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From description to explanation of variations in alfalfa digestibility

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SUMMARY – Feeding value of alfalfa (digestibility, cell wall content, protein content) shows variations due to the genotype and to the environment (year, cut, location, etc.). A part of the genetic variation is not explained by morphological or phenological differences, and could be related to intrinsic traits of the plants. A detailed description of dry matter and fibre degradation in the rumen of fistulated cows showed variations in both extent and rate of degradation. Preliminary data on the histological bases of variations in stem digestibility are presented. Some histological traits evolved along the stem, and others varied with the genotype. Molecular mapping is under study, with the objective of identifying markers associated to this group of growth and digestibility traits (chemical composition, ruminal degradation, histological traits). With this approach, the relationship between these components should be better understood, and molecular markers could be tool kits for alfalfa breeders. The autotetraploidy of this species complicates the approach, but alfalfa takes benefits from the basic studies made on the legume model species, *M. truncatula*.

Key words: *Medicago sativa*, cell wall, histology, *in sacco* degradation, molecular marker.

RESUME – *"De la description à l'explication des variations de digestibilité chez la luzerne". Un grand nombre de travaux a montré que la valeur alimentaire de la luzerne (digestibilité, composition en parois, teneur en protéines) montrait une variabilité génétique et une variabilité induite par le milieu (année, coupe, lieu, etc.). Une partie des variations génétiques ne sont pas expliquées par des différences morphologiques ou phénologiques, et pourraient être liées à des caractéristiques intrinsèques de la plante. Une description précise de la dégradation de la matière sèche et des parois dans le rumen de vaches fistulées a montré que des différences de vitesse de dégradation s'ajoutaient aux différences de dégradabilité déjà connues. Des travaux ont débuté pour rechercher les bases histologiques des variations de digestibilité des tiges. Certains caractères histologiques évoluent le long de la tige, alors que d'autres varient en fonction du génotype. Un travail de cartographie génétique a débuté. L'identification de marqueurs moléculaires liés à cet ensemble des caractères de croissance et de digestibilité (composition biochimique, dégradabilité ruminale ou caractères histologiques) permettrait de mieux comprendre les relations entre ces différents composants de la qualité, et aussi d'offrir de nouveaux outils aux sélectionneurs. L'autotétraploïdie de la luzerne cultivée complique l'approche, mais cette espèce peut bénéficier des travaux fondamentaux menés chez la légumineuse modèle* M. truncatula*.*

Mots-clés : Medicago sativa*, paroi, histologie, dégradabilité* in sacco*, marqueur moléculaire.*

Introduction

In alfalfa, forage quality has two main components, digestibility and protein content. Both of them are positively correlated to the proportion of leaves in the forage. Stem digestibility is also a component of whole plant digestibility (Julier and Huyghe, 1997). Genetic variation for digestibility was described by several authors (Heinrichs *et al*., 1969; Buxton *et al*., 1987; Julier *et al*., 1996). The effects of phenological stages (Kalu and Fick, 1984) or of forage yield (Lemaire and Allirand, 1993) on the decrease of digestibility with growth were described and modelled.

At INRA Lusignan, several trials were run in the past years to describe genetic variation in digestibility, and to correlate digestibility with other biochemical traits or morphological traits (Julier *et al.*, 1999a). Briefly, we showed a large range of variation, up to 4 percent units for varieties with a high yield potential, in digestibility among the varieties already registered. There was a close correlation between enzymatic digestibility and cell wall contents (NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin), but in a multisite experiment, NDF content had the highest broad sense heritability. Digestibility was positively correlated to protein content, but the correlation was not very high. The interaction between variety and environment was significant, but some highly digestible varieties seemed stable over environments. Analysing the within-variety variation for digestibility, we found up to 11 percent

units of differences among genotypes (Julier *et al.*, 2000). A large range of variation is thus available within the breeding populations, so the genetic progress can be achieved with this material. In a diallel and a factorial mating designs, the genetic variance was mainly additive.

More physiological or genetic bases are needed to fully explain the variations in forage quality of alfalfa. We present our results or current programs on this topic. They range in three categories:

(i) An analysis of the degradation of dry matter and cell wall in the rumen of fistulated cows, for 15 cultivars contrasted for their *in vitro* digestibility.

(ii) The histological bases of the variations for digestibility.

(iii) The research of molecular markers associated to phenotypic traits (growth, histology, quality) in a mapping population.

Dry matter and cell wall degradation in the rumen of fistulated cows, for 15 cultivars

The *in sacco* method was used to investigate the phenotypic variation in the kinetics of degradation of dry matter and fibre for 15 alfalfa cultivars grown at Lusignan (France), and to compare these kinetic parameters to laboratory analyses of dry matter solubility, fibre content (NDF) and fibre (NDF) solubility (Julier *et al.*, 1999b). Among the 15 cultivars, NDF ranged from 42.2 to 48.4% and NDF solubility from 27.5 to 31.0%. Bags containing forage samples were incubated for 2, 4, 8, 12, 24, 48, and 72 h in three fistulated Holstein cows. Dry matter and NDF degradation curves were modelled with a sigmoid curve with three parameters. Phenotypic variations were observed for dry matter and NDF disappearance at each time of incubation (Fig. 1), and for the parameters of the degradation kinetics.

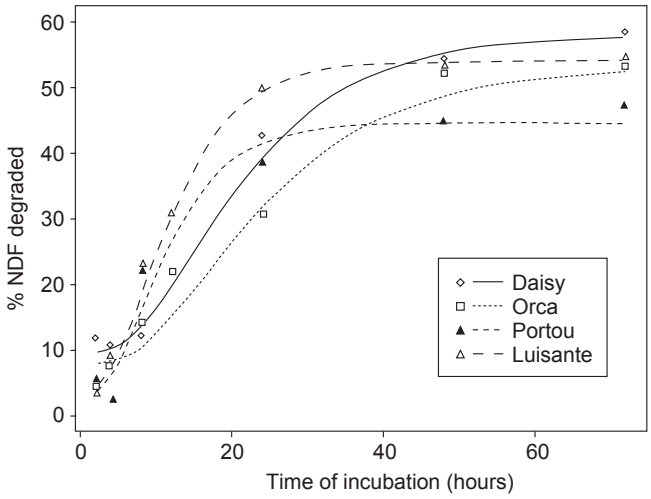

Fig. 1. NDF degradation kinetics for 4 cultivars.

For dry matter degradation, the rapidly degraded fraction ranged from 32.6 to 40.3%, the slowly degradable fraction from 31.2 to 38.4%, the undegradable fraction from 24.7 to 34.4%, and the rate of degradation from 0.11 to 0.19. For NDF degradation, the slowly degradable fraction ranged from 41.6 to 50.5%, the undegradable fraction from 42.0 to 55.4% and the rate of degradation from 0.07 to 0.16.

NDF and dry matter solubility measured in wet chemistry were correlated with dry matter undegradable fraction. The NDF solubility was correlated to the NDF undegradable fraction. The chemical measurement of digestibility, useful for the investigation of variations in energy value in alfalfa were thus validated by the *in sacco* method. But some parameters of degradation (rapidly degraded fraction, rate of degradation of the slowly degraded fraction), that showed some variations, are not related to chemical analyses. The effect of the variations in the parameters of degradation has to be studied on the ruminant performances. The rate of degradation could influence the voluntary intake. See Julier *et al*. (2001) for more details.

Histological bases of the variations for digestibility

Several authors stated that the anatomy of cells and tissues can be as important as their chemical composition in determining the *in vivo* digestibility of the fibres (Akin *et al.*, 1990; Wilson and Mertens, 1995). It is known, in alfalfa, that lignin is mainly located in xylema and sclerenchyma tissues. Vallet *et al*. (1998) described the progress in lignin deposition in the stem. They proposed, in order to compare stems of various cultivars, to consider the physiological age of the stem portions, measured by the distance between the apex and the stem portions.

We analysed the variation for several histological parameters, along the stems and among genotypes. Sampling of 2 cm-long stem portions were placed in a fixative, and 50 m-thick sections were made with a vibratome. Sections were stained with fasga, a polychromatic stain that coloured the lignified tissues in red and the cellulose in blue. Images of the sections were captured and analysed with an image analysis software, Optimas.

With Optimas, programs were written to measure several parameters on the stem sections: surface of the whole section, surface of the pith parenchyma, surface of the cortex, surface of the xylem, proportion of xylem in the cortex and distance between epiderma and parenchyma. On xylem tissue, the surface of the large cells (vessels), the surface of the small cells (fibres) and the mean thickness of the lignified cell walls were quantified. These traits were chosen to describe both the histological evolution along the stem and the genetic variation among plants.

Along the stem, some of the histological traits were related to the evolution in cell wall (NDF) and lignin contents (surface of the entire section, surface of the cortex, surface of pith parenchyma), but others were not related (surface of the small cells of the xylem, thickness of the lignified cell wall of the xylem).

For the histological traits the bottom of the stem showed more genetic variation than the top of the stem, probably because the top of the stem have a common structure of young tissues with an important elongation and no lignification.

In a first study about the genetic variation on histological traits, two genotypes, i.e. two plants, originating from a divergent selection for digestibility were analysed. Some histological traits showed variation among the two genotypes: the surface of the cortex, the distance between the epiderma and the pith parenchyma, the surface of the small and of the large cells of xylem, the surfacic density of the lignified wall of the xylem. Further results are presented by Guines *et al.* (this volume) in a poster.

Molecular markers associated to phenotypic traits (growth, histology, quality)

Genetic progress in alfalfa is very slow, even for relatively heritable traits, because of the allogamy and the tetraploidy of the species. Molecular markers linked to phenotypic traits could help alfalfa breeding. Research of markers of phenotypic traits can be made through two ways: with the bulk segregant analysis (Michelmore *et al.*, 1991) or with a linkage map and the research of QTL. The first way is mainly devoted to mono or oligogenic traits, and the second way is more efficient for quantitative traits.

Theoretical problems of mapping in a polyploid species are studied in several research teams (Wu *et al.*, 1992; Hackett *et al.*, 1998; Ripol *et al.*, 1999; Skinner *et al.*, 2000) on various agronomically important crops (alfalfa, potato, sweet potato, sugar cane, etc.). Theory for research of QTL in tetraploid species was developped by Xie and Xu (2000).

We choose to develop a molecular linkage map, based on dominant AFLP markers and codominant microsatellite markers. The mapping population is a F1 population with 228 plants obtained from the cross of two plants contrasted by their autumn dormancy, forage quality, disease resistance and lodging resistance. F1 plants are under study for agronomic traits. We plan to analyse various quantitative traits, related to growth and quality: plant height, digestibility, etc. Furthermore, plants will be scored for stem growth rate, in order to find the parameters of the physiological model that are genetically variable, and to find QTL of these parameters.

On 180 F1 plants, 187 AFLP markers were obtained. About half of them segregated as simplex markers, a quarter as duplex markers and a quarter were distorted. For each parent, a map with 8 linkage

groups, among the 32 groups expected, was obtained. More markers are needed to complete the map. See the poster of Julier *et al.* (this volume).

Among 36 microsatellite markers obtained at INRA-CNRS of Toulouse (France) on the legume model species *M. truncatula* and provided by T. Huguet, 10 showed polymorphic patterns among the two parents (S. Santoni, INRA Montpellier, pers. comm.). We are currently testing the F1 progeny for the markers with the higher number of alleles. These codominant markers, together with duplex dominant markers, will be very useful to gather the linkage groups in homology groups. Furthermore, the microsatellites should give the opportunity to combine the maps of both parents.

Conclusions and prospects

Genetic progress in traits related to forage production (yield, quality) relies on the ability to explain and understand the physiology and the genetic of the traits. Description of genetic variation for forage yield and forage quality was made in the past years by several groups. With a more detailed description of forage quality by histological traits and traits related to the degradation in the rumen, we should identify the basic components of agronomic traits. For these components, we should be able to find QTL or markers that could help in breeding. More work is needed on the use of molecular markers in breeding schemes of an autotetraploid species.

Research of QTL of forage yield is probably a nonsense because yield is the result of the expression of many elementary traits. We need to identify these elementary traits, test if it exists genetic variation and then look for QTL. Crop physiologists at INRA, Unité d'Ecophysiologie des Plantes Fourragères, at Lusignan are developing a model of alfalfa stem growth after a cut. In a first step, stem elongation model is based on five parameters that take into account the meristem, the elongation zone, and the mature zone, and their relationships (Durand *et al*., 1999). In a following step, a better description of the early phase of regrowth after a cut will be analysed. This phase is partly responsible for the variation in forage yield. In a first attempt, a model with 5 parameters related to three growing zones in the stem was applied to former data of stem elongation. The model fitted correctly to the data, and the values of the parameters were reliable (J.L. Durand, unpublished data). Genetic variation for the parameters of the model will be assessed, and research of QTL of model parameters could be a way of understanding and identifying the genetic bases of variation for forage yield. In future programs, the model should be completed by a module of stem thickening, possibly including tissue and lignin repartition in the stem. At this stage, stem digestibility could be included as a result of the previous traits.

References

Akin, D.E., Rigsby, L.L., Lyon, C.E. and Windham, W.R. (1990). Relationship of tissue digestion to textural strength in bermudagrass and alfalfa stems. *Crop Sci.*, 30: 990-993.

Buxton, D.R., Hornstein, J.S. and Marten, G.C. (1987) Genetic variation for forage quality of alfalfa stems. *Can. J. Plant Sci*., 67: 1057-1067.

Durand, J.L., Shaufele, R. and Gastal, F. (1999). Grass leaf elongation rate as a function of developmental stage and temperature: Morphological analysis and modelling. *Ann. Bot*., 83: 577-588.

Hackett, C.A., Bradshaw, J.E., Meyer, R.C., McNicol, J.W., Milbourne, D. and Waugh, R. (1998). Linkage analysis in tetraploid species: A simulation study. *Genet. Res*., *Camb*., 71: 143-154.

Heinrichs, D.H., Troelsen, J.E. and Warder, F.G. (1969). Variation of chemical constituents and morphological characters within and between alfalfa populations. *Can. J. Plant Sci*., 49: 293-305.

Julier, B., Guy, P., Castillo-Acuna, C., Caubel, G., Ecalle, C., Esquibet, M., Furstoss, V., Huyghe, C., Lavaud, C., Porcheron, A., Pracros, P. and Raynal, G. (1996). Genetic variability for pest resistance and forage quality in perennial diploid and tetraploid lucerne populations (*Medicago sativa* L.). *Euphytica*, 91: 241-250.

Julier, B. and Huyghe, C. (1997). Effect of growth and cultivar on alfalfa digestibility in a multi-site trial. *Agronomie,* 17: 481-489.

Julier, B., Huyghe, C. and Ecalle, C. (1999a). Potential for including the digestibility in breeding of alfalfa. In: *Proc. XIII Eucarpia Medicago spp. Group Meeting*, Perugia, Italy, pp. 125-133.

Julier, B., Lila, M., Furstoss, V., Travers, V. and Huyghe, C. (1999b). Measurement of cell-wall digestibility in lucerne using the filter bag technique. *Anim. Feed Sci. Technol.,* 79: 239-245.

Julier, B., Huyghe, C. and Ecalle, C. (2000). The within-cultivar part of genetic variance is higher for morphological traits and yield than for digestibility and fiber content in alfalfa. *Crop Science*, 40: 365-369.

Julier, B., Emile, J.C., Lila, C. and Huyghe, C. (2001). Phenotypic variation for *in sacco* dry matter and fibre degradation kinetics in lucerne. *Austr. J. Agric. Res*., 52 (in press).

Kalu, B.A. and Fick, G.W. (1984). Morphological stage of development as a predictor of alfalfa herbage quality. *Crop Sci*., 23: 1167-1172.

Lemaire, G. and Allirand, J.M. (1993). Relation entre croissance et qualité de la luzerne: Interaction genotype-mode d'exploitation. *Fourrages,* 134: 183-198.

Michelmore, R.W., Paran, I. and Kecceli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific regions by using segregating populations. *Proc. Natl Acad. Sci. USA*, 88: 9828-9832.

Ripol, M.I., Churchill, G.A., da Silva, J.A.G. and Sorrells, M. (1999). Statistical aspects of genetic mapping in autopolyploids. *Gene*, 235: 31-41.

Skinner, D.Z., Loughin, T. and Obert, D.E. (2000). Segregation and conditional probability association of molecular markers with traits in autotetraploid alfalfa. *Mol. Breed*., 6: 295-306.

Vallet, C., Lemaire, G., Monties, B. and Chabbert, B. (1998). Cell wall fractionation of alfalfa stem in relation to internode development: Biochemistry aspect. *J. Agric. Food Chem*., 46: 3458-3467.

Wilson, J.R. and Mertens, D.R. (1995). Cell wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Sci.,* 35: 251-259.

Wu, K.K., Burnquist, W., Sorrels, M.E., Tew, T.L., Moore, P.H. and Tanksley, S.D. (1992). The detection and estimation of linkage in polyploids using single-dose restriction fragments. *Theor. Appl. Genet*., 83: 294-300.

Xie, C. and Xu, S. (2000). Mapping quantitative trait loci in tetraploid populations. *Genet. Res., Camb*., 76: 105-115.