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Assessment of Symbiotic Effectiveness of Advanced Generation Mutants of Fenugreek (*Trigonella foenum graecum* Linn.) Selected for Higher Yield

Abhay Dashora*, K. Ramkrishna, G.R. Choudhary and Navindra Saini
Department of Plant Breeding and Genetics, SKN College of Agriculture, Rajasthan Agricultural University, Jobner 303 329, India

Abstract: A study was conducted under controlled conditions to assess symbiotic efficiency of eight advanced generation (M_6 to M_8) mutants of fenugreek variety RMt-1, basically selected for higher yield. The mutants significantly differed for the nodule traits, but showed no difference for nitrogen content per plant when inoculated with *Rhizobium meliloti* FR-9. Also, these mutants did not differ significantly for their ability to use combined nitrogen (20 mM KNO_3). Two mutants namely 95-2 and 54-3 identified as nitrate tolerant mutants for nodulation, were evaluated in field under various regimes of fertilizer nitrogen, ranging from no nitrogen to 60 kg N ha^{-1}. The two mutants were stable for nodulation irrespective of application of fertilizer nitrogen. While mutant 54-3 out-yielded parent and mutant 95-2, the biological yield of the three host genotypes was statistically at par.

Key words: Fenugreek, symbiotic efficiency, host mutant, nitrate tolerance, nodulation, *Rhizobium*.

Biological nitrogen fixation by *Rhizobium*-legume symbiosis is regarded as the most efficient agricultural system. Since the symbiosis involves interaction between the two genetic systems, genetic variation in both can be exploited to maximize the rate of biological nitrogen fixation (Dixon and Wheeler, 1986). Fenugreek, primarily a spice, is a leguminous crop, nodulated by *Rhizobium meliloti*. However, systematic approach to improve its symbiotic potential is scanty. Genetic variation exists both for the host and the micro symbiont genotypes for symbiotic effectiveness (Sinha and Ramkrishna, 1996). It has, however, long been suggested that supplementation of variability through induction of mutation in grain legumes to improve symbiotic efficiency may be important (Micke, 1984). It was also pointed out that the ability of mutagenesis to raise natural mutation rates by ten to hundred-fold could account for many mutations, which affect N fixation-related traits.

Under field conditions, the extent of nodulation and symbiotic N fixation are limited by the antagonistic effect of combined nitrogen on nodulation. In the present study, therefore, the experiments were carried out to know: (i) whether the mutants primarily selected for higher yield potential also have associated changes in symbiotic traits, (ii) whether any of the mutants can nodulate in presence of combined nitrogen, and (iii) whether the laboratory results can be extrapolated under field conditions. The present study was

* Department of Plant Breeding & Genetics, Rajasthan College of Agriculture, Udaipur 313 001, India

Table 1. Response of fenugreek variety RMt-1 and its mutants to Rhizobium FR 9 inoculation and combined nitrogen (each figure is mean of three successive generation, i.e., M_6, M_7 and M_8 raised in three replications)

Host genotypes	Nodule traits					Plant traits**			
	Nodule No.	Nodule fresh wt. (mg)	Nodule dry wt. (mg)	Plant fresh wt. (g)		Plant dry wt. (g)		Nitrogen content (mg/plant)	
	FR9	FR9	FR9	FR9	20 mM KNO_3	FR9	20 mM KNO_3	FR9	20 mM KNO3
Uninoculated (# control)				0.233		0.026		0.298	
RMt-1	6.00	15.60	1.70	0.377	0.458	0.043	0.048	1.06	1.47
15-4 (20 KR)*	8.99	20.70	2.14	0.308	0.446	0.032	0.039	0.71	1.30
18-1 (20 KR)	7.22	18.05	1.90	0.346	0.479	0.039	0.051	0.85	1.56
27-4 (20 KR)	10.22	31.89	3.01	0.364	0.493	0.043	0.049	0.92	1.64
72-3 (40 KR)	6.88	19.08	2.04	0.349	0.440	0.034	0.043	0.76	1.37
78-4 (40 KR)	4.44	11.37	1.22	0.347	0.442	0.035	0.046	0.77	1.57
95-2 (40 KR)	8.66	27.64	2.77	0.398	0.482	0.045	0.049	0.89	1.93
97-1 (40 KR)	9.33	21.46	2.08	0.379	0.420	0.046	0.046	0.73	1.65
100-5 (40 KR)	3.55	8.88	0.94	0.374	0.425	0.042	0.042	1.15	1.62
CD at 5%	1.7	4.9	0.5	0.05	0.04	0.01	NS	NS	NS

NS = Non significant; * Figures in parenthesis indicate the dose of gamma rays originally received; ** observed at 40 days of plant growth; # Values indicated are mean over replications and genotypes.

aimed at evaluating eight advanced generation mutant lines of fenugreek variety RMt-1 (primarily selected for higher yield) for symbiotic effectiveness under controlled and field conditions.

Materials and Methods

A laboratory isolate of *Rhizobium meliloti* designated as FR-9 was used (Sinha and Ramkrishna, 1996). The bacteria was maintained on Yeast Extract Mannitol (YEM) agar slant and transferred in YEM broth tubes and cultured for regrowth at 26°C±2°C in an incubator. Aliquotes of 5 mL of late exponential phase culture were used for inoculation. The host genotypes consisted of fenugreek variety RMt-1 and its eight M_6, M_7 and M_8 generation mutants, basically derived from gamma ray treatments and selected for higher yield. For aseptic culture of plants, surface sterilized seeds of different host genotypes were sown 1 cm deep in sterile sand, contained in glass vessels. The inoculation was performed after two days of seedling emergence. Each treatment was replicated thrice. The plants were raised under florescent light (5000 lux) and irrigated with nitrogen-free mineral solution of Thornton (1930) as and when required. In certain treatments the medium was supplied with KNO_3 at a concentration of 20 mM. The study comprised of four treatments, viz., (i) RMt-1 and its mutants

Table 2. Nodulation response and yield potential of fenugreek variety RMt-1 and its nitrate tolerant mutants at various regimes of fertilizer nitrogen under field conditions

Host genotypes	Nitrogen doses	Nodule number	Grain yield (q ha^{-1})	Biological yield (q ha^{-1})
RMt-1	N_0	27.57	8.96	36.88
	N_{20}	19.10	12.92	44.38
	N_{40}	16.90	10.00	38.13
	N_{40+20}	10.70	9.79	42.29
Mutant 54-3	N_0	23.87	15.00	45.21
	N_{20}	17.83	13.13	39.58
	N_{40}	22.47	14.17	34.79
	N_{40+20}	23.90	16.25	43.13
Mutant 95-2	N_0	21.53	11.46	45.21
	N_{20}	29.23	7.91	43.75
	N_{40}	29.80	13.13	47.29
	N_{40+20}	32.97	10.00	45.83
CD at 5%		6.32	25.00	NS

NS = Non-significant.

receiving only FR-9 inoculation, (ii) RMt-1 and its mutants receiving FR-9 inoculation in presence of 20 mM KNO_3 (iii) RMt-1 and its mutants receiving only 20 mM KNO_3 and (iv) RMt-1 and its mutants receiving no inoculation and no nitrogen and hence, served as control. The plants were harvested at 40 days of growth and observations on symbiotic traits were recorded. Symbiotic effectiveness of individual treatment was based on total nitrogen content, determined following Snell and Snell (1939). The data were analyzed as per factorial CRD (Panse and Sukhatme, 1967). On the basis of inference drawn from controlled experimentation, two mutants were identified as nitrate tolerant. These two mutants, along with RMt-1 (parent), were subjected to field trials under different nitrogen levels in a RBD design with three replications. Since fenugreek was cultivated on these plots for past several years the crop was not inoculated. The various nitrogen regimes were: (i) no nitrogen, (ii) 20 kg N ha^{-1}, (iii) 40 kg N ha^{-1}, and (iv) 60 kg N ha^{-1}, out of which 40 kg was applied as basal dose and the rest 20 kg at the time of flowering.

Results and Discussion

Laboratory experiments showed that the mutants significantly differed for nodule traits, but were found statistically at par for nitrogen content per plant (Table 1). In the presence of 20 mM KNO_3 (supplied as sole nitrogen source) no significant difference was observed among the mutants. It was, however, interesting to observe that mutant 95-2 could undergo nodulation even in the presence of 20 mM KNO_3, which is known to completely inhibit nodule formation (Streeter, 1984). The performance of all the mutants was consistent over three generations, indicating the fixation of the genotypes. Mutants 95-2 and RMt-1 along with another nitrate-tolerant mutant 54-3 (identified through another independent study of this laboratory) were subjected to a field trial under different regimes of nitrogen fertilization (Table 2). Variance analysis of data (grain yield, biological yield and nodule number) revealed that while the two mutants were unaffected for nodulation under different nitrogen regimes, the grain yield of mutant 54-3 was significantly higher than RMt-1 or the mutant 95-2. The biological yield, however, was statistically at par among the three host genotypes. The interaction among host and nitrogen treatments was statistically non-significant. The grain yield of the three host genotypes did not follow a particular trend over various nitrogen doses. The crop seems to have less nitrogen requirement. The higher yield of mutant 54-3 than mutant 95-2 may be due to an efficient partitioning of C and N assimilates. From the results of the present study it appears that mutagenised population selected for higher yield also accompany changes in symbiotic traits and more particularly nodulation traits and that a large number of mutants may be screened under laboratory condition for nitrate tolerance. These observations are not unique, because similar findings have been reported for *Pisum sativum* (Micke, 1984). Changes in symbiotic properties after mutagenesis have been reported in pea (Jacobson, 1984), Frenchbean (Brunner and Zapata, 1984; Gautam and Singh, 1989) and fenugreek (Subba Rao, 1986). There is also evidence that mutations conferring nitrate tolerance for nodulation are not rare and that such mutants do possess agronomic superiority over their susceptible counterparts (Brunner and Zapata, 1984).

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