Quality evaluation of Medicago sativa materials belonging to the Italian ecotype "Romagnola"

Torricelli R., Veronesi F., Mazza L., Schiatti F.

in

Delgado I. (ed.), Lloveras J. (ed.).
Quality in lucerne and medics for animal production

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 45

2001
pages 67-71

Quality evaluation of *Medicago sativa* materials belonging to the Italian ecotype 'Romagnola'

R. Torricelli*, L. Mazza, F. Schiatti*** and F. Veronesi***
*Department of Botany and Agroenvironmental Biotechnologies, Università degli Studi di Perugia, Borgo XX giugno 74, 06121 Perugia, Italy
**Consorzio Nazionale Sementi, via Selice 301/A, 48017 Conselice, Ravenna, Italy
***Studio Consulenza Agraria Franco Schiatti, via L. Ariosto 10, 42100 Reggio Emilia, Italy

SUMMARY – On the basis of preliminary results concerning the analysis of lucerne variability for quality traits, it seems worthwhile paying more attention to the variability between entries than within: within entry variability seems quite difficult to handle, even if the absence of a negative correlation of statistical significance between dry matter yield and crude protein content deserve a certain interest. To this respect, a large number of single plants should be analysed to apply selection differentials within lucerne populations and to start with a breeding program. Such a breeding approach could be quite expensive and it is difficult to propose to private breeders in the absence of a clear economic return in terms of higher market prices for higher quality lucerne products.

Key words: Lucerne, variability, crude protein, breeding.

RESUME – *"Evaluation de la qualité de matériels de* Medicago sativa *appartenant à l'écotype italien 'Romagnola'". Sur la base des résultats préliminaires concernant l'analyse de la variabilité de la luzerne pour les traits de qualité, il semble intéressant de se concentrer plus sur la variabilité entre les différentes populations, que celle à l'intérieur de la même population. La variabilité à l'intérieur de la même population semble plutôt difficile à traiter, même si l'absence d'une corrélation négative statistiquement significative entre la production de substance sèche et le contenu en protéines est digne d'attention. À ce propos, un grand nombre de plantes devraient être analysées pour appliquer des différentiels de sélection dans les populations de luzerne et pour commencer un programme d'amélioration génétique. Un tel programme pourrait être plutôt cher à proposer à des cultivateurs privés sans avoir un évident revenu à travers des prix plus élevés pour une luzerne de meilleure qualité.*

Mots-clés : *Luzerne, variabilité, protéine, amélioration génétique.*

Introduction

Lucerne (*Medicago sativa* L., 2n = 4x = 32) is the most important forage legume in temperate climates (Michaud *et al*., 1988) and in Italy it is grown on about 800,000 ha. At the moment, its importance is raising with the increase of public interest in sustainable agriculture because, as reported by McCoy and Echt (1992) alfalfa is a low input energy efficient crop that helps improve soil tilth. Furthermore, it occupies a significant economic position in the animal feed market (i.e. hay, dehydrated forage, pellets and silage products) and deserve a particular interest in the Parmigiano cheese production area of Italy (Torricelli *et al*., 2000).

Since quite a long time ago Italian lucerne breeders clearly shown the importance to transform the still very common local ecotypes in broad-based varieties with the aim of controlling seed quality; in 1995 governmental regulations indicated that ecotypes will be definitively cancelled from the National Register of Varieties in 2002 (Barcaccia *et al*., 1997).

On the basis of the above reported reasons, since 1990 the Consorzio Nazionale Sementi (Co.Na.Se) started a program with the aim to synthesise a broad-based lucerne variety from the 'Romagnola' ecotype, the most diffused lucerne ecotype in Italy. The breeding program was developed through an agreement between Co.Na.Se. and the Plant Breeding Institute of Perugia (actually Genetics and Breeding Section of the Department of Botany and Agroenvironmental Biotechnologies of Perugia). As a result of the program, one of the first example of the practical co-operation between private and public plant breeders in forage legume research in Italy, the lucerne variety 'Classe', obtained through phenotypic selection, has been inscribed in the National Register. The variety is swiftly increasing its diffusion and it is at the moment considered one of the top varieties in its area of adaptation (Emilia Romagna region, north-eastern Italy).

During the development of the program, some information have been collected on quality traits and the present paper is reporting the obtained results, which have to be considered widely introductory.

Materials and methods

The experiments were carried out at Conselice (Emilia Romagna).

Experiments A: Initial evaluation

In 1992-93 a dense stand evaluation trial, planned to evaluate the basic materials, was sown with 'Romagnola' and 7 different varieties ('Boreal', 'Capital', 'Casalina', 'Delta', 'Equipe', 'Giulia' and 'Selene'), according to a randomised block design with 5 replications using 10 m^2 plots and a seeding rate of 40 kg/ha of viable seed. The crop was cut at full bloom twice in 1992 (15/07 and 19/08) and 3 times in 1993 (27/05, 9/07 and 6/08); at each cut, data were collected on dry matter yield (DMY, t/ha). At the second 1993 cut a sample of 1 kg of green matter yield (GMY) was collected in each plot; the material was dried in a ventilated oven at 60ºC for 48 hours and for each sample data have been collected on: crude protein (CP, % on DMY), crude fibre (CF, % on DMY), acid detergent fibre (ADF, % on DMY) and lignin (LI, % on DMY) contents.

Experiment B: Phenotypic evaluation

One thousand seedlings of 'Romagnola' were transplanted in Jiffy pots in December 1991 and maintained in a greenhouse during the winter; 940 plants were space-transplanted in a nursery in April 1992. The plants were evaluated for agronomic traits during the 1992-93 growing seasons and utilised to carry out the phenotypic selection program as reported by Mazza *et al*. (1994).

At the first 1993 cut, 34 plants were randomly chosen; green matter yield for each plant was dried and dry matter utilised to obtain the following data: DMY (g/plant), dry matter content (DMC = DMY/GMY), CP, CF, ashes (A, % on DMY). For the same plants, data related to flowering (F; 1 = late, 5 = early), leafiness (L; 1 = low, 3 = high), growth habit (GH; 1 = erect, 4 = prostrate) and spring regrowth (SR; 1 = minimum, 6 = maximum) were also collected.

In both experiments (A and B), as the ranges of percentages appeared to be quite low, arcsin ÷P transformation was not applied (Little and Hills, 1978; Steel and Torrie, 1980) and actual data were statistically analysed.

Results and discussion

Experiment A

Data relative to total DMY (1992 + 1993 cuts), CP, CF, ADF and LI are reported in Table 1. As already reported by Mazza *et al*. (1994), 'Romagnola' appeared to be the most interesting material for DMY. Looking at CP, the materials showed a range of variation between 19.99% ('Giulia') and 21.70% ('Boreal') and 'Romagnola' appeared characterised by an average CP (20.04%) significantly lower than 'Boreal'. CF ranged between 29.44% ('Selene') and 26.85% ('Delta'); 'Romagnola', with an average CF of 28.83%, was not significantly different from any other entry. 'Romagnola' was characterised by the highest value of ADF (32.19%) while 'Boreal' showed the lowest level (28.89%); for this character 'Romagnola' appeared to be significantly different from 'Delta' and 'Boreal'. Absence of significant differences was shown by LI.

Experiment B

Average values relative to plant evaluation are reported in Table 2; single data were utilised to apply a multiple regression approach, using CP as dependent variable (Y). First of all, a backward elimination procedure (Draper and Smith, 1981) permitted to remove variables DMC and GH due to their small partial-*F* values. A multiple determination coefficient $R^2_{Y.1,2,3,4,5,6}$ = 0.456 was obtained (where 1 = DMY; 2 = F; 3 = L; 4 = SR; 5 = CF; 6 = A); as a consequence, the 6 traits do not permit to explain more than 45.6% of the total variability shown by CP.

Table 1. Experiment A: dry matter yield (DMY, t/ha, total of 1992 + 1993), crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), lignin (LI)

Materials	DMY	CP	CF	ADF	LI
Boreal	15.33 bc	21.70 a	27.75 ab	28.89 c	5.53
Capital	17.61 ab	20.42 ab	28.75 ab	32.29 a	6.42
Casalina	15.73 bc	20.20 b	28.76 ab	31.89 ab	6.35
Delta	13.51 c	21.40 ab	26.85 b	29.50 bc	6.08
Equipe	13.55 c	20.96 ab	28.96 a	31.01 abc	6.09
Giulia	16.41 ab	19.99 b	28.84 ab	31.35 ab	5.88
Romagnola	18.72 a	20.04 b	28.83 ab	32.19 a	6.29
Selene	13.09 c	20.11 b	29.44 a	32.09 a	5.98

[a,b,c]Means followed by the same letter are not significantly different at $P \leq 0{,}05$.

Table 2. Experiment B: averages values and ranges of variation relative to traits collected on spaced plants

Characters	Average ± se	Range	
Dry matter yield (1)	383.79 ± 18.43	180.00	677.00
Flowering (2)	2.46 ± 0.13	1.00	4.30
Leafiness (3)	2.47 ± 0.10	1.00	3.00
Spring regrowth (4)	3.63 ± 0.11	1.80	4.50
Crude fibre (5)	27.53 ± 0.56	20.30	32.87
Ashes (6)	8.56 ± 0.18	5.08	11.72
Dry matter content (7)	79.84 ± 0.33	76.77	83.00
Growth habit (8)	2.12 ± 0.08	1.00	3.30
Crude protein (Y)	19.82 ± 0.27	15.30	22.70

Further information are coming from the analysis of simple correlations through a path coefficient procedure (Wright, 1923; Li, 1955; Dewey and Lu, 1959) whose results are reported in Table 3. The only interesting information seems to be the direct effects on CP of both DMY ($P_{X1.Y}$ = -0.207) and A ($P_{X6.Y}$ = 0.227); even in these cases the effects are of limited intensity.

Conclusion

On the basis of our preliminary results, for quality traits it seems worthwhile to give more attention to the among entries variability than to the within entry variability: within entry variability seems quite difficult to handle, even if the absence of a negative correlation of statistical significance between DMY and CP deserves a certain interest. In this respect, a far larger number of single plants should be analysed to get more clear information and, eventually, to apply selection differentials and start with a breeding program. Such a breeding approach could be quite expensive and it is difficult to propose to private breeders in the absence of a clear economical return in terms of higher market prices for lucerne products of higher quality.

Table 3. Experiment B: path coefficient analysis (independent variables: dry matter yield, flowering, leafiness, spring regrowth, crude fibre, ashes; dependent variable: crude protein)

Dry matter yield (1) vs. Crude protein (Y)	r = -0.110	Flowering (2) vs. Crude protein (Y)	r = -0.136
Direct effect, $P_{X1.Y}$	-0.207	Direct effect, $P_{X2.Y}$	-0.107
Indirect effect via flowering, r_{12} $P_{X2.Y}$	-0.015	Indirect effect via dry matter yield, r_{12} $P_{X1.Y}$	-0.030
Indirect effect via leafiness, r_{13} $P_{X3.Y}$	-0.037	Indirect effect via leafiness, r_{23} $P_{X3.Y}$	-0.034
Indirect effect via spring regrowth, r_{14} $P_{X4.Y}$	0.090	Indirect effect via spring regrowth, r_{24} $P_{X4.Y}$	0.070
Indirect effect via crude fibre, r_{15} $P_{X5.Y}$	0.022	Indirect effect via crude fibre, r_{25} $P_{X5.Y}$	-0.073
Indirect effect via ashes, r_{16} $P_{X6.Y}$	0.037	Indirect effect via ashes, r_{26} $P_{X6.Y}$	0.011
Total	-0.110	Total	-0.163

Leafiness (3) vs. Crude protein (Y)	r = -0.127	Spring regrowth (4) vs. Crude protein(Y)	r = 0.244
Direct effect, $P_{X3.Y}$	-0.194	Direct effect, $P_{X4.Y}$	0.265
Indirect effect via dry matter yield, r_{13} $P_{X1.Y}$	-0.040	Indirect effect via dry matter yield, r_{14} $P_{X1.Y}$	-0.071
Indirect effect via flowering, r_{23} $P_{X2.Y}$	-0.019	Indirect effect via flowering, r_{24} $P_{X2.Y}$	-0.028
Indirect effect via spring regrowth, r_{34} $P_{X4.Y}$	0.054	Indirect effect via leafiness, r_{34} $P_{X3.Y}$	-0.040
Indirect effect via crude fibre, r_{35} $P_{X5.Y}$	0.047	Indirect effect via crude fibre, r_{45} $P_{X5.Y}$	0.187
Indirect effect via ashes, r_{36} $P_{X6.Y}$	0.025	Indirect effect via ashes, r_{46} $P_{X6.Y}$	-0.069
Total	-0.127	Total	0.244

Crude fibre (5) vs. Crude protein (Y)	r = -0.594	Ashes (6) vs. Crude protein (Y)	r = -0.026
Direct effect, $P_{X5.Y}$	-0.561	Direct effect, $P_{X6.Y}$	0.227
Indirect effect via dry matter yield, r_{15} $P_{X1.Y}$	0.008	Indirect effect via dry matter yield, $r_{16}P_{X1.Y}$	-0.034
Indirect effect via flowering, r_{25} $P_{X2.Y}$	-0.014	Indirect effect via flowering, r_{26} $P_{X2.Y}$	-0.005
Indirect effect via leafiness, r_{35} $P_{X3.Y}$	0.016	Indirect effect via leafiness, r_{36} $P_{X3.Y}$	-0.021
Indirect effect via spring regrowth, r_{45} $P_{X4.Y}$	-0.088	Indirect effect via spring regrowth, r_{46} $P_{X6.Y}$	-0.081
Indirect effect via ashes, r_{56} $P_{X6.Y}$	0.045	Indirect effect via crude fibre, r_{56} $P_{X5.Y}$	-0.112
Total	-0.594	Total	-0.026

References

Barcaccia, G., Falcinelli, M., Papa, R., Pellicoro, A., Tavoletti, S. and Veronesi, F. (1997). Genomic variability estimation and agronomic evaluation of cultivated lucerne populations of central Italy. In: *Proceedings of the XX Meeting of Eucarpia Fodder Crops and Amenity Grasses Section,* Staszewski Z., Mlyniec W. and Osinki R. (eds), Radzikow (Poland), 7-10 October 1996, pp. 48-56.

Dewey, D.R. and Lu, K.H. (1959). A correlation and path-coefficient analysis of components of crested wheatgrass seed production. *Agron. J.,* 51: 515-518.

Draper, N.R. and Smith, H. (1981). *Applied Regression Analysis*. John Wiley and Sons, New York, 709 pp.

Li, C.C. (1955). *Population Genetics.* University of Chicago Press, pp. 144-187.

Little, M.T. and Hills, F.J. (1978). *Agricultural Experimentation*. John Wiley and Sons, New York, pp. 350.

Mazza, L., Schiatti, F., Falcinelli, M., Torricelli, R. and Veronesi, F. (1994). A new lucerne variety coming from the Italian ecotype "Romagnola" through phenotypic selection. In: *Proceedings of the Meeting "Management and Breeding of Perennial Lucerne for Diversified Purposes",* Lusignan (France), 4-8 September 1994. FAO, Rome, pp. 195-197.

McCoy, T.J. and Echt, C.S. (1992). Chromosome manipulations and genetic analysis in *Medicago*. *Plant Breeding Reviews*, 10: 167-197.

Michaud, R., Lehman, W.F. and Rumbaugh, M.D. (1988). World distribution and historical development. In: *Alfalfa and Alfalfa Improvement*, Hanson, A.A. (ed.). ASA, CSSA, SSSA Publishers, Madison, Wisconsin, pp. 25-91.

Steel, G.D. and Torrie, J.H. (1980). *Principles and Procedures of Statistics. A Biometrical Approach*. McGraw-Hill Book Company, New York, pp. 633.

Torricelli, R., Mazza, L., Falcinelli, M. and Veronesi, F. (2000). Development of lucerne varieties coming from the Italian ecotype 'Romagnola'. In: *Lucerne and Medics for the XXI Century. Proceedings of the XIII Eucarpia Medicago* spp. *Group Meeting*, Veronesi F. and Rosellini, D. (eds), Perugia (Italy), 13-16 September 1999, pp. 321-324.

Wright, S. (1923). The theory of path coefficients. *Genetics,* 8: 239-255.