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A multiple-trait breeding program for improving the symbiosis for N_2 fixation between *Medicago sativa* L. and *Rhizobium meliloti*

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Summary The goal of breeding alfalfa for increased N_2 fixation potential is addressed. A chronological progression of breeding, physiological, microbiological, and plant pathological research is described. Studies describing the interrelationships among plant morphological, plant physiological, and *Rhizobium* effectiveness traits are summarized. It was concluded that N_2 fixation in alfalfa is affected by coordinated responses among many physiological and biochemical traits. The simultaneous improvement of many factors in the symbiosis requires a comprehensive multiple-step breeding program. The current program includes selection in the glasshouse for seedling vigor, *Rhizobium* preference, shoot growth, nodule mass, root growth, nitrogenase (as measured by acetylene reduction), and nodule enzyme activity. The inclusion of additional selection traits is anticipated. Field evaluations of N_2 fixation potential of alfalfa populations are made with ^{15}N isotope dilution techniques. Plant germplasm sources used in the breeding program include several heterogeneous populations which have good combining ability and pest resistance when they are intercrossed. Significant progress has been made in achieving the goal of breeding alfalfa for improved N_2 fixation.

Program establishment

Our N_2 fixation breeding project has been in progress for about 9 years. It is a multidisciplinary research effort involving scientists with expertise in plant breeding, plant physiology, soil microbiology, biochemistry, agronomy, and plant pathology. More than 25 faculty, post-doctoral scientists and graduate students have participated in the research. Our goals have been to: 1) develop an in-depth understanding of N_2 fixation in alfalfa, 2) breed alfalfa with an improved capability for N_2 fixation, and 3) develop strategies to apply N_2 fixation information and improved germplasm to agricultural production systems.[1]

We have approached breeding for increased N_2 fixation from the viewpoint that the symbiosis between alfalfa and *Rhizobium meliloti* has analogies to a host:pathogen association[15]. In the symbiosis the plant provides an energy source and a suitable environment for the bacteria, while the bacteria provide a source of usable N for the plant. In all selection experiments, mixtures of *Rhizobium* strains are used as inoculum and heterogeneous plant populations are used as the host. Selection experiments are conducted under nil-N conditions in the

Table 1. Mean effect of 2 cycles of recurrent, bidirectional selection for four traits in two alfalfa populations (adapted from Viands *et al.*[20])

	Characteristics associated with N_2 fixation			
Selection trait and subpopulation	Shoot dry weight (g/plant)	Acetylene reduction rate (μmoles C_2H_2/ plant/h)	Nodule* mass (score)	Fibrous* root (score)
Original unselected	0.99	0.91	3.45	2.94
Shoot dry weight				
High	1.54	1.29	3.89	3.12
Low	0.80	0.63	3.13	2.68
Nodule mass score				
High	1.46	1.36	4.09	3.47
Low	0.99	0.67	3.08	2.54
Fibrous root score				
High	1.25	1.41	4.06	3.89
Low	0.97	0.68	3.13	2.44
Nitrogenase activity (C_2H_2) per plant				
High	1.39	1.38	4.03	3.14
Low	0.99	0.61	3.07	2.60
LSD 0.05	0.17	0.21	0.25	0.28

* Nodule mass and fibrous root traits evaluated by visual scores 1 = least amount, to 5 = greatest amount.

glasshouse and acetylene reduction (AR) procedures are used as the measure of nitrogenase activity (NA)[11,14,20].

Bidirectional recurrent selection

Our first studies[14] demonstrated that alfalfa genotypes (clonal propagules) varied for NA and that the relative differences among genotypes were reproducible. Nitrogenase activity was positively correlated with shoot weight, root weight, number of fibrous roots, and nodule mass. Subsequent studies[20] using different alfalfa populations demonstrated that shoot weight, fibrous root score, nodule mass score, and NA each responded to bidirectional selection (Table 1). Results of stepwise regression analysis indicated that only nodule mass explained a significant amount of the variation for NA. Fifty to 65% of the variability for NA was unexplained by the traits measured. We hypothesized that host-Rhizobium interactions were important sources of the unexplained variability in NA.

Field studies, using ^{15}N isotope dilution procedures[9], were conducted with alfalfa subpopulations from the glasshouse selection

Table 2. Proportion of nitrogen from fixation (N_{sy}), mg of nitrogen fixed per plant, and herbage mass averaged over three harvests in the field for two alfalfa populations after one cycle of bidirectional selection from nitrogenase activity (NA) per plant (adapted from Heichel *et al.*[9])

Subpopulation	N_{sy} (%)	N fixed/ plant (mg)	Herbage mass (g/plant)
MnNC-4 (unselected)	72.0	5.6	0.20
MnNC-5 (high NA)	68.0	6.8	0.26
MnNC-5 (low NA)	62.2	5.6	0.24
LSD 0.05	10.8	1.4	0.07
MnPL-6 (unselected)	78.9	13.4	0.46
MnPL-7 (high NA)	69.4	7.7	0.32
MnPL-7 (low NA)	59.8	8.9	0.40
LSD 0.05	9.9	1.2	0.07

N_{sy} and N fixed/plant estimated by ^{15}N isotope dilution method.

experiments (Table 2). Rankings for proportion of nitrogen fixed (N_{sy}) for the subpopulations were consistent with the results of glasshouse selection for low and high levels of NA. However, both the high and low NA subpopulations failed to yield more than the unselected parental populations. It was concluded that inbreeding depression had occurred during selection for individual physiological traits.

Three cycles of recurrent selection for multiple traits associated with increased N_2 fixation were conducted in the same two alfalfa populations during a subsequent glasshouse selection program. Supplemental N was added to the soil during field evaluations to mimic soil solution nitrate levels frequently encountered in midwest U.S. soils by seedling alfalfa. The selected and unselected (original) subpopulations from each population and the cross between the two selected subpopulations were evaluated in the field for N_2 fixation at 0 to 100 kg/ha supplemental N from fertilization. Inbreeding effects in both the MnNC and MnPL populations were evident by a lack of response in yield from selection (Table 3). MnPL-10 showed an increase in percent N from fixation compared to MnPL-6, when both were compared under high soil N. This change in proportion of N fixed was not associated with an increase in either amount of N fixed or yield. The cross between the two selected subpopulations showed an increased amount of fixed N compared to the parent populations at both levels of soil N and increased yield at 100 kg/ha supplemental N. These results demonstrated: 1) the usefulness of alfalfa population crosses to overcome inbreeding effects, 2) the importance of considering soil N when evaluating N_2 fixation of alfalfa populations, and 3) the importance of evaluating plant selections made in the glass-

Table 3. A comparison of the proportion of nitrogen from fixation (N_{sy}), mg of nitrogen fixed per plant, and herbage mass for subpopulations of two alfalfa populations before and after 3 cycles of recurrent selection for enhanced nitrogenase activity and the cross between the two selected subpopulations (Heichel unpublished data)

	N_{sy} (%)		N fixed/ plant (mg)		Herbage mass (g/plant)	
Entry	0 N	100 kg N/ha	0 N	100 kg N/ha	0 N	100 kg N/ha
MnNC-4 (unselected)	77.1[a]	63.0[a]	34.6[b]	25.0[d]	1.93[a]	1.72[b]
MnNC-7 (3rd cycle selection)	76.7[a]	59.0[a]	36.1[ab]	26.6[cd]	1.91[a]	1.80[b]
MnPL-6 (unselected)	75.6[a]	52.2[b]	39.2[ab]	30.4[bc]	1.83[a]	1.80[b]
MnPL-10 (3rd cycle selection)	71.2[a]	62.5[a]	38.2[ab]	31.8[b]	1.71[a]	1.72[b]
MnNC-7 X MnPL-10	76.8[a]	63.2[a]	41.0[a]	40.3[a]	1.86[a]	2.04[a]
Average soil N levels over entries	75.5	60.0	37.8	30.8	1.85	1.82

N_{sy} and N fixed/plant estimated by ^{15}N isotope dilution method.
Numbers in a column followed by the same letter do not differ significantly at the 5% level.

Table 4. A comparison of nodule occupancy by indigenous and antibiotic-resistant mutant rhizobial strains on subpopulations of two alfalfa populations before and after 3 cycles of recurrent selection for enhanced nitrogenase activity and on the cross between the two selected subpopulations (adapted from Hardarson *et al.*[7])

	Rhizobial strain occupancy		
Entry	Indigenous	102F51 $str^r a$	102F77 spc^r
MnNC-4 (unselected)	46 b	12 a	41 b
MnNC-7 (3rd cycle)	26 a	23 b	49 b
MnPL-6 (unselected)	34 ab	12 a	54 b
MnPL-10 (3rd cycle)	39 ab	31 b	28 a
MnNC-7 X MnPL-10	29 a	15 ab	55 b

Numbers in a column followed by the same letter do not differ significantly at the 5% level.

house for N_2 fixation in the field using ^{15}N isotope dilution methodology.

Host selection for physiological and morphological traits associated with N_2 fixation modified host-Rhizobium compatibility (Table 4)[7]. The third cycle selection from both the MnNC and MnPL alfalfa populations had greater nodule occupancy of Rhizobium strain 102F51 than the unselected populations. Strain 102F51 was the most effective strain in the mixture of strains used during the plant selection program for increased NA. The MnNC and MnPL populations had different responses to strain 102F77. The MnNC-7 x MnPL-10 cross performed

similarly to the MnNC-7 subpopulations. In companion experiments, indigenous Rhizobium strains were isolated from nodules of field-grown plants of the MnNC and MnPL subpopulations shown in Table 2. The effectiveness of these strains was measured as herbage dry weight of 8-week-old seedlings of the unrelated cultivar 'Saranac' at ON. Strains of *R. meliloti* isolated from alfalfa subpopulations selected for traits associated with high N_2 fixation were about 10% more effective than were strains isolated from subpopulations selected for traits associated with low N_2 fixation. These data indicated that alfalfa subpopulations selected for traits associated with increased N_2 fixation exhibited a preference for the more effective Rhizobium strains. Based on these observations we included preferential Rhizobium strain selection in our breeding program for improving N_2 fixation. This was done by using a mixture of Rhizobium strains with high and low effectiveness as inoculum and then only selecting the most vigorous plants. This assumes that plants which primarily nodulate with low effectiveness strains will be discarded.

Nodule enzymes

Our research has shown that plants with improved N_2 fixation capabilities often do not show increased plant growth. One possibility is that the plants may be limited in their ability to assimilate the increased amounts of fixed N. This prompted us to investigate the nodule enzymes of N and carbon assimilation in the MnNC and MnPL subpopulations selected for high and low NA[6]. Glutamate synthase (GOGAT) activity and phosphoenolpyruvate carboxylase (PEPC) activity were both correlated with NA in the eight MnNC subpopulations ($r = 0.63^{**}$ and 0.42^{*}, respectively), but not in the eight MnPL subpopulations ($r = 0.34$ and 0.10, respectively). Correlations among activities of nodule enzymes for MnNC-4, MnNC-7, MnPL-6, MnPL-10, and the MnNC-7 x MnPL-10 cross are presented in Table 5. Reasons why there were significant associations among NA and nodule enzymes in the MnNC population and in the population cross, but not in the MnPL population, were not evident. The differences between alfalfa populations appeared to be genetically controlled. We decided to explore the value of using measurements of GOGAT and PEPC activities as selection criteria in our N_2 fixation breeding program.

One cycle of bidirectional selection was conducted for GOGAT and PEPC activities in each of six alfalfa populations[11]. Before selection for nodule enzyme activities the six populations were selected

for large plants, large nodule mass and high NA. Compared to the unselected populations the GOGAT and PEPC activity levels were increased 7 and 5%, respectively, by that selection. Selection for high GOGAT and PEPC activity further increased enzyme activity about 6 and 7%, respectively. Selection for low GOGAT and PEPC activity decreased enzyme activity about 8 and 11%, respectively as compared to the unselected subpopulations. Subpopulations resulting from selection for low enzyme activity had reduced plant weight as compared to unselected subpopulations. Selection for high enzyme activity did not affect plant weight. NA activity was not affected by selection for nodule enzyme activity. It appeared that an optimum level of nodule PEPC and GOGAT activity is required to maintain yield and decreases below the optimum level results in lower yields and poorer plant performance.

Partitioning of soil and fixed nitrogen

When initiating our breeding program we assumed that improvement of N_2 fixation would automatically increase forage and protein yields. However, improvements in these attributes have not been routinely observed in our selected populations under field conditions. We concluded that selection for increased N_2 fixation should be accompanied by selection for yield traits to utilize the increased capability to symbiotically fix N. In order to verify this hypothesis we conducted two cycles of selection in three alfalfa populations for increased amounts of forage, roots, and crowns measured during the autumn. We also selected for increased concentration of N stored in the tap root. Our intent was to breed an alfalfa that could be used as a source of residual N in a crop rotation[2]. The data presented in Table 6 illustrate that it was possible to increase root and crown mass, as well as N concentration. In two of the populations, increases in whole plant N concentration were accompanied by small increases in N_2 fixation capacity. Although we did not specifically select for increased N_2 fixation, the slight improvements observed in the two populations fostered optimism for the success of breeding programs combining selection for increased N_2 fixation and increased N yield.

We have frequently observed that soil N affects the amount of N that alfalfa derived from N_2 fixation (Table 3). A substantial amount of total N in legumes often comes from soil N^{10}. We observed that selection for increased concentration of root N (Table 6) resulted in increased N_2 fixation in the BIC and SW Comp populations and an increased uptake of soil N in the UC Cargo population. Conversely,

Table 5. Correlation coefficients between nitrogenase activity (NA) and activities of the nodule enzymes, glutamate synthase (GOGAT), and phosphoenolpyruvate carboxylase (PEPC), for subpopulations of two alfalfa populations before and after 3 cycles of recurrent selection for enhanced NA and for the cross between the two selected subpopulations (adapted from Groat *et al.*[6])

Entry	Correlation coefficient		
	NA-GOGAT	NA-PEPC	GOGAT-PEPC
MnNC-4 (unselected)	0.90**	0.80*	0.81*
MnNC-7 (3rd cycle)	0.95**	0.93**	0.95**
Mn-PL-6 (unselected)	0.24	0.47	0.90**
MnPL-10 (3rd cycle)	− 0.10	0.51	0.23
MnNC-7 × MnPL-10	0.75**	0.66**	0.91**

*, ** indicate significance at the 0.05 and 0.01 level of probability, respectively. 6 df for all entries except the cross which had 14 df.

Table 6. Responses of three non-winterdormant alfalfa populations to 2 cycles of recurrent selection for increased root mass, crown mass, and percent root nitrogen during October of the seeding year (adapted from Heichel and Barnes[10]).

Entry	Experiment I		Experiment II	
	Root mass (g/plot)	Crown mass (g/plot)	Whole plant N concentration (% N)	Changes in N_2 fixation capacity (%)
BIC-7 (unselected	52	25	2.68	
MN BIC-7, N2 (2nd cycle)	54 (+ 4%)	25 (0%)	2.89 (+ 8%)	+ 6
SW Comp (unselected)	46	20	2.56	
MN SW Comp, N2 (2nd cycle)	60 (+ 30%)	26 (+ 30%)	2.79 (+ 9%)	+ 3
UC Cargo (unselected)	32	17	2.76	
MN UC Cargo, N2 (2nd cycle)	51 (+ 59%)	24 (+ 41%)	2.83 (+ 3%)	− 7
Agate (winterdormant control)	42	19	2.55	
Baker (winterdormant control)	34	15	1.92	

Experiment I planted 5/16, harvested 7/22 and 10/2 – data presented are from the last harvest. Experiment II planted 5/14, harvested 7/7, 8/6, 9/10 and 10/22 – data presented are mean of last two harvests.

selection for increased N_2 fixation (Table 2) increased the percent of plant N from fixation and decreased the N derived from soil.

We demonstrated that much of the total plant N in alfalfa was derived from soil N through nitrate reductase activity (NRA)[16]. In order to better understand the relationships among plant genotypes, N_2 fixation, and soil N uptake we recently initiated studies of leaf NRA[12]. Significant variation was found among plants within all alfalfa

populations studied to date for levels of both constitutive and inducible leaf NRA. NRA was not correlated with NA, or with either nodule GOGAT or PEPC activities. The effect of high and low levels of NRA on forage yield in high N_2 fixing alfalfa populations is being evaluated.

Host-Rhizobium associations

Symbiotic N_2 fixation should be optimized when the most effective Rhizobium infects the roots of the most productive plant genotype. In alfalfa the host-Rhizobium compatibility appears to be conditioned by the genomes of both the plant and bacterium (Table 4). Therefore, we concluded that it was important to utilize adapted, highly effective Rhizobium strains when selecting high N_2-fixing plants. Through the cooperation of many scientists we developed a collection of *R. meliloti* isolates from nodules of cultivars grown in each of 36 states in the USA[5]. The collection is being evaluated for physiological characteristics and effectiveness on different germplasm sources. Isolates have been evaluated for their ability to grow in culture at various pH levels. All isolates grew between pH 6.5 and 8.0, 21% grew at pH 5.5 and 17% grew at pH 4.5. Tolerance to low pH appeared to be related to geographic origin of the isolates. It was curious that all isolates from Kansas were tolerant to low pH's because Kansas soils are generally not acidic. The original source of alfalfa germplasm and inoculum in Kansas reportedly is from 'Chilean' sources in California. Based on the present distribution of acid tolerant Rhizobium strains in the USA and the distribution of alfalfa cultivars tracing to Kansas Common germplasm there appears to be an association between Rhizobium characteristics and alfalfa germplasm adaptation. This example illustrates the need for a better understanding of factors affecting host-Rhizobium associations and their importance in N_2 fixation and plant adaptation.

We have isolated one non-nodulation and three ineffective nodulation plant traits in alfalfa[13,18]. These have been useful as non N_2 fixing controls in ^{15}N isotope dilution studies[3], and for use in basic studies of plant physiological processes[15,17]. Studies are currently underway to use these plant traits to develop host-controlled genetic systems that prohibit effective nodulation by indigenous strains so that introduced strains (inoculum) can be more competitive. We are currently searching for Rhizobium strains that can overcome the

effects of the host genes that condition these ineffective or non-nodulation traits.

Associated agronomic traits

Breeding for improved N_2 fixation has not proved to be an end unto itself. It needs to be part of a breeding program that also considers improved yields and pest resistance. We have reported that selection for several physiological traits often reduced yield because of inbreeding. For this reason we have chosen several heterogenous germplasm sources as our base populations. These populations produce heterosis for forage yield when used in strain crosses, are well-adapted to Minnesota, have good agronomic performance, and have high levels of pest resistance.

Pest resistance is important in alfalfa breeding. In our earliest studies[14,20] we developed several populations with good N_2 fixation characteristics that were not useful because they proved to be susceptible to one or more important pests. This was especially true for bacterial wilt caused by *Corynebacterium insidiosum* (McCull.) H. L. Jens. We demonstrated an increase in susceptibiliity to bacterial wilt in subpopulations selected for improved N_2 fixation[19]. The apparent association between genes conditioning resistance to bacterial wilt and those influencing nodulation illustrates how breeding for one character may affect the expression of other traits. We also have observed that N_2 fixation capacity can be influenced by temperature[20], dormancy caused by daylength fluctuations[8], and soil moisture deficits[4]. We have concluded from our studies that physiological and biochemical processes involved in plant growth and development exhibit coordinated responses in N_2 fixation in response to many biotic and abiotic agents in the environment.

Current breeding program (January 1, 1984)

We have learned that breeding for improved N_2 fixation involves many plant traits in addition to NA. Our breeding program is continually undergoing changes as we learn more about the host-Rhizobium symbiosis. Our objective is to simultaneously improve many factors in the symbiosis. This dictates the need for a comprehensive multiple-step breeding program. The present breeding program includes the following steps:

Step No.	Objective	Methods	Approximate plant age (weeks)	Select plants (% original populations)
1	Planting	Glasshouse, nil-N sand benches, 3–4 seeds/hill on 10 x 10 cm centers.	0	100
2	Rhizobium inoculation	Mixture of high and low effectiveness strains	1	–
3	Selection for seedling vigor.	Thin to largest seedling/ hill.	4	33
4	Uniform plant growth	Cut off shoots at early flowering stage.	8	–
5a)	Selection for plant vigor, nodulation	Dig plants in groups of 25 at early bud stage. Select 10–12 plants with largest shoot growth, nodule mass and root mass.	13	–
b)	Selection for high nitrogenase activity	Cut off tops of selected plants from step 5a and evaluate crown and root system for NA. Select 5 or 6 plants ranking highest in NA.	–	7
6	Replanting selected plants	Transplant plants selected from step 5b to sand benches like step 1.	17	–
7	Uniform plant growth	Cut off shoots at early flowering stage.	22	–
8	Selection for high nodule enzyme activities	Remove nodules and analyze for GOGAT, PEPC and total soluble protein. Select best 2 or 3 plants from each 5 or 6 plant group in step 5b.	26	3
9	Replanting selected	Store plants from step 8 at 5°C until nodule	29	–

	plants	enzyme activities are determined. Transplant selected plants to pots of soil.		
10	Cross-pollinate selected plants.	Allow plants to regrow for about 4 weeks then cut off shoots to provide uniformity in flowering 4 weeks later. Intercross seed from 80–120 plants for each population.	37	–
11	Harvest seed	Bulk harvest intercrossed seed from each population or from crosses between populations.	42	–
12	Field evaluation for N_2 fixation	Unselected populations and selected subpopulations are tested for % N and quantity of N from N_2 fixation by either ^{15}N isotope dilution or difference methods.	–	–

The present breeding program utilizes many ideas that have resulted from the multidiscipline approach of our research team. We anticipate that additional traits will be included when current studies are concluded. Some of the additional traits may be selection for NRA, selection for enzymes which are involved with transporting N compounds from the roots to shoots, and selection for yield traits that utilize the increased N_2 fixing ability of the alfalfa plant. We have concluded that breeding for increased N_2 fixation capability is complex, but we are optimistic that it will be successful.

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References

1 Barnes D K, Heichel G H, Vance C P, Viands D R and Hardarson G 1981 Successes and problems encountered while breeding for enhanced N_2 fixation in alfalfa. pp 233–248. *In* Genetic Engineering of Nitrogen Fixation and Conservation of Fixed Nitrogen. Eds. J M Lyons *et al.* Plenum Press, New York.

2 Barnes D K, Sheaffer C C nd Heichel G H 1983 Breeding alfalfa for improved residual nitrogen production. Agron. Abst. 54–55.
3 Boller B C and Heichel G H 1983 Photosynthate partitioning in relation to N_2 fixation capability of alfalfa. Crop Sci. 23, 655–659.
4 Carter P R and Sheaffer C C 1983 Alfalfa response to soil water deficits. III. Nodulation and N_2 fixation. Crop Sci. 23, 985–990.
5 Ellis W R and Barnes D K 1983 Characteristics of *Rhizobium meliloti* isolates from alfalfa in the U.S. Proceed. Eighteenth Central Alfalfa Improvement Conf. pp 15–16.
6 Groat R G, Vance C P and Barnes D K 1984 Host plant nodule enzymes associated with selection for increased N_2 fixation in alfalfa. Crop Sci. 24, 895–898.
7 Hardarson G, Heichel G H, Barnes D K and Vance C P 1982 Rhizobial strain preference of alfalfa populations selected for characteristics associated with N_2 fixation. Crop Sci. 22, 55–58.
8 Heichel G H, Barnes D K and Vance C P 1983 Nitrogen fixation of alfalfa in the seeding year. Crop Sci. 21, 330–335.
9 Heichel G H, Barnes D K and Vance C P 1981 Evaluating elite alfalfa lines for N_2 fixation under field conditions, pp 217–232. *In* Genetic Engineering of Nitrogen Fixation and Conservation of Fixed Nitrogen. Eds. J M Lyons *et al.* Plenum Press, New York.
10 Heichel G H and Barnes D K 1984 Opportunities for meeting crop nitrogen needs from symbiotic nitrogen fixation. pp. 49–59. *In* Organic Farming. Eds. D F Bezdicek and J F Power. Special Publication. American Society of Agronomy. Madison, Wisconsin.
11 Jessen D L, Barnes D K and Vance C P 1983 Selection for gluatamate synthase and phosphoenolpyruvate carboxylase enzyme activities in alfalfa nodules. Agron. Abst. 68.
12 Nelson D L, Vance C P, Barnes D K and Heichel G H 1983 Variability for nitrate reductase enzyme activity (NRA) in alfalfa. Proceed. Eighteenth Central Alfalfa Improvement Conf. pp 14–15.
13 Peterson M A and Barnes D K 1981 Inheritance of ineffective nodulation and non-nodulation traits in alfalfa. Crop Sci. 21, 611–616.
14 Seetin M W and Barnes D K 1977 Variation among alfalfa genotypes for rate of acetylene reduction. Crop Sci. 17, 783–787.
15 Vance C P 1983 *Rhizobium* infection and nodulation: A beneficial plant disease? Annu. Rev. Microbiol. 37, 399–424.
16 Vance C P and Heichel G H 1981 Nitrate assimilation during vegetative regrowth of alfalfa. Plant Physiol. 68, 1052–1056.
17 Vance C P, Heichel G H and Barnes D K 1984 Nitrogen and carbon assimilation in *Medicago sativa* L. mediated by host plant genes for nodule effectiveness. *In* Advances in Nitrogen Fixation Research. Eds. C Veeger and W E Newton. Nyhoff Publ., The Hague/ Pudoc, Wageningen. pp 565–571.
18 Vance C P and Johnson L E B 1983 Plant determined ineffective nodules in alfalfa (*Medicago sativa* L.): structural and biochemical comparisons. Can. J. Bot. 61, 93–106.
19 Viands D R, Barnes D K and Frosheiser F I 1980 An association between resistance to bacterial wilt and nitrogen fixation in alfalfa. Crop Sci. 20, 699–703.
20 Viands D R, Barnes D K and Heichel G H 1981 Nitrogen fixation in alfalfa – response to bidirectional selection for associated characteristics. USDA Tech. Bull. 1643. 18 p.