higher than 3.0. Five clusters of only two QTL were also observed, four of which had at least one QTL with a LOD value higher than 3.7. Out of the observed QTLs for cell wall digestibility traits DINAGZ, IVNDFD and SNDFD, 28 putative QTL had a LOD value higher than or equal to 2.7, and 10 ranged between 2.0 and 2.7.

NDF content – Two QTL for NDF content were observed in top cross, the LOD values of which were lower than 2.7. Three QTL were observed in RILs *per se*, two of which had high LOD values and

TABLE 4 - *Putative QTLs identified for digestibility traits from 131 RILs in F288 x F271, experimented in 1998, 99, 00 (7 locations) for their per se value, and experimented in 1999, 00 (6 locations) for their top cross value (QTLs were italicized when LOD were lower than 2.7, add is the estimated additive effect of the QTL, and favorable parental line increased the feeding value of the trait, ie increased digestibility).*

Evaluation type	trait	chr-pos	bin	support interval	lod	R^2	add	fav line
top-cross	*IVDMD*	*1-266*	*1.10*	*254-286*	*2.2*	*7.5*	*0.23*	*F288*
	IVDMD	*2-0*	*2.01*	*0-2*	*2.1*	*7.2*	*0.20*	*F271*
	IVDMD	*2-84*	*2.05*	*74-90*	*2.2*	*7.4*	*0.22*	*F288*
	IVDMD	6-186	6.06	158-196	5.6	17.8	0.46	F288
	IVDMD	8-36	8.05	26-44	3.7	12.2	0.30	F288
	DINAGZ	2-132	2.08	120-142	3.6	12.0	0.30	F288
	DINAGZ	3-146	3.06	134-168	2.7	8.9	0.24	F288
	DINAGZ	*6-14*	*6.01*	*6-24*	*2.0*	*6.7*	*0.21*	*F288*
	DINAGZ	6-164	6.06	150-196	6.8	21.2	0.57	F288
	DINAGZ	8-38	8.05	26-44	3.6	12.0	0.26	F288
	IVNDFD	2-166	2.08	152-182	3.6	12.8	0.57	F288
	IVNDFD	*3-142*	*3.05*	*134-174*	*2.4*	*8.0*	*0.34*	*F288*
	IVNDFD	6-186	6.06	158-194	7.2	22.4	0.74	F288
	IVNDFD	*9-126*	*9.02*	*108-136*	*2.4*	*8.2*	*0.33*	*F271*
	SNDFD	2-132	2.08	118-146	2.9	9.8	0.30	F288
	SNDFD	3-146	3.06	136-166	4.0	13.2	0.44	F288
	SNDFD	6-184	6.06	154-196	5.2	16.8	0.45	F288
RIL *per se*	*IVDMD*	*1-138*	*1.04*	*126-146*	*2.2*	*7.5*	*0.39*	*F288*
	IVDMD	3-56	3.03	38-74	2.9	9.8	0.65	F288
	IVDMD	*3-136*	*3.05*	*132-148*	*2.4*	*8.0*	*0.43*	*F288*
	IVDMD	6-186	6.06	176-194	10.9	31.7	1.29	F288
	IVDMD	9-102	9.02	98-112	4.9	15.7	0.61	F271
	DINAGZ	*1-64*	*1.02*	*32-100*	*2.1*	*7.5*	*0.86*	*F288*
	DINAGZ	*2-130*	*2.08*	*120-146*	*2.0*	*6.7*	*0.51*	*F288*
	DINAGZ	3-142	3.05	136-150	6.6	20.7	1.02	F288
	DINAGZ	4-210	4.09	206-216	2.8	9.3	0.57	F288
	DINAGZ	6-20	6.01	12-24	4.2	13.8	0.69	F288
	DINAGZ	6-182	6.06	158-192	8.6	26.0	1.26	F288
	DINAGZ	*9-112*	*9.02*	*68-134*	*2.0*	*6.6*	*0.57*	*F271*
	IVNDFD	1-92	1.02	82-118	3.1	10.3	0.95	F288
	IVNDFD	3-136	3.05	132-150	2.0	6.6	0.68	F288
	IVNDFD	6-184	6.06	160-190	14.6	40.2	2.62	F288
	IVNDFD	9-100	9.02	78-102	4.1	13.4	0.96	F271
heterosis	*IVDMD*	*3-166*	*3.08*	*152-180*	*2.4*	*8.1*	*0.26*	*F271*
	IVDMD	*8-36*	*8.05*	*24-44*	*2.5*	*8.5*	*0.25*	*F288*
	DINAGZ	1-92	1.02	82-110	2.7	9.1	0.24	F271
	DINAGZ	*1-300*	*1.11*	*288-304*	*2.4*	*8.4*	*0.23*	*F288*
	DINAGZ	5-162	5.05	144-172	5.2	16.7	0.36	F288
	DINAGZ	*6-182*	*6.06*	*146-200*	*2.2*	*7.5*	*0.23*	*F271*
	DINAGZ	8-40	8.05	32-44	5.4	17.2	0.29	F288
	IVNDFD	1-116	1.03	106-128	4.6	15.0	0.43	F271
	IVNDFD	6-170	6.06	156-190	7.1	22.1	0.58	F271
	IVNDFD	*9-102*	*9.02*	*98-114*	*2.4*	*8.0*	*0.24*	*F288*

explained at least 15% of the phenotypic variation. Except in position 1-96, the allele which increases NDF content originated from F271. Two QTL for NDF content heterosis were observed, each of them originating from one parent, with significant epistatic effects between them. None of the observed QTL were common between top cross, RILs *per se* or heterosis effect. QTL for NDF, which is the sum of different components making up the cell wall, were observed in the same locations as QTL for Hcell/NDF, Cell/NDF, ADL or KL/NDF, except one in position 3-56, which had a LOD value equal to 5.1. This last QTL colocalized with a QTL of starch content (BARRIÈRE et al., 2002), and logically a QTL for IVDMD. Starch and NDF are the most important complementary components of the maize plant at silage harvest, and these results illustrate the value of dissecting traits into their elementary parts before QTL analysis for feeding value traits.

Hemicellulose / NDF content – Three QTL for Hcell/NDF in top cross, and four in RILs *per se*, were observed. Two QTL, located in positions 2-74 and 6-168 were common in RILs *per se* and top cross. In both RILs *per se* and top cross, the QTL located in position 6-168 explained more than 24% of the phenotypic variation, and had a LOD value higher than 7.9. For RILs *per se*, epistatic effects were significant between QTL located in positions 6-168 and 4-38. Except in position 9-114, the allele which increases Hcell/NDF originated from F288. Two QTL for Hcell/NDF heterosis were observed, each of them originating from one parent, in positions where no QTL for Hcell/NDF were observed in RILs *per se* or top cross.

Cellulose / NDF content – Six QTL for Cell/NDF in RILs *per se* were observed, two of which were located in positions 2-66 and 6-166 and were common with the two QTL observed in top cross. Except in position 9-156, alleles increasing Cell/NDF originated from F271. The two QTL observed in positions 2-66 and 6-166 for Cell/NDF were common with QTL detected for Hcell/NDF, in both top cross and RILs *per se*, and F271 alleles increasing the content for Cell/NDF decreased the content of Hcell/NDF. Epistatic effects were significant in RILs *per se* between Cell/NDF QTL in positions 6-166 and 1-134. Only one QTL for Cell/NDF heterosis was observed, in position 3-172 which was in the same support interval as the QTL for Hcell/NDF heterosis observed in position 3-144. Once more, the allele which increased heterosis for Cell/NDF decreased heterosis for Hcell/NDF.

ADL / NDF content – Six QTL for ADL/NDF were observed in top cross and four in RILs *per se*. Two QTL, located in positions 3-144 and 6-188 were common in RILs *per se* and top cross. The QTL located in position 6-188 explained more than 18 and 20% of the phenotypic variation respectively in top cross and RILs *per se*. This QTL for ADL/NDF observed in position 6-188 was in the same support interval as the QTL observed also in top cross and RILs *per se* for Hcell/NDF and Cell/NDF in position 6-166. Except in position 9-110, alleles increasing lignin content in both top cross and RILs *per se* originated from F271. The QTL of ADL/NDF, located in position 9-110, was in the same interval support as a QTL for Hcell//NDF, possibly illustrating again a cluster of genes involved in cell wall biogenesis. Five QTL for ADL/NDF heterosis were observed, only one of which, located in position 6-22, was common with a QTL for ADL/NDF in RILs *per se*. But the F271 allele, which increased ADL/NDF in RIL *per se*, decreased heterosis for ADL/NDF.

Klason lignin content – Only one QTL for KL/NDF was observed in top cross, in position 9-130 where no QTL for ADL/NDF were observed in top cross, but in an interval support just close to the one of a QTL for ADL/NDF in RILs *per se*. Three QTL for KL/NDF were observed in RILs *per se*, two of which were common to QTL observed for ADL/NDF. The common QTL in position 6-184 explained 26.1% of the phenotypic variation for KL/NDF, and respectively 20.4% for ADL/NDF. Three QTL for KL/NDF heterosis were observed, one of which, located in position 8-20, was in the same interval support as a QTL for ADL/NDF heterosis, and one was located in position 6-164, with an interval support in which numerous QTL related to lignification were observed. Epistatic effects were significant between KL/NDF heterosis QTL in positions 1-134 and 8-20. Klason lignin and ADL did not completely represent the same part of lignification in maize plants, since QTL for KL/NDF, but not for ADL/NDF, were observed in bins 1.02 and 1.04. Conversely, seven QTL for ADL/NDF were observed in bins where no QTL for KL/NDF were observed. Thus, ADL could be more related to the resistant core part of lignin than Klason lignin.

In vitro dry matter digestibility – Five QTL for

IVDMD were found in RILs *per se* and five were also noted in top cross, but only one, located in position 6-186, was common to RILs *per se* and top cross. Such great differences in QTL observed in lines and hybrids could be related to the confused status of the IVDMD trait, which is related to both cell wall characteristics, and contents in crude protein, starch and soluble carbohydrates, which are greatly subject to heterosis effects. In this way, positions 1-138 and 3-56 supported both QTL for starch content and IVDMD in RILs *per se* (BARRIÈRE *et al.*, 2002). Two QTL were observed for IVDMD heterosis, one of which was also observed in top cross. In top cross, epistatic effects were significant between IVDMD QTL located in positions 6-186 and 8-36. Only one QTL for IVDMD, located in bin 2.05, appeared here in a similar bin as a QTL previously observed by LÜBBERSTEDT *et al.* (1997b, 1998) in a different genetic background.

DINAGZ cell wall digestibility – Seven QTL for DINAGZ were observed in RILs *per se*, and five QTL in top cross. Only one QTL observed in top cross was not observed in RIL *per se*. Three QTL out of five in top cross, and three QTL out of seven in RILs *per se*, were observed at positions where QTL for ADL/NDF were also observed respectively in top cross or RILs *per se*. Moreover, two other QTL in RILs *per se* were observed at positions where QTL for ADFL/NDF were observed in top cross. The QTL for DINAGZ observed only in top cross and located in position 8-38, had a high LOD value and explained 12% of the phenotypic variation observed between hybrids. Similarly, one of the QTL observed only in RILs *per se* and located in position 4-210 had a LOD value higher than 2.7 and explained 9.3% of the phenotypic variation. Five QTL were observed for DINAGZ heterosis, two of which had a LOD value higher than 5.0. These two last QTL were located in positions 5-162 and 8-40, where QTL for ADL/NDF heterosis and DINAGZ heterosis were respectively observed.

IVNDFD cell wall digestibility – Four QTL were observed for IVNDFD both in top cross and in RILs *per se*, but only two were common in lines and hybrids, located in positions 3-142 and 6-186, where QTL were also observed for DINAGZ and ADL/NDF. In RILs *per se*, all QTL observed for IVNDFD were also observed for DINAGZ (but the opposite was not true). In top cross, the QTL observed for IVNDFD in position 9-126 was also observed for DINAGZ in RILs *per se*, but the other QTL observed in position 2-166 with a high LOD value, and having significant epistatic effects with the QTL in position 6-186, could be original. However, it could also correspond, with a false location estimate, to the QTL for DINAGZ and ADL located in position 2-132. Three QTL for IVNDFD heterosis were observed, two of which were in positions where QTL for IVNDFD were also observed in top cross and RILs *per se*. The third, with a LOD value equal to 4.6, was located in position 1-116, where QTL for KL/NDF, but not ADL/NDF, were observed in both top cross and RILs *per se*.

SNDFD digestibility – Only three QTL were found for this tentative estimate of *in vivo* NDF digestibility, in positions where QTL for DINAGZ or IVNDFD were also observed. The only value of this trait is to confirm the *in vitro* results relative to cattle feeding.

Colocalisations between QTL and genes involved in the cell wall biogenesis

Tentative colocalisations and candidate genes, were mainly investigated using data available in the MaizeDB. The brown-midrib gene bm2 is located in bin 1.11, where we observed QTL for ADL/NDF and DINAGZ heterosis. In bins 3.05 and 3.06, where we observed QTL for ADL/NDF and cell wall digestibility, the transcription factor myb2 and the lax-midrib1 gene are located. QTL for ADL/NDF and DINAGZ heterosis, in position 5-162, were in a similar bin (bin 5.05) as the phenylalanine ammonia lyase 1 (pal1) gene involved in the early beginning of the lignin biosynthesis pathway. The cellulose synthase genes cesa4a and cesa4b are in bin 2.06, where we observed a QTL for Cell/NDF. Moreover, HOLLAND *et al.* (2000) located one gene of cellulose synthase catalytic sub-unit in bin 6.05, in which we observed QTL for Cell/NDF. Possibly indicating a cluster of different genes involved in cell wall biogenesis, QTL for Hcell/NDF and/or Cell/NDF were observed in bin 4.05, in which are located the brown-midrib gene bm3 and a pal homolog. QTL were also observed in bin 9.07, in a location overlapping that of the brown-midrib gene bm4.

DISCUSSION AND CONCLUSIONS

As previously discussed (BARRIÈRE *et al.*, 2002), QTL analysis must be considered carefully i) when the number of RIL is low, ii) when QTL effects are

estimated from the same data as used for QTL mapping, especially when the number of investigated RILs is under 200 and when the trait is of moderate heritability iii) and because, even with large sample sizes, the power of QTL detection is only moderate for QTLs with small effects (MELCHINGER *et al.*, 1998; UTZ *et al.*, 2000). However, results should be considered with some confidence, because LOD values were often higher than 2.7, and because QTL were obtained for traits, the estimates of which were obtained independently (ADL and KL, ADL and DINAGZ, ...).

As expected, QTL identified for digestibility and lignification traits in RILs *per se* were more numerous than QTL found in top cross, and this could be strongly related to the lowest genetic variance between hybrids, all top crossed by the same testor line. Moreover, all QTL found in top cross were not always found for heterosis or in RILs *per se*. Four major clusters of QTL were observed for digestibility and lignification traits. These were located in descending order for LOD values and percentage of explained phenotypic variation in positions 6-182 (bin 6.06), 3-142 (bin 3.05), 9-112 (bin 9.02) and 8-24 (bin 8.05). Bin 6.06 was also greatly involved in cell wall content and composition because QTL for NDF, Hcell/NDF and Cell/NDF were also found in position 6-166, with overlapping support intervals. Out of these four clusters, the one located in position 8-24 appeared to be more greatly involved in heterotic effects than in direct additive effects, both for digestibility traits and lignin content. Similarly, the two QTL, found in position 5-162 (bin 5.05) with LOD values equal respectively to 4.1 and 5.2, were only observed for heterosis effect in ADL/NDF and DINAGZ. More QTL were observed for DINAGZ than for IVNDFD, and DINAGZ was probably a more complete breakdown of the quantitative digestibility traits into their underlying mendelian traits, despite the fact that LOD values, when found, were higher for IVNDFD than for DINAGZ. The QTL observed did not make it possible to draw any conclusions about the relationship between cell wall composition in Hcell/NDF or Cell/NDF and lignin content or cell wall digestibility. However, QTL for Hcell/NDF were more frequently clustered than QTL for Cell/NDF with cell wall digestibility QTL, which is possibly related to linkage of genes involved in ferulic acid cross-linking between arabinoxylan residues of hemicellulose and lignin. Moreover, ADL/NDF was the lignin part the most involved in the indigestibility of the cell wall, whereas KL/NDF was a more overall approach to lignin content in the cell wall and was less related to cell wall digestibility.

There were no QTL for digestibility traits in a similar position as QTL for yield (BARRIÈRE *et al.*, 2002). However, a QTL of yield could be very close to a QTL of DINAGZ in bin 6.01, but the favorable allele originated for both traits from the same line (F288). The building of an ideal line, based on the high yielding RIL n° 143, and gathering all the favorable putative QTLs (alleles) for cell wall digestibility, but also for starch and crude protein contents, yield, and earliness found in the RILs between F288 and F271, required the use of five other RILs. This objective will thus demand more than one generation of line crossing and marker assisted selection, but very significant improvements are expected.

There could be a risk of antagonistic effect between digestibility traits and borer resistance. According to the MaizeDB, and according to Schön *et al.* (1993), BOHN *et al.* (1996), BOHN *et al.* (2000) and CARDINAL *et al.* (2001), QTL for European corn damage or sugarcane borer damage were observed, often in independent QTL mapping, in bins 1.11, 2.08, 3.05/06, 5.05, 7.04 and 9.06, where QTL related to lignin content or digestibility were also observed in this analysis. The relationship between borer resistance and silage maize feeding has to be further investigated, but BEEGHLY *et al.* (1997) showed genetic correlations ranging from –0.19 to –0.47 between lignin content of the stalk and second generation of corn borer damage.

Further investigations are required to verify the consistency of this QTL mapping, especially using other maize germplasm, such as very digestible lines (for example F4) (MÉCHIN *et al.*, 2000), or lines tolerant to borers such as B52 or DE811. But lines with a high digestibility are often of poor agronomic value, because this trait, which was not simultaneously taken into account during years of grain maize breeding, was lost in elite modern lines, and is available now in old lines or old ecotypes. QTL identification allows then a targeted introgression in elite lines of small chromosomal areas, which are of interest for feeding value traits. Moreover, knowledge of the lignin biosynthesis pathway is increasing rapidly, particularly through gene silencing, while genes greatly involved in cell wall digestibility, including transcription factors, are being identified. Alleles of interest will soon be identified in genetic resources, and marker assisted selection will soon be extensively used in forage maize breeding.

ACKNOWLEDGMENTS - We are grateful to L. Moreau and H.F. Utz for their help and advice in QTL analysis and computing with PLABQTL. We are also grateful to O. Argillier, O. Bouchard and V. Méchin, who contributed to the preliminary QTL mapping in this RIL progeny. We thank the breeding companies involved in the ProMaïs – INRA network "DINAG" that contribute to the funding of this forage maize research.

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