J. Mycopathol. Res. **42**(2) : 219-223 (2004)

Study on the genetic variation for acetylene reduction rate, nodulation and other characters in alfalfa (*Medicago sativa*)

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This investigation was carried out with six parental clones of alfalfa (*Medicago sativa* L.) viz. Saranac, MnNC-4, vernal, MnNC-632, Sonara and Roamerj and five strains of *Rhizobium mltiloti* Dang., including four single strains and one commercial inoculant, 'JIBANU SAR' : to determine the general (GCA) and specific (SCA) combining abilities for nodule number per plant, acetylene reduction rate, plant dry weight, N content (%) and total plant N. the most effective strain of *Rhizobium* was BKV-27, while BKV-32 was the poorest. Diallel analysis of the six-parent half diallel cross revealed that mean squares of both GCA and SCA were highly significant. The GCA variance components were higher than SCA for nodule number per plant, total plant N, total plant weight. This suggested that the major portion of the total genetic variation was additive. Recurrent selection for these characters should be possible. Both additive and non-additive gene effects were equally important for acetylene reduction rate with *Rhizobium* strains BKV-47, BKV-27 and BKV-98. Reciprocal recurrent selection methods might be necessary to improve this character with these single *Rhizobium* strains. Combining ability analysis for each of the parental clone indicated that colne-3 had consistantly high and positive GCA effects over all *Rhizobium* treatments. Clone-5 was on the reverse side with negative GCA effects in all cases. Other parental clones failed to produce consistantly positive GCA effects across all the *Rhizobium* strains. Differential expression of gene effects for alfalfa genotypes with different strains of *Rhizobium* was evident.

Key words : Genetic variation, alfalfa, *Rhizobium,* nodulation, GCA, SCA

INTRODUCTION

The relative ability and efficiency for a symbiotic relationship between a leguminous plant and a root nodule bacteria is determined by the genetic factors of both the partners. Symbiotic N_2 fixation is enhanced in legume when effective and highly competitive strains of *Rhizobium* successfully nodulate host plants. Variation among alfalfa (*Medicago sativa* L.) cultivars and *Rhizobium meliloti* Dang. strains in their ability to fix N have been reported by several workers (Burton, 1964, 1967 ; Bordeleau and Antoum, 1977). Gibson (1962) reported variation in the effectiveness of nodulation among a wide range of alfalfa cultivars inoculated with different *Rhizobium* strain. Seetin and Barnes (1977) demonstrated that the acetylene reduction rate of selected alfalfa clones inoculated with commercial *Rhizobium* inoculum was genetically controlled. It has now been acertained that 'nod' genes plays important roles in different stages of nodule formation.

The objective of this study was to determine the genetic variances and their interactions with *Rhizobium* strains for nodule number, rate of acetylene reduction, plant dry weight, plant N content (%) and total N of whole plant in six parent half diallel cross of alfalfa. In order to eliminate the possible perplexity of the strain mixture in estimating the genetic variance, the experiment was carried out with potted plants inoculated with one of the four strains of *Rhizobium* or a composite commercially prepared charcoal-peat-based inoculum 'JIBANU SAR'.

MATERIALS AND METHODS

The six parental clones of alfalfa used in this study were selected from commercial cultivars 'MnNC-4' (clone-1), 'Sonara' (clone-2), Sarance (clone-3), 'Roamer' (clone-4), Vernal (clone-5) and 'MnNC-632 (clone-6). Seeds of the 15 single crosses were produced by hand crossing in the green house during the winter 1998-99. The clones are self-incompatible. All the F_1 progenies were assumed to be cross-pollinated. *Rhizobium* strains BKV-27, BKV-32, BKV-47, BKV-98 and JIBANU SAR were obtained from the Nodule Research Laboratory, Bidhan Chandra Krishi Viswavidyalaya. The commercial inoculant was a mixture of several *Rhizobium* strains.

Alfalfa seeds were surface sterilized in 0.2% mercuric chloride, and thoroughly washed in several changes of distilled water. The seeds were germinated in petri dishes and planted singly in plastic pots (5×5×50 cm) filled with vermiculite. Four pots arranged in a row represented each entry per replication. The experiment was planted in a split-plot design with four replications. The 15 single crosses were subplots with *Rhizobium* strain as whole plots. The germinated seeds were inoculated with one of the five strains of *Rhizobium* immediately after planting. Except for JIBANU SAR (the charcoal-peat-base inoculum), inoculation was done by pipetting into each pot 1 ml of *Rhizobium* suspension containing approximately 10^7 cells. Plants were supplied with N-free nutrient solution (Bhaduri 1951) in alternate days. The pots were covered with thin sheet of clean plastic wrap to prevent contamination among treatments. Seedlings grew through small holes punched in the wrap.

The alfalfa plants were grown in the greenhouse with 16 ± ½ hour photoperiod and 23°C for 8 weeks. At harvest, the four plants from each replication were bulked and separated into shoots and roots. Nodule number per pant was counted. The rate of acetylene reduction was determined as described by Hardy *et al.* (1973). The roots were incubated in presence of acetylene for 1 h at room temperature. The amount of ethylene produced was alalysed by gas chromatography. Plant dry weight was determined after drying at 100°C for 24 h. The dry sample of the whole plant was ground in a grinding mechine with a 1 mm screen. Nitrogen content (%) was determined form, a duplicate sample from each entry by Coleman model 29A Nitrogen Analyser. Total nitrogen of the whole plant was obtained by multiplying plant dry weight with percentage N content.

Griffing's (1956) Model-1, Method-4 was used for combining ability analysis. All data were later submitted to computer programme for the analysis of combining abilities by strain interactions.

RESULTS AND DISCUSSION

The differences among the *Rhizobium* treatments showed highly significant value. The most effective strain BKV-27 produced the highest acetylene reduction rate and total plant N (Table 1). On the country, plants inoulated with BKV-32 had the lowest acetylene reduction rate, plant dry weight and total plant N.

Table 1 : Mean for different characters of Alfalfa inoculated with one of the five different *Rhizobium* treatments

Rhizobium	Nodule number/ Plant	Acetylene reduction/µ mole/pt/hr.	Total dry wt.	N Content%	Total Plant N/µg
BKV-47	56.4	4.82	0.78	3.52	27.4
BKV-27	81.6	5.37	0.87	3.40	29.5
BKV-98	60.5	5.03	0.92	3.63	33.3
JIBANU SAR	41.8	3.85	0.69	2.94	17.0
BKV-32	39.2	1.93	0.58	2.96	17.1
L.S.D.(0.05)	16.3	0.489	0.114	0.369	1.08

Table 2 : Mean squares for five characters of Alfalfa

Source of variation	df	Nodule number	Acetylene reduction	Total dry	N Content	Total Plant N
Rhizobium	4	51.2***	8.642***	1.407***	5.618***	41.29***
Error-a	15	0.68	0.018	0.006	0.008	0.62
Alfalfa genotypes	14	44.86***	5.859***	3.671***	0.314***	31.42***
GCA	5	141.50***	14.216***	9.246***	0.365***	74.26***
SCA	9	7.42***	0.905***	0.483***	0.302***	6.03***
Genotype x *Rhizobium*	56	12.09***	1.085*	0.210*	0.071*	1.81*
CGA x Strains	20	2.432***	1.272*	0.169***	0.094***	2.16***
SCA x Strains	36	3.043***	0.968*	0.182*	0.042*	1.70*
Error-b	210	0.792	0.26	0.035	0.005	0.694

*, *** significant at 0.05 and 0.001 probability level, rrespectively.

Table 3 : Mean squares of general (GCA) and specific (SCA) combining abilities for five characters of Alfalfa inoculated with one of the *Rhizobium* strain

Rhizobium strain	Source of variance	df	Nodule number	Acetylene reduction	Total dry wt.	N Content	Total Plant N
BKV-47	GCA	5	23.026**	6.375***	0.4253***	0.0465***	4.625***
	SCA	9	3.055**	1.921*	0.0256**	0.0393***	0.346***
	Error	42	0.902	0.700***	0.0060	0.0023	0.076
	GCA : SCA‡		7.83	7.26	5.62	0.35	5.60
BKV-27	GCA	5	27.499**	2.026***	0.6200***	0.08659***	5.016***
	SCA	9	3.624***	1.250***	0.0789***	0.0617***	0.952***
	Error	42	0.876	0.649	0.0089	0.0044	1.000
	GCA : SCA		5.88	4.83	1.51	0.26	1.67
BKV-98	GCA	5	19.361**	6.417***	0.582***	0.1462***	3.972***
	SCA	9	4.321***	0.911***	0.927	0.0709***	0.312**
	Error	42	0.746	0.426	0.0084	0.0124	0.058
	GCA : SCA		4.59	3.58	1.70	1.18	5.20
JIBANU SAR	GCA	5	16.302**	5.952***	0.5820***	0.5298***	3.872***
	SCA	9	2.092***	1.350***	0.0363***	0.2215***	0.678**
	Error	42	0.493	0.632	0.0077	0.0082	0.090
	GCA : SCA		3.69	2.57	5.69	0.72	5.82
BKV-98	GCA	5	12.805***	3.596***	0.6821***	0.2092***	3.990***
	SCA	9	.506**	1.958**	0.0802**	0.1840***	0.722**
	Error	42	0.483	0.826	0.0123	0.0217	0.055
	GCA : SCA		2.04	1.94	2.32	0.34	1.80

*, *** Significant at 0.05 and 0.001 probability level, rrespectively.

‡ Calculated from $\sum_i g_i^2/(P-1) : \frac{2}{P(P-3)} \sum_{i>j} S_{ij}^2$

Table 4 : Average GCA effect general (δ^2 gi) and specific ((δ^2 Si) combining ability effect variance for Acetylene reduction rate of six parental Alfalfa clones inoculated with one of five *Rhizobium* treaments.

	Rhizobium strain														
Parental colnes	BKV-47 GCA		SCA	BKV-27 GCA		SCA	BKV-98 GCA		SCA	JIBANU SAR GCA		SCA	BKV-32 GCA		SCA
	Effect	Var.	Var.	Effect	Var.	Var.	Effect	Var.	Var.	Effect	Var.	Var.	Effect	Var.	Var.
1	0.034	–0.001	0.123	–0.182	0.032	0.29	0.289	0.085	0.161	–0.473	0.245	0.129	0.072	0.010	0.238
2	0.226	0.062	0.405	0.063	0.001	0.063	–0.370	0.145	0.183	0.270	0.081	0.190	0.033	–0.001	0.243
3	0.536	0.287	0.092	0.598	0.359	0.151	0.548	0.296	0.327	0.923	0.832	0.025	0.468	0.219	0.045
4	0.466	0.198	0.348	–0.194	0.039	0.146	0.249	0.067	0.498	–0.389	0.163	0.099	–0.127	0.023	0.209
5	–1.084	1.172	0.285	–0.394	0.158	0.098	–0.909	0.825	0.089	–0.695	0.479	0.036	–0.472	0.234	0.092
6	–0.142	0.023	0.618	0.218	0.056	0.116	0.210	0.045	0.249	0.387	0.148	0.075	0.056	0.001	0.082
S.E. (CGA Effect)	0.064			0.054			0.051			0.063			0.042		

The combined analysis (Table 2) showed that the general combining ability (GCA) and specific combining ability (SCA) and their interactions with *Rhizobium* strains were highly significant for all characters. This highly significant interaction between GCA and SCA with *Rhizobium* strains indicated variable experssion of gene effects for alfalfa genotypes in symbiosis with different strains of *Rhizobium*.

Both the GCA and SCA mean squares were highly significant for all the plant characters in each *Rhizobium* treatment (Table 3). Comparing the magnitudes of variance components of GCA and SCA, the GCA were higher than SCA for total plant dry weight, total plant N and nodule number per plant. These results suggested a preponderance of additive gene effects. Selection for these characters associated with high N_2 fixing rate might be achieved by using simple recurrent selection. However, both additive and non-additive gene effects were equally important for acetylene reduction rate with *Rhizobium* strains BKV-27, BKV-98 and BKV-47. Therefore, when these *Rhizobium* strains would be used as an inoculum, a more complicated selection method such as reciprocal recurrent selection may be necessary to improve this character.

The alfalfa genotypes X *Rhizobium* strains interaction was reflected in the estimate of GCA effect variance of acetylene reduction rate for each parental clone within each treatment (Table 4). Parental clone 3 was the best general combiner, with consistently high GCA effects on all the five *Rhizobium* treatments. None of the other parental clone showed such consistant positive GCA effect across all the *Rhizobium* strains. On the other extreme, clone 5 had negative GCA effect in all cases. The general (δ^2 gi) and specific (δ^2 si) combining ability effect variances provided a further comparison of the parental clones for their relative importance in both general and specific performance. For instance clone 3 had the highest values of GCA but also had GCA effect variances greater than SCA effect variances except with *Rhizobium* strain BKV-98, where GCA and SCA variance were about equal. This indicated the additive gene effect of clone-3 to their progenies. Clone-6 exhibited high GCA effect with *Rhizobium* strains BKV-27, BKV-98 and 'JIBANU SAR', but it attained this high average performance in different ways. With 'JIBANU SAR', relatively high GCA and low SCA effect variances were obtained, indicating that clone 6 transmitted its high performance uniformly to all its F_1 progenies. With BKV-27 and BKV-98, SCA effect varience were higher than GCA effect variance indicating that specific combinations of clone-6 with other clones gave higher or lower performance than excepted.

The significant interaction of alfalfa genotypes and *Rhizobium* strains obtained in this study indicated that the estimate of genetic variances for the alfalfa genotypes could very from one *Rhizobium* to another. Moreover the use of single strain of *Rhizobium* was reported to produce higher forage yield than those inoculated with composite inoculum in alfalfa (Bordeleau and Antoum, 1977), subclover (*Trifolium subterraneum* L.) (Jones *et al.*, 1978) and berseem (*T. alexandrinum* L.) (Poi and Kabi, 1979). However, Heichel and Vance (1979) suggested that evaluation of symbiotic effectiveness with mixture of strains might be more realistic than the use single strain for crops as genetically diverse as alfalfa. In this study, it is clear that taking maximum use of genetical variability among the genotypes of alfalfa and strains of *Rhizobium meliloti* Dang. substantial improvement of N_2 fixing rate in alfalfa could be attained. Moreover as this experiment was carried out under greenhouse conditions, there was no competition with other strains of *Rhizobium*. In practice, the selected *Rhizobium* strains must be able to compete successfully against those inffective strains in the field. Therefore, further work is needed to assess the competitive ability of these *Rhizobium* strains.

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(Accepted for publication July 20, 2004)