BREEDING FOR INCREASED FORAGE YIELD AND QUALITY IN ALFALFA

D A. Phillips, M. W. Demment, and L. R. Teuber
Department of Agronomy and Range Science
University of California, Davis, CA 95616

Previous attempts to select directly for increased forage yield and quality in alfalfa have not been notably successful (Elliott et al., 1972). At least two factors are responsible for that situation. First, larger plants generally contain a higher proportion of undesirable quality components, such as lignin, to support their increased weight. Second, it is easier and certainly profitable for seed suppliers to develop new disease-resistant alfalfa varieties. Although disease-resistant alfalfa varieties contribute significantly to agricultural productivity, there is little evidence that, in the absence of the pathogen, such plants produce more forage than related, susceptible cultivars. In contrast, some grasses that have been selected for physiological vigor, rather than for disease resistance, show higher yields under disease-free conditions (Wilson and Jones, 1982).

Since 1979 our group has been testing whether it is possible to develop alfalfa plants that produce more forage with similar or improved quality traits. This report describes the very promising results we have obtained thus far in this project and indicates how we will be extending our present results to field tests over the next 5 years.

Our approach to improving alfalfa requires an understanding of how the plant assimilates nitrogen (N), a mineral element crucial for protein synthesis in this protein-rich plant. Nitrogen in alfalfa comes either from direct fixation of atmospheric N_2 gas by Rhizobium bacteria in the alfalfa root nodules or from assimilation of mineral N compounds such as nitrate and ammonium in the soil. A high level of soil N inhibits N_2 fixation, but in most field environments alfalfa plants probably use N from both soil nitrate and N_2. In addition, the amount of soil nitrate available to an alfalfa crop can change drastically during the life of a normal stand, because fertilizer N carryover depends greatly on the previous crop. For these reasons it makes sense to have alfalfa plants that can use both N_2 and soil N efficiently.

RESULTS

In 1979 we observed that individual alfalfa plants performed quite differently on various N sources (Sheehy et al., 1980). That test was done by making stem cuttings from 35 different alfalfa plants and comparing various physiological traits of genetically identical plantlets (cuttings) grown separately on either N_2 or NH_4NO_3. Our conclusion from those experiments was that many alfalfa plants grew well on one source of N but not on the other. That observation suggested that superior alfalfa varieties might be developed by selecting plants that grow well under both N_2-dependent and NH_4NO_3-dependent conditions.

In 1980 we screened over 100 different plants of Hairy Peruvian for their capacity to grow on N_2 and on NH_4NO_3 under greenhouse conditions. By establishing the plants initially with Rhizobium meliloti 102F28 in the absence of NH_4NO_3 (nil-N), the plants formed root nodules and fixed N_2. The shoot of every plant was clipped on the first day a single floret opened. Forage from the seedling growth period was discarded, and plants were maintained during the first regrowth on the nil-N nutrient solution. When each plant flowered for a second time, the shoot was clipped, weighed, and analyzed for reduced N concentration (crude protein). On the same day, the plant was given the first daily treatment with 8 mM NH_4NO_3, which stopped N_2 fixation in the root nodules. When each plant flowered for a third time, the shoot was harvested again, weighed and analyzed for crude protein content. This procedure allowed us to measure plant growth under both N_2- and NH_4NO_3-dependent growth conditions. ONLY THOSE PLANTS THAT PRODUCED A HIGH FORAGE DRY WEIGHT WITH HIGH FORAGE N CONCENTRATION (CRUDE PROTEIN) ON BOTH N_2 AND NH_4NO_3 WERE SELECTED AS PARENTS FOR THE NEXT GENERATION. From 122 original Hairy Peruvian plants, only five plants were chosen (Phillips et al., 1982). Those five plants were intercrossed randomly without emasculation during the winter months to produce the cycle-1 population, which was subjected to the same selection procedure in 1981. Plants selected in 1981 were intercrossed in the same manner to produce a second generation of improved alfalfa, Hairy Peruvian 32 (HP32), from the original Hairy Peruvian (HP) population.

In 1982 it was possible to assess the breeding progress made by comparing HP and HP32 in the greenhouse under the original conditions used for selection. Those tests showed that we had increased forage dry matter and N assimilation in plants grown under the controlled N conditions used during the selection process (Table 1). Calculations from those data established the realized heritabilities for the various traits and indicated the promising potential of the selection protocol (Teuber et al., 1984).

Table 1. The effect of plant breeding on forage yield and crude protein content in alfalfa grown under greenhouse conditions. Initial populations of Hairy Peruvian and Moapa 69 were improved through two generations of selection for high forage dry weight and high forage protein concentration on both 0 and 8 mM NH_4NO_3 to produce Hairy Peruvian 32 and Moapa 69-32. Original and improved populations were tested separately on N_2 and NH_4NO_3 nitrogen sources to assess the breeding progress. Values below represent the percent improvement of the selected population over Hairy Peruvian or Moapa 69.

Improved alfalfa population	Nitrogen source			
	NH_4NO_3		N_2	
	Dry weight	Protein	Dry weight	Protein
	----------% change----------			
Hairy Peruvian 32	+42*	+45*	+69*	+81*
Moapa 69-32	+57	+54	+28*	+27

* - Breeding effect significant at $P < 0.05$

Although the 1982 tests of HP32 established that we had improved forage dry matter and N assimilation by 40 to 80% under greenhouse conditions (Table 1), a number of critical questions remained. First, how had we affected plant growth under N conditions more typical of those found in various soil environments? Second, did HP32 respond only in the presence of the Rhizobium strain used during the selection process? Third, how had we affected normal parameters of forage quality in the material? Fourth, was HP32 better than HP under field conditions? Finally, could the technique be applied to common agronomically important cultivars which already had been subjected to intense selection for other traits? We have spent the past 3 years answering some of those questions, and we have detailed plans for the next 5 years to complete the necessary experiments and field trials. Here we provide a brief synopsis of relevant information.

Detailed studies under controlled conditions in which a range of NO_3^- concentrations with R. meliloti 102F28 were tested on both HP and HP32 showed that HP32 was significantly superior to HP at all NO_3^- levels (Table 2). Because isotopically-labeled NO_3^- was used in these studies, it was possible to separate total plant N into fractions derived from N_2 and NO_3^-. Those data showed that across all NO_3^- treatments HP32 fixed an average of 38% more N_2 than HP (Phillips et al., 1985b). Taken as a whole, the data clearly indicate that HP32 performs better than HP with the 1-2 mM NO_3^- plus Rhizobium conditions that one would expect to find in most field environments.

Using microbiologically-controlled conditions in the greenhouse, we found that HP32 assimilated more forage dry matter and N than HP with every strain of Rhizobium meliloti tested (Table 3). HP32 produced the largest increase in N_2 fixation (62%) with strain 102F28, which had been present during the selection procedure, but even strain 414, a California isolate which had never been exposed to HP32, was induced to fix 55% more N_2 in HP32 than in HP. This is a critical point because mediocre rhizobia, like strain 414, are present in the soil of most alfalfa fields. Normally such strains are difficult to replace by inoculation with new rhizobia, and the demonstration that plant breeding can be used to improve the symbiotic performance of mediocre indigenous strains is an important step forward (Phillips et al., 1985a,b).

Table 2 Forage yield and crude protein content of Hairy Peruvian 32 tested under greenhouse conditions with Rhizobium meliloti 102F28 and various concentrations of KNO_3. The improved Hairy Peruvian 32 and the original Hairy Peruvian were compared over three growth cycles with harvests at 20% flowering to produce the values below. There was no significant difference in the total growth period of the two populations averaged across all nitrate treatments. Improvements in forage yield and protein content produced by breeding were calculated relative to the performance of the original Hairy Peruvian with the same NO_3^- treatment.

Solution N available	Forage dry wt.	Improvement in dry wt.	Forage protein	Improvement in protein
mM NO_3^-	g/plant	%	g/plant	%
0	8.04	+34*	1.72	+43
	8.47	+37*	1.76	+40*
2	8.41	+18*	1.70	+24*
8	13.87	+25*	2.91	+29*

- Breeding effect significant at $P < 0.05$.

Table 3. Forage yield and crude protein content of Hairy Peruvian 32 tested under greenhouse conditions with various strains of Rhizobium meliloti in the absence of NH_4NO_3. The improved Hairy Peruvian 32 and the original Hairy Peruvian were compared over three growth cycles with harvests at 20% flowering to produce the values below. There was no significant difference in the total growth period of the two populations averaged across all bacterial treatments. Improvements in forage yield and protein content produced by breeding were calculated relative to the performance of the original Hairy Peruvian with the same Rhizobium treatment.

Rhizobium strain	Source of bacteria	Forage dry wt.	Improvement in dry wt.	Forage protein	Improvement in protein
		g/plant	%	g/plant	%
102F28	Nitragin Co.	11.13	+58*	2.21	+62*
102F65	Nitragin Co.	9.11	+24*	1.90	+36
414	CA soil	8.21	+50*	1.65	+55*
445	CA soi	10.47	+16	2.20	+19*

* - Breeding effect significant at $P < 0.05$.

Laboratory analyses showed that our breeding efforts had a generally favorable effect on various measures of forage quality (Tables 4 and 5). TDN was calculated on the basis of ADF according to an equation developed with California alfalfa (Bath, 1985). Apparently the dual selection for both dry matter assimilation and crude protein concentration avoided the problem of developing larger plants with more low quality components needed to support a larger stem. When HP and HP32 were compared carefully, it was observed that HP32 had a significantly larger number of stems on each plant than HP (Y. Kapulnik et al., manuscript submitted). Thus the HP32 plants can produce more forage without increasing the size (and thereby decreasing the quality) of each individual stem.

Table 4. The effect of plant breeding on forage quality factors in Hairy Peruvian for plants grown under greenhouse conditions. Hairy Peruvian (HP) and the improved population Hairy Peruvian 32 (HP32) were compared in one harvest under 0 and 8 mM NH_4NO_3 growth conditions similar to those used during the selection and breeding process. Plants were treated with Rhizobium meliloti 102F28 bacteria to allow N_2 fixation to occur in the absence of NH_4NO_3 and were harvested at 20% flowering (Demment et al., manuscript submitted).

Forage trait	Nitrogen source			
	NH_4NO_3		N_2	
	HP	HP32	HP	HP32
TIVDDM† (g/plant)	.80	1.09*	.49	.84*
TDN (%)	58.58	58.72	57.72	
Leaf crude protein (%)	31.80	32.75	26.75	28.50*
Leaf lignin (%)	2.56	2.52	3.03	
Stem crude protein (%)	15.50	15.87	10.56	10.50
Stem lignin (%)	9.13	8.54	9.36	

* - Breeding effect significant at $P < 0.05$.
† - TIVDDM = total in vitro digestible dry matter; TDN = total digestible nutrients.

Table 5 The effect of plant breeding on alfalfa forage yield and quality under field conditions during the first year of a preliminary study. Hairy Peruvian and the improved Hairy Peruvian 32 were sown in the spring at Davis, California. Data are mean values per cutting, averaged across the first two cuttings. Seeds were inoculated before planting with a peat mixture of Rhizobium meliloti 102F28.

Alfalfa population	Forage dry matter	Forage crude protein	TIVDDM	TDN	IVDDM†	ADF
	----- kg/ha -----			--------- % ---------		
Hairy Peruvian	2610	454	1970	58.30	75.36	30.29
Hairy Peruvian 32	2880	557	2250	60.00	78.17	
Breeding effect:	+10%	+22%	+14%	+3%	+4%	-9%

† - IVDDM = in vitro digestible dry matter; ADF = acid-detergent fiber

No conclusive data are yet available from field trials. However, preliminary results from two harvests during the seeding year at one field location show that HP32 produced 10% more forage dry matter and 22% more crude protein than HP (Table 5). Forage quality of HP32 from those harvests was just as good or better than HP for all traits analyzed.

The very promising results obtained by applying our selection protocol to a small population of Hairy Peruvian encouraged us to test the breeding technique on larger populations of the agronomic cultivar Moapa 69. During 1983 and 1984 the selection procedure was used with 2500 and 2000 plants, respectively. Tests run under greenhouse conditions in 1985 showed that Moapa 69-32 produced 25-55% more forage and crude protein than Moapa 69 under both N_2- and NH_4NO_3-dependent growth conditions (Table 1). Also during 1985 a third cycle of selection on 200 plants of Moapa 69-32 was completed.

A final evaluation of the materials produced in this work requires extensive field testing. In 1986 we will establish replicated field trials at various locations in California to assess our progress. Those tests will evaluate improvements made through breeding for increased forage quality by comparing the starting populations, the selected populations, and highly productive alfalfa cultivars in common use. We hope to determine through 4 to 5 years of field tests whether the promising results obtained with our selection technique under controlled conditions are supported by positive field data.

CONCLUSIONS

A very promising screening technique has been developed to breed alfalfa for increased forage yield and quality. During 7 years of work under greenhouse conditions it has been established that the improved alfalfa populations produce more dry matter and crude protein under all levels of soil N. The improved plants also increased N_2 fixation by all strains of Rhizobium bacteria tested. One year of preliminary data shows that the improved plants also produced more forage dry matter and crude protein under field conditions. Beginning in 1986, field trials will be started to test these materials in suitable locations throughout California.

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