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Genetic control of quality traits of lucerne (*Medicago sativa* L.)

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Abstract. An important objective in lucerne breeding is the improvement of feeding value. An understanding of the inheritance of digestibility and cell wall related traits would facilitate the breeding of varieties with higher feeding value. The aim of this experiment was first to determine the genetic control of dry matter digestibility and related biochemical and morphological traits, and to assess phenotypic and genetic correlations between these traits. Quantitative genetic parameters were estimated for enzymatic solubility, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), protein content, plant height, and leaf-to-stem ratio. A 7×7 diallel design with reciprocals and without selfing among parents from different populations and a 7×7 factorial design within the 'Flamande' population were studied. In the diallel study, effects due to general combining ability (GCA) were higher than those due to specific combining ability (SCA) for all characters tested. In the factorial study, the F_1 progeny effect was significant for all characters. The male effect was highly significant for all traits and higher than the female effect except for plant height. The additive variance was higher than the dominance variance for all characters except for plant height. The inheritance was predominantly additive. The highest narrow-sense heritabilities were found for NDF and ADF and leaf-to-stem ratio. As a consequence, NDF or ADF would be more efficient selection criteria than enzymatic solubility in a breeding program for improved feeding value. In both mating designs, NDF, ADF, and ADL were positively correlated with one another and negatively with enzymatic solubility and protein content.

Additional keywords: combining ability, additive variance, dominance variance, heritability, within- and among-population genetic variation.

Introduction

Lucerne is a high-yielding forage with a high feeding value. The main limitation to its use is the low digestibility of the bottom portion of stems, at pre-flower maturity (Buxton *et al.* 1987). Breeding efforts have emphasised the improvement of digestibility. Genetic variation in digestibility has been reported in lucerne (Wilson *et al.* 1978; Buxton *et al.* 1987; Julier *et al.* 2000). Because large genetic differences exist in digestibility and other quality traits, progress may be achieved through breeding. The expected genetic gain in a breeding population depends on the inheritance and the range of variation of these characters. However little information is available on the inheritance of forage quality in lucerne.

Genetic studies have been carried out on *in vitro* dry matter digestibility (IVDMD), neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin contents, and protein degradability. Neff and Simon (1986) reported that narrow-sense heritability for *in vitro* organic matter digestibility (IVOMD) ranged from 0.84 in the seeding year to 0 in the second year, in a diallel involving 12 non-inbred lucerne clones as parents. In a parent *v.* polycross-progeny comparison, they observed narrow-sense heritabilities for IVOMD ranging from 0.43 to 0.80. Hill and Barnes (1977) reported relatively large estimates of broad-sense heritability for lignin (0.44) and protein (0.64) contents but extremely low estimates were obtained for IVDMD (0.079). Broad-sense heritability estimates for ADF and NDF were intermediate (0.22–0.34). Selection for greater protein content or reduced ADF, NDF, or lignin content would be recommended over selection for greater IVDMD in a breeding program for improving nutritive value of lucerne. Inheritance for protein degradability was mainly additive with a narrow-sense heritability of 0.22 (Rooney *et al.* 1994, 1997).

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A better understanding of the genetic control of digestibility, NDF, ADF, and lignin contents would contribute to develop lucerne varieties with higher digestibility.

The objective of this experiment was first to determine the type of gene action controlling dry matter digestibility, NDF, ADF, acid detergent lignin (ADL), and protein contents. The second objective was to determine phenotypic and genetic correlations between these traits.

Materials and methods

Two mating designs were used in this experiment to assess the genetic control of traits. The first one was a 7 × 7 diallel design with reciprocals and without selfing, and the second one a 7 × 7 factorial design also called North Carolina design II (NCII) (Comstock and Robinson 1952).

Diallel design

In 1996, 42 F_1 full-sib families were obtained by crossing 7 plants with reciprocals. Parental plants were chosen from 7 different varieties. Three of them were Flemish types (Europe-1, Luzelle-3, Orca-4), one was a non-dormant variety from Spain (Aragon-5), one was a Provence type (Magali-2), and the last two were *falcata* populations from Russia (Krasnokutskaya-6) and from East of France (Malzeville-7). For the diallel crosses one plant was randomly taken from each variety. Crosses were performed without emasculation. Selfing was avoided or was minimum because the allopollen is favoured in lucerne. The F_1 progenies with poor vigour were eliminated. The F_1 germinated seeds derived from these crosses were grown in a greenhouse for 2 months. In April 1997 the F_1 progenies were transplanted in 2 locations: Lusignan (Vienne, 46°36′N, annual rainfall 700 mm), located in the centre-west of France; and Capelle-en-Pévèle (50°30′N, annual rainfall 817 mm), in the north of France. In each location the experimental design consisted of a complete randomised block with 3 replicates. Each plot consisted of a single row with 10 plants, spaced 10 cm apart and 70 cm between plots. At both locations, the F_1 progenies were harvested at full bloom at different times: at Lusignan in autumn 1997 and in the first 2 cuts of 1998 and 1999, and at Capelle-en-Pévèle in the first 2 cuts of 1998. In total, 7 harvests were analysed over 3 years. Each plot was cut and 500 g of fresh forage was sampled, dried at 60°C, and ground to pass through a 1-mm sieve. Plant height was scored prior to each cut. On dried samples, enzymatic solubility (Lila *et al.* 1986), NDF, ADF, ADL (Goering and Van Soest 1970), protein content, and leaf-to-stem ratio were predicted by near infrared spectroscopy. The prediction equations were tested for accuracy by the standard error of cross validation (SECV) and the coefficient of determination (R^2). These values were, respectively, 1.48 and 0.92 for NDF, 1.49 and 0.90 for ADF, 0.52 and 0.76 for ADL, 2.11 and 0.84 for enzymatic solubility, 0.92 and 0.94 for protein content, and 0.18 and 0.76 for leaf to stem ratio.

Statistical analysis of the diallel design

Because the 7 parental plants were chosen from different populations and 1 plant per variety was used in making the crosses, the data were analysed as a fixed effect model (Baker, 1978). The diallel design was analysed according to Griffing's method 3, model I (1956) using the DIALLEL-SAS 1 of Zhang and Kang (1997). Variances across harvests tested with Bartlett's test were homogeneous. The model used in the DIALLEL-SAS 1 program was:

$$y_{ijkl} = \mu + g_i + g_j + s_{ij} + r_{ij} + h_k + b_l + b(h)_{lk} + gh_{ik} + gh_{jk} + sh_{ijk} + rh_{ijk} + e_{ijkl}$$

where μ is the mean of the population in the experiment, g_i is the general combining ability (GCA) effect for the *i*th parent, g_j is the GCA effect for the *j*th parent, s_{ij} is the specific compatibility (SCA) effect for the *ij*th F_1, r_{ij} are the reciprocal effects for the *ij*th or *ji*th F_1 (with $r_{ij} = m_i + m_j + n_{ij}$, where m_i is the maternal effect of the i_{th} parent, m_j is the maternal effect of the *j*th parent, n_{ij} is the non-maternal effect of the *ij*th or *ji*th F_1 progeny), h_k is the effect of *k*th harvest, b_l is the effect of *l*th block, $b(h)_{lk}$ is the effect of *l*th block within *k*th harvest, gh_{ik} is the interaction between GCA effect for the *i*th parent and *k*th harvest, gh_{jk} is the interaction between GCA effect for the *j*th parent and *k*th harvest, sh_{ijk} is the interaction between SCA effect for the *ij*th F_1 and harvest *k*, rh_{ijk} is the interaction between reciprocal effects for the *ij*th F_1 and harvest *k*, and e_{ijkl} is the residual error.

The model used to assess the F_1 progeny effect was:

$$y_{ijk} = \mu + h_i + b_j + b(h)_{ij} + f_k + (fh)_{ik} + e_{ijk}$$

where μ is the population mean, h_i is the effect of *i*th harvest, b_j is the effect of *j*th block, $b(h)_{ij}$ is the effect of *j*th block within *i*th harvest, f_k is the effect of *k*th F_1 progeny, $(fh)_{ik}$ is the interaction between F_1 progeny and *i*th harvest, and e_{ijk} is the residual effect. The terms h_i and b_j were tested against $b(h)_{ij}$, and $b(h)_{ij}$ was tested against the residual error.

Phenotypic correlations were calculated between pairs of traits using the CORR procedure of SAS (1988) on the mean value of F_1 progeny for each harvest. Genetic correlations were calculated using the MANOVA option of the GLM procedure of SAS with the variance/covariance matrix of genotype and the variance/covariance matrix of the interaction between genotype and harvest.

Factorial design

In 1996, 7 male parental plants and 7 female parental plants were crossed manually in a factorial design according to a North Carolina design II. The 14 parental plants were randomly sampled from within the 'Flamande' population. In 1997 the 49 F_1 half-sib progenies were established in 2 locations in France (the same as in the diallel design) in a complete randomised block design with 3 replicates. In total, 7 harvests were conducted over 3 years. The experimental design and the measurements were the same as in the diallel design.

Statistical analysis of the factorial design

Because the parental plants were randomly sampled from one population, the factorial design was analysed with F_1 progeny as a random effect. The first step of the factorial analysis was to test the F_1 progeny effect using the GLM procedure of SAS (1988). Variances across harvest tested with Bartlett's test were homogeneous. The model used was:

$$Y_{ijk} = \mu + h_i + b_j + b(h)_{ij} + f_k + (fh)_{ik} + e_{ijk}$$

where Y_{ijk} is the observed trait value in the experiment, μ is the grand mean, h_i is the effect of the *i*th harvest, b_j is the effect of the *j*th block, $b(h)_{ij}$ is the effect of the *j*th block within *i*th harvest, f_k is the effect of the *k*th F_1 progeny, $(fh)_{ik}$ is the interaction between *k*th F_1 progeny and *i*th harvest, and e_{ijk} is the residual error. As in the diallel design, harvests and blocks were tested against the block × harvest interaction, and block within harvest was tested against the residual error. For the factorial analysis the model used was:

$$Y_{ijkl} = \mu + h_k + b_l + b(h)_{lk} + m_i + f_j + mf_{ij} + mh_{ik} + fh_{jk} + mfh_{ijk} + e_{ijkl}$$

Table 1. Mean squares for the analysis of variance for the diallel design
GCA, general combining ability; SCA, specific combining ability; REC, reciprocals

Source of variation	d.f.	Enzymatic solubility (%)	NDF (%)	ADF (%)	ADL (%)	Protein content (%)	Leaf/stem	Plant height (cm)
Harvest	6	1923***	2379***	1683***	211***	1132***	4.25***	35781***
Block	2	4.8	0.3	3.5	0.2	4.5	0.052	76.6
Block (harvest)	12	23.3***	24.2***	17.9***	0.8***	4.7***	0.18***	195.5**
F_1 progeny	41	17.0***	21.0***	16.2***	0.9***	9.8***	0.05***	588.3***
GCA	6	62.0***	77.7***	60.0***	3.6***	42.7***	0.18***	2787***
SCA	14	5.9	6.3	4.4	0.4*	5.5***	0.02*	366***
REC	21	10.5***	13.0***	10.1***	0.5***	2.7**	0.03***	161**
F_1 progeny × harvest	246	7.5***	8.8***	6.6***	0.4***	2.7	0.02***	131.0***
GCA × harvest	36	21.2***	24.6***	18.3***	1.2***	8.5***	0.07***	444.9***
SCA × harvest	84	5.6	6.8	5.05	0.3	1.8	0.01	84
REC × harvest	126	4.4	5.1	3.9	0.2	1.3	0.01	61.9
Error	548[A]	3.8	4.7	3.5	0.2	1.3	0.01	71.7
Mean		67	41.8	31.3	7.5	18.6	0.68	72.2

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for significance of mean squares.
[A]d.f. of error should have been 574, but there were missing values due to one parent missing in one location.

where μ is the grand mean, h_k is the effect of the kth harvest, b_l is the effect of lth block, $b(h)_{lk}$ is the effect of the lth block within kth harvest, m_i is the effect of the ith male parent, f_j is the effect of the jth female parent, mf_{ij} is the interaction between ith male and jth female parents, mh_{ik} is the interaction between ith male and kth harvest, fh_{jk} is the interaction between jth female and kth harvest, mfh_{ijk} is the interaction between male, female and kth harvest, and e_{ijkl} is the residual effect. Variances for F_1 progeny, male, female, and male × female interaction were calculated from the expected mean squares using the VARCOMP procedure of SAS (1988). Additive (σ^2_A) and dominance (σ^2_D) variance based on an autotetraploid model were calculated according to Wricke and Weber (1986):

$$\sigma^2_A = 2(\sigma^2_M + \sigma^2_F) - 2/3(\sigma^2_{MF}) \text{ and } \sigma^2_D = 6\,\sigma^2_{MF}$$

where σ^2_M is the variance due to males, σ^2_F is the variance due to females, and σ^2_{FM} is the variance due to male × female interaction. The standard errors (s.e.) of variances for the random effects in the model were estimated according to Becker (1975) as:

$$\text{s.e.}(\sigma^2_g) = \left(\frac{2}{K^2} \Sigma_i \left(\frac{MS^2_i}{f_i + 2} \right) \right)^{1/2}$$

where MS_i is the mean square of effect i used to estimate the variance component g, f_i is the number of degrees of freedom corresponding to the effect i, and K is the coefficient of σ^2_g in the expected mean square of g. The narrow-sense heritability was calculated according to Wricke and Weber (1986):

$$h_N^2 = (\sigma^2_A + 1/3\,\sigma^2_D)/(\sigma^2_A + \sigma^2_D + \sigma^2_E)$$

Phenotypic correlations were calculated using the CORR procedure of SAS (1988) on the mean value of F_1 progeny in each condition. Genetic correlations were calculated using the MANOVA option of the GLM procedure of SAS with the variance/covariance matrix of genotype and the variance/covariance matrix of the interaction between genotype and harvest.

Results

Diallel design

In the diallel analysis the F_1 progeny effect was significant for all characters (Table 1). All traits except protein content exhibited significant genotype × harvest interaction. This interaction was mainly due to the GCA component. The GCA effect was significant for all the characters. The SCA effect was significant for plant height, leaf-to-stem ratio, protein content, and ADL, but not for NDF, ADF, and enzymatic solubility. For all characters, the mean squares for GCA were larger than for SCA. Reciprocal effects were significant for all characters. The presence of reciprocal effects resulted from maternal effects, meaning that the direction of the cross is important. These maternal effects were due to Orca-4, Aragon-5, and Malzeville-7, which showed significant maternal effects (Table 2). Orca-4 showed significant positive maternal effect for NDF, ADF, ADL, and plant height and negative for enzymatic solubility and leaf-to-stem ratio. Aragon-5 and Malzeville-7 showed a significant negative maternal effect for NDF, ADF, ADL, and plant height, and a positive maternal effect for enzymatic solubility. Aragon-5 showed significant positive maternal effect for leaf-to-stem ratio.

GCA estimates (Table 3) of Europe-1 and Orca-4 were positive and significant for NDF, ADF, and ADL and negative for enzymatic solubility, protein content, and leaf-to-stem ratio. Aragon-5 showed negative and significant GCA for NDF, ADF, and ADL, and positive for enzymatic solubility, protein content and leaf-to-stem ratio.

Enzymatic solubility showed negative and highly significant genetic and phenotypic correlations (Table 4) with plant height, NDF, ADF, and ADL and positive with leaf-to-stem ratio and protein content. Plant height was

Table 2. Maternal effects for the seven parents in the diallel cross

	Enzymatic solubility (%)	NDF (%)	ADF (%)	ADL (%)	Protein content (%)	Leaf/stem	Plant height (cm)
Europe-1	0.04	0.013	–0.008	–0.008	–0.03	–0.001	0.46
Magali-2	–0.03	0.040	0.026	0.010	0.03	–0.004	0.81
Luzelle-3	–0.06	0.040	0.050	0.040	0.12	0.002	–0.11
Orca-4	–0.57***	0.060***	0.49 ***	0.10***	–0.12	–0.024**	1.59**
Aragon-5	0.35***	–0.44***	–0.41***	–0.07***	–0.016	0.026***	–0.18
Krasnokutskaya-6	–0.07	0.14	0.12	0.02	–0.046	–0.001	–0.66
Malzeville-7	0.34**	–0.37**	–0.27**	–0.09***	0.31***	0.002	–1.90***
s.e.	0.1	0.1	0.1	0.03	0.07	0.06	0.5

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for maternal effects significantly different from 0.

Table 3. General combining ability (GCA) effects for the seven parents in the diallel cross

	Enzymatic solubility (%)	NDF (%)	ADF (%)	ADL (%)	Protein content (%)	Leaf/stem	Plant height (cm)
Europe-1	–0.84***	0.96***	0.85***	0.21***	–0.64***	–0.03***	4.82***
Magali-2	0.17	–0.25	–0.23	–0.01	–0.14	–0.008	2.88***
Luzelle-3	–0.08	0.003	–0.003	–0.06	0.09***	–0.006	–3.03***
Orca-4	–0.62***	0.71***	0.62***	0.18***	–0.55***	–0.03***	3.18***
Aragon-5	0.85***	–0.94***	–0.83***	–0.12***	0.22**	0.06***	0.60
Krasnokutskaya-6	0.25	–0.29*	–0.25	–0.13***	0.67***	0.02*	–4.97***
Malzeville-7	0.28*	–0.19	–0.12	–0.06*	0.34***	0.004	–3.49***
s.e.	0.13	0.14	0.13	0.03	0.07	0.007	0.6

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for GCA significantly different from 0.

Table 4. Phenotypic and genetic (*in italics*) correlations among F_1 progenies in the diallel cross

	NDF	ADF	ADL	Protein content	Leaf/stem	Plant height
Enzymatic solubility	–0.86*** *–0.99*	–0.84*** *–0.89*	–0.45*** *–0.90*	0.48*** *0.63*	0.68*** *0.93*	–0.80*** *–0.55*
NDF		0.98*** *0.99*	0.82*** *0.90*	–0.59*** *–0.58*	–0.88*** *–0.93*	0.62*** *0.55*
ADF			0.83*** *0.89*	–0.48*** *–0.55*	–0.88*** *–0.93*	0.56*** *0.52*
ADL				–0.56*** *–0.82*	–0.82*** *–0.75*	0.21*** *0.82*
Protein content					0.56**** *0.42*	–0.51*** *–0.83*
Leaf/stem						–0.46*** *–0.41*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for significance of phenotypic correlations.

positively correlated with NDF, ADF, and ADL, and negatively with leaf-to-stem ratio, protein content, and enzymatic solubility. NDF, ADF, ADL, and enzymatic solubility were positively and strongly correlated with each other. Genetic and phenotypic correlations were similar except for the correlations between plant height and ADL, protein content and plant height, enzymatic solubility and plant height, and between enzymatic solubility and ADL.

Factorial design

In the factorial analysis (Table 5) the F_1 progeny effect was significant for all characters tested. The female effect was highly significant for all characters except for the protein content and the male effect was highly significant for all traits. According to the mean squares, female effect was larger than male effect for plant height and for leaf-to-stem

Table 5. Mean squares for the analysis of variance for the 7×7 factorial cross
F, female effect; M, male effect; F × M, interaction between male and female.

Source of variation	d.f.	Enzymatic solubility (%)	NDF (%)	ADF (%)	ADL (%)	Protein content (%)	Leaf/stem	Plant height (cm)
Harvest	6	2543.5***	3447***	2450.71***	293.8***	1120***	6.70***	39986.4***
Block	2	1	0.76	1.3	0.072	14.9	0.016	110.5
Block (harvest)	12	22.02***	35.2***	30.9***	1.5***	7.9***	0.064***	505.6***
F_1 progeny	48	18.7***	27.02***	20.7***	0.79***	3.6***	0.070***	134.6***
F	6	46.2***	61.1***	53.2***	1.80***	2.5*	0.24***	347.8***
M	6	71.9***	118.1***	84.1***	3.2***	17.5***	0.21***	279.7***
F × M	36	4.08	5.2*	3.9*	0.17	1.3	0.013*	79.6*
F_1 progeny × harvest	288	3.6*	4.2*	3.3*	0.20*	1.1	0.013	65.8**
F × harvest	36	5.8**	6.3**	5.03**	0.33**	1.1	0.017**	197.3***
M × harvest	36	4.2	5.2*	3.7	0.27*	2.1***	0.015*	93.1**
F × M × harvest	216	3.2	3.6	2.8	0.17	1	0.007	40.5
Error	665[A]	3.04	3.5	2.7	0.17	1.00	0.009	49.5
Mean		65.7	43.4	32.6	7.9	16.8	0.67	81.9

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for significance of mean squares.
[A]d.f. of error should have been 670 but there were missing data.

Table 6. Phenotypic and genetic (*in italics*) correlations among F_1 progeny for the 7 × 7 factorial cross

	NDF	ADF	ADL	Protein content	Leaf/stem	Plant height
Enzymatic solubility	–0.89*** *–0.99*	–0.85*** *–0.99*	–0.50*** *–0.95*	0.59*** *0.77*	0.66*** *0.92*	–0.74*** *–0.80*
NDF		0.98*** *0.99*	0.81*** *0.97*	–0.71*** *–0.76*	–0.89*** *–0.91*	0.53*** *0.79*
ADF			0.84*** *0.97*	–0.60*** *–0.76*	–0.90*** *–0.91*	0.43*** *0.79*
ADL				–0.60*** *–0.87*	–0.92*** *–0.90*	0.17*** *0.71*
Protein content					0.71*** *0.68*	–0.49*** *–0.61*
Leaf/stem						–0.28*** *–0.97*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for significance of phenotypic correlations.

ratio. The female × male interaction was significant for plant height, NDF, ADF, and leaf-to-stem ratio. The F_1 progeny × harvest interaction was significant for all traits except for protein content and leaf-to-stem ratio but mean squares of the interaction were low.

Enzymatic solubility showed negative and highly significant phenotypic correlations (Table 6) with plant height, NDF, ADF, and ADL, and positive correlation with leaf-to-stem ratio and protein content. Plant height was positively and highly correlated with NDF and ADF. As in the diallel design, genetic and phenotypic correlations were similar except for the correlation between plant height and ADL, ADL and enzymatic solubility, plant height and leaf-to-stem ratio, leaf-to-stem ratio and enzymatic solubility, and between ADL and protein content. In 3 harvests (Capelle-en-Pévèle in 1998: first cut and second cut), plant height showed different and lower phenotypic correlations with ADL and leaf-to-stem ratio than in the others harvests.

The male variance component was higher than the female variance component for protein content, NDF, ADF, ADL, enzymatic solubility, and plant height (Table 7). The female × male interaction was lower than female and male effects for all characters tested, except for protein content where male variance was lower than female × male interaction. The additive variance was larger than the dominance variance for all characters except for plant height. The error variance was large for all traits. Because of this large error variance the estimates of the narrow-sense heritability were relatively low, with the lowest one for plant height (0.09) and protein content (0.18). The highest narrow-sense heritabilities were found for NDF (0.39), ADF (0.38), leaf-to-stem ratio (0.37), and enzymatic solubility (0.33).

Discussion

The diallel analysis gave information on GCA, SCA, and reciprocal effects among full-sib families. In addition the

Table 7. Estimates of genetic variances and narrow-sense heritability in the factorial analysis

Standard errors (s.e.) are given in parentheses. σ^2_G, variance among F_1 progenies; σ^2_F, female variance; σ^2_M, male variance; $\sigma^2_{F\times M}$, female × male variance; σ^2_E, residual variance; σ^2_A, additive variance; σ^2_D, dominance variance; h^2_N, narrow-sense heritability

	σ^2_G	σ^2_F	σ^2_M	$\sigma^2_{F\times M}$	σ^2_E	σ^2_A	σ^2_D	h^2_N
Enzymatic solubility	0.730 (0.18)	0.271 (0.16)	0.456 (0.25)	0.041 (0.0068)	2.950 (0.16)	1.43	0.246	0.33
NDF	1.090 (0.26)	0.366 (0.21)	0.761 (0.40)	0.074 (0.0024)	3.450 (0.19)	2.20	0.444	0.39
ADF	0.830 (0.19)	0.323 (0.18)	0.541 (0.29)	0.052 (0.0066)	2.680 (0.15)	1.69	0.312	0.38
ADL	0.030 (0.0075)	0.010 (0.0062)	0.020 (0.011)	0.000 (0.0003)	0.170 (0.009)	0.06	0	0.26
Protein content	0.120 (0.035)	0.007 (0.0091)	0.100 (0.06)	0.014 (0.0021)	1.003 (0.055)	0.20	0.084	0.18
Leaf/stem	0.003 (0.0006)	0.001 (0.0008)	0.001 (0.0007)	0.0002 (0.00002)	0.009 (0.0005)	0.01	0.001	0.37
Plant height	3.240 (1.32)	0.780 (1.24)	1.060 (0.95)	1.540 (2.67)	49.40 (2.67)	2.65	9.240	0.09

factorial analysis gave information on genetic variance components and made it possible to calculate narrow-sense heritability among half-sib families. The diallel analysis showed that GCA effects were significant and larger than SCA effects for all characters, indicating a mainly additive inheritance. Similarly, in the factorial analysis, additive variance was larger than dominance variance for all characters except for plant height. The nature of gene action for digestibility traits was similar within one population and among plants randomly selected from different populations. Previous studies of heritability for digestibility have reported that most genetic effects were additive (Neff and Simon 1986). Chaverra Gil *et al.* (1967) found significant and positive GCA and SCA effects for *in vitro* dry matter digestibility, with GCA values higher than SCA. This was in agreement with Hill and Barnes (1977) who reported significant GCA effects for ADF, leaf-to-stem ratio, and protein content.

The presence of reciprocal effects resulted from maternal effects. Before initiating a breeding program, maternal effects could be controlled in an experimental design with reciprocals, and parental plants with positive maternal effect could be selected, but it would be very time consuming. In a breeding program for the production of synthetic varieties, after 3 generations of multiplication, the determination of maternal effects is almost impossible.

In the factorial design, genetic variance within-population was the highest one for plant height, NDF, and ADF. Julier *et al.* (2000) and Crochemore *et al.* (1996) reported higher within-population variance than among-population genetic variance for morphological traits in lucerne. In their study, genetic variance in Flemish populations was observed for quality traits and was as large as genetic variance among populations. The within-cultivar variance accounted for 31–70% of the total genetic variance for quality traits (Julier *et al.* 2000).

In our experiment, enzymatic solubility was positively correlated with leaf-to-stem ratio and protein content and negatively with NDF, ADF, ADL, and plant height. NDF, ADF, and ADL were positively correlated with each other and negatively with protein content. This was in agreement with results reported by Hill and Barnes (1977). Genetic correlations were negative between plant height and digestibility (–0.55 in the diallel analysis and –0.80 in the factorial analysis). Julier *et al.* (2000) reported a relatively low correlation ranging from 0.14 to 0.55 for dry matter yield and NDF content. Julier and Huyghe (1997) reported no significant correlation between forage yield and digestibility at harvest. However, Chaverra Gil *et al.* (1967) and Shenk and Elliott (1970) mentioned that the development of high yielding, highly digestible cultivars was difficult because of the negative relationship between digestibility and forage yield.

Narrow-sense heritabilities were calculated for all characters tested in the factorial design. Low estimates of heritability were obtained for plant height (0.09) and protein content (0.18) because of a narrow genetic variability in the material under study. Estimates of narrow-sense heritability were 0.39 for NDF and 0.38 for ADF and slightly lower for enzymatic solubility (0.33) and ADL (0.26). Hill and Barnes (1977) reported high estimates of broad-sense heritability for lignin (0.48) and protein (0.64) and extremely low estimates for IVDMD (0.079). Those estimates were obtained from an analysis of a set of 4 five-parents diallel crosses from 2 lucerne cultivars. They concluded that given this low estimate of heritability for IVDMD, no response to selection with any breeding method could be expected. This was in agreement with results of Kellogg *et al.* (1976), but not with those of Shenk and Elliott (1970) and Thomas *et al.* (1968). Shenk and Elliott (1970) obtained a response to selection for 6-h *in vitro* dry matter disappearance after 2 cycles of selection for improved nutritive value. In breeding for improved feeding value of lucerne, Hill and Barnes (1977) recommended the selection for greater protein content or reduced ADF, NDF, or lignin content over the selection for greater IVDMD. Jung *et al.* (1994) reported that selection for low lignin was more effective in altering cell wall degradation than selecting for IVDMD.

From our experiments, narrow-sense heritabilities for NDF, ADF, ADL, and enzymatic solubility indicated that NDF or ADF would be more efficient selection criteria than enzymatic solubility or ADL in breeding programs. Although narrow-sense heritability was quite low for enzymatic solubility, NDF, ADF, and ADL, the potential for

improvement of nutritive value exists, given the additive nature of gene action. The large variance error observed could be reduced by improving the sampling method or by increasing the number of blocks.

In this study, spaced plants were used, and the results will be applied in a dense stand situation. B. Julier (1997, unpublished data) showed that there was a high correlation between quality traits in dense *v.* spaced plants.

The within-population genetic variation for quality traits and the additive nature of gene action in a breeding program for improving nutritive value have to be exploited without reducing forage yield.

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