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Interaction between alfalfa cultivars and *Rhizobium* strains for nitrogen Fixation

G.-Y. Tan* and W.-K. Tan*

Plant Science Department, University of Alberta, Edmonton, Canada

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Summary. Two experiments were conducted in the greenhouse to study the interaction between alfalfa cultivars (*Medicago sativa* L. and *M. falcata* L.) and strains of *Rhizobium meliloti* Dang. for acetylene reduction rate, plant height and dry weights of shoot, root and whole plant. Fifteen alfalfa cultivars were inoculated with 10 strains of *Rhizobium* in Experiment I. Variance component analysis revealed that more than 30% of the total variance was due to alfalfa cultivars for acetylene reduction rate and 26% was accounted for by *Rhizobium* strains. More than 36% of the total variation was attributed to the interaction between alfalfa cultivars and *Rhizobium* strains for this character. Twenty-five host cultivars and 11 *Rhizobium* strains were included in Experiment II. The results also showed that the interaction of alfalfa cultivars and *Rhizobium* strains contributed the largest portion of the total variation for dry weights of shoot, root and whole plant and acetylene reduction rate. The results clearly demonstrated that the non-additive effects were the major component of variation for these characters associated with nitrogen fixation in alfalfa. Therefore, an effective way of improving nitrogen fixation in alfalfa is to select for a favourable combination of specific *Rhizobium* strains and alfalfa cultivars.

Key words: Nitrogen fixation – Alfalfa cultivars – *Rhizobium* strains – Acetylene reduction

Introduction

Symbiotic nitrogen fixation involves a complex interaction between host plant, nodule bacteria, and the environment. A fully effective combination depends upon the genetic compatibility between the host plant and the *Rhizobium* strain. Variation between alfalfa (*Medicago sativa* L.) cultivars and *Rhizobium* strains in their abilities to fix nitrogen have been reported by several workers (Gibson 1962; Bordeleau et al. 1977; Tan and Tan 1981). Significant interactions between *Rhizobium* strain and alfalfa cultivars have also been reported (Gibson 1962; Brockwell and Hely 1966; Gassar et al. 1972). Seetin and Barnes (1977); Duhigg et al. (1978) and Tan (1981) showed that acetylene reduction rate and other characters associated with nitrogen fixation in alfalfa were genetically controlled. Tan (1981) further found that the estimate of genetic variance for acetylene reduction rate in alfalfa genotypes could vary from one *Rhizobium* strain to another.

The objective of this study was to determine the variation of nitrogen fixing characters in alfalfa cultivars inoculated by a number of strains of *Rhizobium meliloti* Dang. The characters measured included: acetylene reduction rate, dry weight of shoot, root and whole plant and plant height. The relative importance of the three sources of component variation (general combining ability of alfalfa cultivars, general combining ability of *Rhizobium* strains and specific combining ability of their interactions) contributing to the total variation for these characters were also determined.

Materials and methods

Alfalfa cultivars and strains of *Rhizobium* used in Experiments I and II are given in Tables 1–4. Most alfalfa cultivars were obtained from breeder seeds. Three *Rhizobium* strains (NRG 43, NRG 61 and NRG 118) were provided by Dr. W. A. Rice, while six other strains (A2, A3, A4, V3, S14 and 3DOa8) were obtained from Dr. L. M. Bordeleau. Strain RM9 was supplied by Dr. F. D. Cook. The commercial peat base inoculum 'Nitragin' was given by Dr. J. C. Burton.

* Present address: Cocoa and Coconut Research Institute, P.O. Box 1846, Rabaul, Papua, New Guinea

Alfalfa seeds were surface-sterilized by immersing in 95% (v/v) ethanol for 15 min, 0.2% mercuric chloride solution for 3 min, followed by five consecutive washes in distilled water. Thereafter, they were germinated aseptically on petri dishes containing agar. After germination, seedlings with 1 to 2 cm of radicle were planted individually in rectangular plastic pots (5×5×15 cm) filled with vermiculite. The experiments were planted in a split-plot design with four replications in Experiment I and three replications in Experiment II. The *Rhizobium* strains were main plots and alfalfa cultivars were the sub-plots. Inoculation of the seedlings with each *Rhizobium* strain was done immediately after planting. Each germinated seed received approximately 10^7 viable *Rhizobium*. Plants were supplied with a nitrogen-free solution (Hoagland and Arnon 1950) on alternate days. In order to prevent contamination among treatments, a thin sheet of clear plastic wrap was used to cover the top surface of the pot, leaving small holes for seedlings to grow through.

The experiments were conducted in a greenhouse at a temperature of 23 °C and 16½ h photoperiod. The seedlings were kept for 10 weeks after inoculation. At harvest, five seedlings from each replication were bulked and separated into shoot and root. The rate of acetylene reduction was determined as described by Hardy et al. (1973). The dry weight of plant parts was determined after drying at 100 °C for 24 h. Plant height from the vermiculite surface to the tip of the plant also measured in Experiment II.

The split-plot experimental design was similar to the factorial design which enabled the partition of the total variation into three main sources, i.e. general combining abilities (GCA) of alfalfa cultivars and *Rhizobium* strain and specific combining ability of alfalfa cultivar × *Rhizobium* strain interaction. The variance component analysis and the estimate of the relative importance of the three component variations contributing to the total variation were computed according to the method given by Snedecor and Cochran (1967). For the interpretation of results, differences in GCA are regarded as resulting primarily from the additive effects and the differences in SCA as being attributable to the non-additive effects (Mytton 1978).

Results and discussion

Significant differences were found among alfalfa cultivars for all the characters measured in both experiments I and II (Tables 1 and 3). Alfalfa cultivars such as 'DuPuits', 'Saranac' and 'Thor' (in both experiments), 'Alfa' and 'Angus' (in Experiment I) and 'Algonquin' and 'Arc' (in Experiment II), produced vigorous seedlings with higher plant dry weight and a greater acetylene reduction rate than the rest of the cultivars. Alfalfa cultivars from Western Canada including 'Roamer', 'Drylander', 'Rangelander' and 'Anik' (*M. falcata*), had a very low acetylene reduction rate. Similar results have been reported from alfalfa with *M. falcata* and genotypes derived from the hybrids between *M. sativa* and *M. falcata* (Erdman and Means 1953; Gibson 1962; Leach 1968; Tan and Tan 1981).

Significant differences among the *Rhizobium* strains were obtained in both experiments (Tables 2 and 4). Among all *Rhizobium* strains, NRG61, NRG118 and S14 were the three most effective strains as evidenced by high dry weight of host plant and acetylene reduction rate. At the other extreme, RM9 was the poorest strain in both experiments. The commercial inoculum Nitragin (assumed to be a composite of several *Rhizobium* strains) exhibited an above average effectiveness in Experiment II.

The analysis of variance for all the characters are given in Tables 5 and 6. Mean squares for general combining abilities of alfalfa cultivars and *Rhizobium* strains and specific combining ability for their interaction were significant for all the characters.

Table 1. Means of four alfalfa characters averaged over ten different *Rhizobium* treatments in Experiment I

Alfalfa cultivar	Shoot dry wt mg	Root dry wt mg	Plant dry wt mg	Acetylene reduction μ moles/pt/h
'Alfa'	934	444	1,378	4.86
'Angus'	1,101	524	1,625	5.58
'Anik'	297	112	409	1.86
'Arc'	810	410	1,120	2.25
'Beaver'	746	310	1,056	3.90
'Du Puits'	906	407	1,313	5.88
'Drylander'	659	257	916	2.94
'Grimm'	800	318	1,118	2.19
'Iroquis'	724	334	1,058	4.56
'Ladak'	654	276	930	4.02
'Rambler'	696	260	956	3.90
'Ramsey'	682	398	1,080	3.90
'Saranac'	849	421	1,370	5.22
'Thor'	917	427	1,344	5.10
'Vernal'	737	371	1,108	4.14
Mean	767	351	1,118	4.02
LSD (0.01)	52	36	108	0.24

Table 2. Means of four alfalfa characters expressed under 10 different *Rhizobium* treatments, averaged over 15 alfalfa cultivars in Experiment I

Rhizobium strain	Alfalfa character			
	Shoot dry wt mg	Root dry wt mg	Plant dry wt mg	Acetylene reduction μ moles/pt/h
A2	590	314	904	4.11
A3	778	404	1,182	4.71
A4	826	382	1,208	3.33
V3	633	239	872	2.55
S14	902	368	1,270	5.67
3DOaB	713	302	1,019	2.73
NRG43	584	345	929	3.39
NRG61	1,067	471	1,538	5.85
NRG118	1,006	395	1,401	5.73
RM9	578	291	869	2.07
Mean	767	351	1,118	4.01
LSD (0.01)	75	31	80	0.36

Table 3. Means of five alfalfa characters, averaged over 11 different *Rhizobium* treatments in Experiment II

Alfalfa cultivars	Shoot dry wt mg	Root dry wt mg	Plant dry wt mg	Height cm	Acetylene reduction µ mole/pt/h
'Algonquin'	533	207	740	29.6	3.12
'Angus'	531	200	731	30.5	2.64
'Anik'	248	86	334	20.7	1.74
'Apollo'	510	198	708	29.6	2.88
'Arc'	589	238	827	30.1	3.15
'Beaver'	463	164	627	28.2	2.34
'Chimo'	466	187	653	28.5	2.46
'Citation'	503	205	708	29.2	2.37
'Drylander'	480	153	633	28.5	1.83
'Du Puits'	569	203	772	30.6	3.87
'Honeoye'	525	149	674	29.5	2.85
'Iroquois'	514	185	699	29.9	2.61
'Kane'	498	197	695	28.0	2.94
'Ladak'	509	198	707	30.8	2.04
'Marathon'	533	247	780	27.2	2.80
'Nugget'	454	186	640	28.7	2.22
'Rambler'	499	127	626	20.9	2.31
'Ramsey'	393	225	618	28.5	2.40
'Rangelander'	337	115	452	25.0	2.04
'Roamer'	361	109	470	27.2	1.74
'Saranac'	637	259	896	30.9	3.40
'Spredor'	489	154	643	30.4	3.00
'Thor'	523	222	745	29.6	3.15
'Vernal'	470	162	632	29.0	2.55
Mean	486	180	667	28.3	2.60
LSD (0.01)	50	13	51	0.7	0.14

Table 4. Means of five alfalfa characters expressed under 11 *Rhizobium* treatments, averaged over 24 alfalfa cultivars

Rhizobium strain	Alfalfa character				
	Shoot dry wt mg	Root dry wt mg	Plant dry wt mg	Height cm	Acetylene reduction µ mole/pt/h
A2	465	175	640	29.8	2.61
A3	635	186	821	30.2	2.85
A4	562	100	662	26.7	2.40
S14	552	249	801	31.9	3.90
V3	363	126	489	25.4	2.61
3DOaB	374	181	555	29.4	2.34
NRG43	480	258	738	29.4	2.40
NRG61	513	277	799	30.7	2.88
NRG118	638	216	854	29.9	3.06
RM9	173	61	234	20.0	1.71
Nitragin	593	157	750	29.1	2.79
Mean	486	180	667	28.4	2.61
LSD (0.01)	60	14	48	0.8	0.34

Table 5. Mean squares of general combining abilities of alfalfa cultivars and *Rhizobium* strains, specific combining ability of their interactions and variance component analysis of four alfalfa characters in Experiment I

Source of variation	df	Shoot dry wt	Root dry wt	Plant dry wt	Acetylene reduction
GCA (*Rhizobium* strains)	9	2.4322***	0.2996***	5.968***	37.25***
Error 'a'	27	0.0145	0.0026	0.017	0.32
GCA (Alfalfa cultivars)	14	1.6597***	0.4383***	4.659***	23.73***
SCA (Strains × cultivars)	126	0.1285***	0.0403***	0.417***	3.43***
Error 'b'	420	0.0126	0.0061	0.051	0.28
σ^2_s		0.0383	0.0043	0.093	0.56
σ^2_c		0.0382	0.099	0.106	0.50
σ^2_{sxc}		0.0289	0.0085	0.091	0.78
σ^2_e		0.0126	0.0061	0.051	0.28

*** Significant at the 0.001 level of probability

Table 6. Mean squares of general combining abilities of alfalfa cultivars and *Rhizobium* strains, specific combining ability of their interactions and variance component analysis of four alfalfa characters in Experiment II

Source of variation	df	Shoot dry wt	Root dry wt	Plant dry wt	Acetylene reduction	Plant height
GCA (*Rhizobium* strains)	10	2.565***	2.2436***	3.440***	26.407***	3,292.65***
Error 'a'	20	0.007	0.0004	0.004	0.251	1.28
GCA (Alfalfa cultivars)	23	0.940***	0.1383	1.813***	14.335***	181.75***
SCA (Strains × cultivars)	230	0.198	0.0173***	0.278***	4.180***	28.07***
Error 'b'	506	0.013	0.0009	0.014	0.101	2.70
σ^2_s		0.032	0.0031	0.044	0.306	45.36
σ^2_c		0.022	0.0036	0.046	0.307	4.65
σ^2_{sxc}		0.061	0.0054	0.088	1.359	8.45
σ^2_e		0.013	0.0009	0.014	0.101	2.70

*** Significant at the 0.001 level of probability

Table 7. The percentage of total variation contributed by each source of variation for the alfalfa characters measured in both Experiments I and II

Source of variation	Shoot dry wt	Root dry wt	Plant dry wt	Acetylene reduction	Plant height
Experiment I	%				
Rhizobium strain	32.4	14.9	27.2	26.2	–
Alfalfa cultivars	32.3	34.3	31.0	23.6	–
Strains × cultivars	24.4	29.5	26.7	36.7	–
Error	10.6	21.1	15.0	13.2	–
Experiment II					
Rhizobium strains	25.1	23.8	22.8	14.7	74.1
Alfalfa cultivars	17.2	27.6	24.1	14.8	7.6
Strains × cultivars	47.0	42.5	45.6	65.5	13.8
Error	10.5	6.9	7-3	4.8	4.4

Estimates of variance components from GCA and SCA for all characters in Experiments I and II are also given in Tables 5 and 6, respectively. In Experiment I, the variance components for GCA of alfalfa cultivars were higher than the SCA variance components for shoot, root and plant dry weight. However, for acetylene reduction rate the variance component of SCA was higher than the GCA of either alfalfa cultivars or *Rhizobium* strains. In Experiment II, the variance components for the SCA of alfalfa cultivar × *Rhizobium* strain interactions were much higher than the general effects for all characters except for plant height.

Information on the relative contribution of the three components to the total variance is given in Table 7. In Experiment I, the results showed that alfalfa cultivars accounted for 31 percent of the total variance for plant dry weight and 32.3 and 34.3% for shoot and root dry weight, respectively. *Rhizobium* strains contributed a similar amount as did alfalfa cultivars to shoot dry weight (32%) but only 14.9% for root dry weight. More than 36% of the total variations for acetylene reduction rate was attributed to alfalfa cultivar × *Rhizobium* strain interaction. Thus, the non-additive effect was more important than the additive effects for these characters.

In Experiment II, the GCA of *Rhizobium* strains was higher than the GCA of alfalfa cultivars for shoot dry weight, while the reverse was true for plant and root dry weights. For plant height, the GCA of *Rhizobium* strain accounted for more than 74% of the total variance. The SCA contributed the largest portion of the total variation for dry weights of shoot, root and whole plant (47.0, 41.5 and 45.6%, respectively). More than 65% of the total variation for acetylene reduction rate was due to the specific effects. Similar results have been reported in *Vicia faba* where the specific effect accounted for 70% of the total variation in dry matter production when *R. leguminosarum* was used as an inoculum (Mytton et al. 1977).

In both Experiments I and II, the variances due to error terms were relatively small for all characters except for root dry weight in Experiment I (Table 7). This does not imply that environments have no effect on the association between *Rhizobium* strains and alfalfa cultivars. Burton and Wilson (1939) indicated that environmental conditions might obscure the specific influence of the host plant. Hardarson and Jones (1979) also reported that temperature had considerable influence on the host preference for *Rhizobium* strain with white clover (*Trifolium repens* L.).

Symbiotic nitrogen fixation varies in response to changes in *Rhizobium* strain and host plant. Some workers (Burton 1964, 1972) considered that selection should concentrate on the variation between strains of *Rhizobium* rather than differences between host plants. However, Nutman (1946) and Aughtrey (1948) showed that genetic factors in the host plant were equally important as *Rhizobium* strain in determining the effectiveness of symbiotic nitrogen fixation. The present study demonstrated that N_2-fixation capability of alfalfa was partly controlled by the host genome and partly by the genome of microsymbiont. For any symbiosis to be highly effective, the factors in both *Rhizobium* strain and host plant must be fully compatible. As pointed out by Mytton (1978) where there was a large specific component of variation only a portion of the total genetic variation was considered if selection was practised on either the host plant or the *Rhizobium* strain alone. Consequently, this could seriously limit the genetic advance under selection. The present studies clearly demonstrated that the non-additive effect was the major component of variation for acetylene reduction rate in alfalfa. Tan (1981) also reported that there were differential expressions of gene effects for alfalfa genotype in symbiosis with different strains of *Rhizobium*.

Therefore, the most rapid genetic advance for improving nitrogen fixation in alfalfa could be achieved by selecting specific host-strain combinations.

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References

Aughtry JD Jr (1948) Effect of genetic factors in *Medicago* on symbiosis with *Rhizobium*. Cornell Univ Agric Exp Stn, Mem 280

Bordeleau LM, Antoun H, Lachance RA (1977) Effects des souches de *Rhizobium meliloti* et des coupes successive de la luzerne (*Medicago sativa*) sur la fixation symbiotique d'axote. Can J Plant Sci 57:433–439

Brockwell J, Hely FW (1966) Symbiotic characteristics of *Rhizobium meliloti:* an appraisal of the systematic treatment of nodulation and nitrogen fixation interactions between hosts and rhizobia of diverse origins. Aust J Agric Res 17:885–899

Burton JC (1964) The *Rhizobium* – legume association. In: Gilmour GM, Allen ON (eds) Microbiology and soil fertility. Proc Biol Coll, Oregon State University Press, Corvallis Ore, pp 107–134

Burton JC (1972) Nodulation and symbiotic nitrogen fixation. In: Hanson CH (ed) Alfalfa science and technology. Agronomy 15, Am Soc Agron, Madison Wis, pp 229–246

Burton JC, Wilson PW (1939) Host plant specificity among the *Medicago* in association with root-nodule bacteria. Soil Sci 47:293–303

Duhigg P, Melton B, Baltensperger A (1978) Selection for acetylene reduction rates in 'Mesilla' alfalfa. Crop Sci 18: 813–816

Erdman LW, Means UM (1953) Strain variation of *Rhizobium meliloti* on three varieties of *Medicago sativa.* Agron J 45: 625–629

Gasser H, Guy P, Obaton M, Sikora I (1972) Efficiency of *Rhizobium meliloti* strains and their effects on alfalfa cultivars. Can J Plant Sci 52:444–448

Gibson AH (1962) Genetic variation in the effectiveness of nodulation of lucerne varieties. Aust J Agric Res 13: 388–399

Hardarson G, Jones DG (1979) Effect of temperature on competition amongst strains of *Rhizobium trifolii* for nodulation of two white clover varieties. Ann Appl Biol 92: 229–236

Hardy RWF, Burns RC, Holsten RD (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol Biochem 5:47–81

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circ 347, Calif Agric Exp Stn, Berkeley Calif

Leach GJ (1968) The effectiveness of nodulation of a wide range of lucerne cultivars. Aust J Exp Agric Anim Husb 8:323–326

Mytton LR (1978) Some problems and potentialities in breeding for improved white clover – rhizobium (*Trifolium repens – Rhizobium trifolii*) symbiosis. Ann Appl Biol 88: 445–448

Mytton LR, El-Sherbeeny MH, Lawes DA (1977) Symbiotic variability in *Vicia faba*. 3. Genetic effects of host plant, *Rhizobium* strain and of host × strain interaction. Euphytica 26:785–791

Nutman PS (1946) Genetic factors concerned in the symbiosis of clover and nodule bacteria. Nature 57:463–465

Seetin MW, Barnes DK (1977) Variation among alfalfa genotypes for rate of acetylene reduction. Crop Sci 17:783–787

Snedecor GW, Cochran WG (1967) Statistical methods. The Iowa State University Press, Ames Iowa

Tan G-Y (1981) Genetic variation for acetylene reduction rate and other characters in alfalfa. Crop Sci 21:485–488

Tan G-Y, Tan W-K (1981) Net assimilation rate and relative nitrogen assimilation rate in relation to the dry matter production of alfalfa cultivars. Plant Soil 59:185–192