Biol Fertil Soils (1992) 14:293–299

Biology and Fertility of Soils

Effect of nitrogen levels and *Rhizobium* strains on symbiotic N_2 fixation and grain yield of *Phaseolus vulgaris* L. genotypes in normal and saline-sodic soils

Raman Rai

Department of Microbiology, Narandra Deva University of Agriculture and Technology, Kumarganj-224229, Faizabad (U.P.), India

Received October 14, 1991

Summary. Following screening, selection, characterization, and symbiotic N_2 fixation with 12,5, 25.0, and 40.0 mg N kg^{-1} in normal and saline-sodic soils, only two *Phaseolus vulgaris* genotypes (HUR 137 and VL 63) and two *Rhizobium* spp. strains (ND 1 and ND 2) produced maximum nodulation, nitrogenase activity, plant N contents, and grain yields in saline-sodic soil, with 12.5 mg N kg^{-1}, compared with the other strains. However, interactions between strains (USDA 2689, USDA 2674, and ND 5) and genotypes (PDR 14, HUR 15, and HUR 138) were significant and resulted in more nodulation, and greater plant N contents, nitrogenase activity, and grain yields in normal soils with 12.5 mg N kg^{-1} compared with salt-tolerant strains. Higher levels of N inhibited nodulation and nitrogenase activity without affecting grain yields. To achieve high crop yields from saline-sodic and normal soils in the plains area, simultaneous selection of favourably interacting symbionts is necessary for N economy, so that bean yields can be increased by the application of an active symbiotic system.

Key words: *Rhizobium* spp. – Symbiotic N_2 fixation – Nitrogenase activity – Salt tolerance – Saline-sodic soils – Acetylene reduction assay – ARA

Phaseolus vulgaris is traditionally a crop of the temperate zones of India, but recently, breeders have developed genotypes which are grown in eastern parts of India, especially on the plains. The response by phaseolus beans to inoculation with *Rhizobium* spp. is variable, either because of different climatic or edaphic factors or on account of differences between cultivars (Rennie and Kemp 1983). It is well known that phaseolus beans are sensitive to higher temperatures and that the response to inoculation is variable (Duque et al. 1985). Larger harvests in inoculated compared to fertilizer N treatments have shown that while inorganic N can stimulate dry matter production the yields are not economic (Duque et al. 1985).

It has been reported (Westerman et al. 1985) that mineral N has synergistic effects on N_2 fixation and N partitioning in the vegetative parts of *P. vulgaris*. Furthermore, not only plant cultivars but also bacterial strains (*Rhizobium* spp.) affect the remobilization of N from vegetative structures (Neves et al. 1985).

Salinity and sodicitiy are the most widespread soil constraints in agriculture, and salt-affected areas in India and elsewhere are increasing each year. Excess salts in soil adversely affect survival, growth, nodulation, N_2 fixation, and the legume – *Rhizobium* symbiosis (Rai 1987). With these harsh there is a better chance of improving symbiotic N_2 fixation through a simultaneous selection of both plant genotypes and *Rhizobium* spp. strains (Rai 1983; Rai and Prasad 1983; Rai et al. 1985). In the present investigation suitable *P. vulgaris* genotypes and their *Rhizobium* spp. strains were screened in order to select combinations that produced higher yields in normal and salt-affected soils of U.P. with an economic use of N fertilizer.

Materials and methods

Soil used

Normal and salt-affected soil samples were collected from the Varanasi and Faizabad districts of U.P., India, and analysed for their physical and chemical properties (Jackson 1978). The normal soil of this plains area was a sandy loam with pH 7.5 (1:2 sol: water), organic C 0.46%, total N 0.05%, total P 1190 kg ha^{-1}, available P (Olsen's) 83.8 kg ha^{-1}, total K 1140 kg ha^{-1}, available K (ammonium acetate) 150 kg ha^{-1}, cation exchange capacity at 9.7 mEq 100 g^{-1}; DTPA-extractable Zn 3.8 kg ha^{-1}, Fe 42.4 kg ha^{-1}, Cu 1.02 kg ha^{-1}, clay 20.9%, sand 46.2%, and silt 32.9%. The salt-affected soil had a pH of 8.8, electrical conductivity of 5.2 mmhos cm^{-1}, organic C of 0.2%, E.S.P. of 22.2, exchangeable Na of 6.9 mEq 100 g^{-1}, K of 1.0 mEq 100 g^{-1}, Ca of 0.4 mEq 100 g^{-1}, and Mg of 0.6 mEq 100 g^{-1}, with 20.5% sand, 61.0% silt, and 18.7% clay.

Isolation of Phaseolus vulgaris Rhizobium spp. strains

More than 130 isolates were obtained from well-developed pinkish nodules on plant samples collected from different parts of India. Methods described by Vincent 1970 were used, with yeast extract mannital agar medium. The isolates were inoculated on N-free Burk's medium to

check for contamination by free N_2-fixers, and were characterized on the basis of morphological and plant infection tests (Vincent 1970). Out of 130 isolates, 55 proved effective and 7 were selected as most effective; these were designated *P. vulgaris Rhizobium* spp. strains ND 1, ND 2, NDR 3, ND 4, ND 5, FND 9, and M2ND 99.

Screening of P. vulgaris genotypes tolerant of saline-sodic soil and nodulating in plains soil (pot culture experiment 1, 1988–1989)

Thirty-five varieties of *P vulgaris* were tested for germination, growth, nodulation, and grain yield in saline-sodic and normal soils (data not presented). Only two genotypes, HUR 137 and VL 63, were found suitable for the saline-sodic soil, and three genotypes, PDR 4, HUR 15 and HUR 138, were found most suitable for the normal soil; used for further experiments.

Growth and multiplication of P. vulgaris Rhizobium spp. strains in saline-sodic and normal plains soils

Rhizobium spp. strains ND 1, ND 2, NDR 3, ND 4, ND 5, FND 9, and M2ND 99 and two reference strains (USDA 2689 and USDA 2674) were tested for survival, growth, and multiplication in sterilised saline-sodic and normal soils (Hartle and Alexander 1983) and populations were determined thereafter by dilution and plate-counting techniques. The results showed that out of nine (two reference) strains only two, ND 1, and ND 2, proliferated appreciably in the saline-sodic soil while strains USDA 2689, USDA 2674, and ND 5 reached maximum populations in the normal soil, and these five strains were used for further experiments.

Effect of sodium salts on growth of Rhizobium spp. strains

The effects of NaCl, Na_2SO_4, and $NaHCO_3$ (0–100 mEq of Cl^-, SO_4^{-2} and HCO_3^-) on growth (optical density at 625 nm) of strains was assessed in yeast extract mannitol liqued medium. For this growth study, cultures were grown for 10 days at 28 °C with the respective salt (five replicates). The pH of the medium was maintained regularly at 8.8 by 1 *N* NaOH.

Pot culture experiment 2 (1989–1990)

To evaluate the effect of strains ND 1 and ND 2 on symbiotic N_2 fixation, nodulation, nitrogenase activity, N content of plant parts, and grain yield of *P. vulgaris* genotypes HUR 137 and VL 63 with different amounts of applied N (12.5, 25.0, and 40.0 mg kg^{-1} soil) in saline-sodic soil, a pot culture experiment was conducted in the 3rd week of October, using 15 kg sterilized (autoclaved) saline-sodic soil per pot. Five replicatesfor each strain, genotype, and N level were used in a completely random block design. Contamination was successfully avoided during the experiment because the uninoculated plants were consistently nodule-free. For a microbiological control, seeds of the salt-tolerant genotypes HUR 137 and VL 63 were surface-sterilized with 90% ethanol and mercuric chloride for 5 and 3 min, respectively, followed by 10 rinses in sterile water. These surface-sterilized seeds were inoculated with each salt-tolerant strain, using 2 ml broth containing approximately 6×10^6 cells ml^{-1}.

The inoculum level was assessed at the time of sowing as 10^7 cells per seed. Autoclaved cell suspensions of strains were used in the uninoculated treatments and three plants were raised in each pot. The plants were grown in the glasshouse and watered when needed. N (12.5, 25.0 and 40.0 mg kg^{-1} soil), P (20.0 mg as P_2O_5 kg^{-1} soil), and K_2O (10 mg kg^{-1} soil) were added in the form of urea, single superphosphate, and potassium chloride. Zn, Mo, and Fe were also added at the rate of 6.0 mg kg^{-1} soil in the form of $ZnSO_4$, $NaMoO_4$, and $FeSO_4$.

Thirty-five days after sowing, nodule numbers and dry weights, plant dry weights, nitrogenase activity (Rai 1987), N contents of plant parts (roots, stem, leaves, and total plant; Liao 1981), grain yields at maturity, and N contents of seeds were recorded.

Nitrogenase activity

Nitrogenase activity was determined using the acetylene reduction assay in a continuous flow system (Rai 1987) with 12% acetylene in air at a rate of 100 ml min^{-1}. Samples were taken after 5 min of incubation. Acetylene and ethylene were determined by an Amil-Nucon-44 gas chromatograph equipped with a hydrogen flame ionization detector and a 50-cm stainless steel column (0.32 cm external diameter) filled with poropak N (80–100 mesh), operated at 40 °C with a carrier gas (N_2) flow rate of 40 ml min^{-1}.

Pot culture experiment 3 (1989–1990)

With some modifications, this experiment was conducted in the same manner as experiment 2 (1989–1990) and the *P. vulgaris* genotypes PDR 14, HUR 15, and HUR 138 were used to evaluate the effects of N levels (12.5, 25.0, and 40.0 mg kg^{-1} soil) and *Rhizobium* spp. strain (USDA 2689, USDA 2674, and ND 5) on nodulation, plant dry weights, nitrogenase activity, N contents of plant parts, seed N, and grain yields in sterilized normal soil. Five replicates were used for each treatment in a completely random block design. Grain yields were recorded at maturity and seed N contents were determined by a micro-Kjeldahl method.

Results

Growth and survival of Rhizobium spp. strains in saline-sodic and normal soils

Out of 130 *P. vulgaris Rhizobium* spp. isolates, only seven strains proved suitable for nodulation and growth in saline-sodic and normal soils. Changes in the populations of seven strains of *P. vulgaris* plus two reference strains after incubation for 10, 20, and 30 days are presented in Table 1. The count was highest for strain USDA 2689 (28.6×10^6 g^{-1} soil) followed by strains USDA 2674 and ND 5 in sterilized normal soil. Significant growth and survival of other strains such as ND 1 and ND 2 were also recorded, the count being highest for strain ND 1 (19.8×10^6 g^{-1} soil) followed by ND 2 (17.6×10^6 g^{-1} soil) in sterilized saline-sodic soil. *Rhizobium* spp. strains NDR 3, ND 5, FND 9, USDA 2689, and USDA 2674 did not survive in saline-sodic soil and populations declined after 10 days.

Table 1. Growth and survival of *Phaseolus vulgaris Rhizobium* spp. strains in sterilized saline-sodic and normal plains soils

Strain	Cell count $\times 10^6$ g^{-1} soil					
	Normal soil			Saline-sodic soil		
	10 days	20 days	30 days	10 days	20 days	30 days
ND 1	12.3	16.2	22.9	9.6	14.7	19.8
ND 2	10.6	14.5	21.3	8.3	11.8	17.6
NDR 3	5.7	6.9	10.5	1.2	1.0	0.9
ND 4	4.2	5.6	9.9	1.9	1.6	0.6
ND 5	18.6	22.9	27.3	1.6	0.8	0.2
FND 9	6.7	8.3	12.6	1.3	0.5	0.0
M2ND 99	4.9	6.7	9.8	2.8	3.2	3.5
USDA 2689	19.6	22.7	28.6	1.9	0.7	0.2
USDA 2674	17.5	21.9	27.9	1.3	1.0	0.0
LSD ($P = 0.05$)	0.2	0.3	0.3	0.02	0.1	0.2

Means of five replicate cultures after 10, 20, and 30 days of growth. LSD, least significant difference

Effect of three salts on growth of saline-sodic-tolerant strains

The growth of saline-sodic soil-tolerant *Rhizobium* spp. strains (ND 1 and ND 2) in yeast extract mannitol medium (initial pH 7.5) supplemented with different concentrations of Cl^-, SO_4^{2-}, and HCO_3^- at pH 8.8 was observed. Among the salts tested, bicarbonate was found to be the most toxic to the strains, followed by sulphate and chloride (Table 2). The strains showed reasonable growth at all concentrations of the three salts, but responded differently to different concentrations of Cl^{-1}, SO_4^{2-}, and HCO_3^-. Greater growth was oberved with strain ND 2, followed by ND 1, at different concentrations of the various salts.

Screening of P. vulgaris genotypes

On the basis of germination and nodulation with native *Rhizobium* spp. strains in unsterilized saline-sodic and normal plains soils with 15 mg N kg^{-1}, out of 35 genotypes, only 2, HUR 137 and VL 63, showed active nodulation and plant growth in saline-sodic soil. Moreover, on the basis of results recorded (data not incorporated) in the year 1988–1989, only three genotypes produced maximum numbers and weights of and a high level of nitrogenase activity in normal soil. The five genotypes were thus selected for further study.

Interactions between strain, genotype, and N levels on nodulation and nitrogenase activity in saline-sodic soil

The effects of salt-tolerant *Rhizobium* spp. strains on the numbers and dry weight of nodules, plant dry weights, and nitrogenase activity in nodules of the two salt-tolerant genotypes of *P. vulgaris* at three levels of applied N in sterilized saline-sodic soil are shown in Table 3. Differences among salt-tolerant strains were evident in the nitrogenase activity observed at different levels of applied N. The highest level of nitrogenase activity was observed at 12.5 mg N kg^{-1} soil and higher N rates (25.0 or 40. mg kg^{-1} soil) reduced or inhibited nitrogenase activity and numbers and dry weights of nodules. Significant increases in the number and dry weight of nodules, plant dry weights and nitrogenase activity in nodules were produced by an interaction between genotype VL 63 and strain ND 1, followed by HUR 137×ND 2 and HUR 137×ND 1 interactions. However, these interactions between genotypes and strains were less productive with the higher levels of fertilizer N. Table 3 also shows that the experimental soil was free of contamination because the uninoculated treatments did not produce any nodules.

Table 2. Effect of three salts on growth (optical density) of saline-sodic-tolerant strains grown for 5 and 10 days

Salt		*Rhizobium* spp. strain			
		ND 1		ND 2	
		5 days	10 days	5 days	10 days
Cl^-	0	0.45	0.68	0.47	0.70
	50	0.42	0.65	0.45	0.68
	75	0.40	0.61	0.42	0.63
	100	0.35	0.52	0.37	0.55
SO_4^{2-}	0	0.45	0.68	0.47	0.70
	50	0.39	0.52	0.41	0.64
	75	0.30	0.48	0.32	0.54
	100	0.25	0.33	0.28	0.42
HCO_3^-	0	0.45	0.68	0.47	0.70
	50	0.32	0.45	0.35	0.56
	75	0.28	0.36	0.29	0.45
	100	0.21	0.28	0.24	0.31
LSD ($P = 0.05$)		0.015	0.063	0.021	0.061

0 salt, broth used as control; LSD, least significant difference

Effects of interactions among strains, genotypes, and N levels on N contents of plant parts and grain yields in sterilized saline-sodic soil

Data on the effects of strains, genotypes, and N levels on the N content of various plant parts, grain yields and seed N contents (Table 4) indicate that with the application of fertilizer N less N accumulated in stems compared to roots or leaves in the uninoculated treatments. However, the application of high levels of N did not significantly increase total plant N in relation to the cultivars inoculated with strains supplied with a low level of N (12.5 mg kg^{-1} soil). No synergistic effect between the uptake of mineral N and N_2 fixation was observed. Interactions between *Rhizobium* strains and genotypes at higher levels of applied N (40.0 mg kg^{-1} soil) gave significantly higher seed yields and seed N levels compared to the uninoculated control. The N concentrations in various plant parts and in the seeds showed significant variations among genotypes and strains at different levels of applied N.

Effects of interactions among strains, and genotypes, and N levels on nodulation and symbiotic N_2 fixation in sterilized normal soil

Table 5 shows numbers and dry weights of nodules, plant dry weights, and nitrogenase activity for plant – *Rhizobium* spp. combinations as affected by different concentrations of applied N in sterilized soil. Different combinations of *Rhizobium* spp. strains and plant genotypes produced significant differences in the number and dry weight of nodules, plant dry weights, and nitrogenase activity at 12.5 mg N kg^{-1} soil. Greater increases in the number of nodules and in the other measurements were observed with the interaction between genotype HUR 128 and USDA 2674, followed by HUR 15×USDA 2689, and PDR 14×ND 5. The results also showed that the application of fertilizer N at 25.0 and 40.0 mg kg^{-1} soil reduced nodulation and nitrogenase activity. Data presented in Tables 3 and 5 clearly indicate that nodulation and symbiotic N_2 fixation were lower in sterilized saline-sodic soil than in normal soil.

Effects of interactions among strains, genotypes, and N levels on the N content of different plant parts and grain yields in normal soil

Table 6 shows the effects of strains, genotypes, and applied N levels on N contents of different plant parts, grain yields, and seed N contents. The pattern of N distribution and accumulation in plant parts was the same as that observed in the saline-sodic soil (Table 4). Significant differences were observed in accumulated N levels in various plant parts. Howver, all the genotypes showed a significant increase in grain yield at 40.0 mg N kg^{-1} soil compared with 12.5 or 25.0 mg kg^{-1} soil and compared with the uninoculated treatments. The strains differed significantly in N contents of plant parts and in grain yields although there were differences among genotypes also. The

Table 3. Effect of N levels and *Rhizobium* strains on nodulation, plant dry weight, and nitrogenase activity of *Phaseolus vulgaris* genotypes in sterilized saline-sodic soil

Genotype	*Rhizobium* spp. strain	Fertilizer N (mg kg^{-1} soil)	Nodules (no. $plant^{-1}$)	Dry weight of nodules (mg $plant^{-1}$)	Mean dry weight of three plants (g)	Nitrogenase activity (μmol C_2H_4 h^{-1} g^{-1} nodules)
HUR 137	Uninoculated	12.5	0.0	0.0	2.75g	0.0
		25.0	0.0	0.0	5.92f	0.0
		40.0	0.0	0.0	9.34	0.0
HUR 137	ND 1	12.5	3.9bc	9.39c	10.95cde	132.78b
		25.0	1.2ef	2.62e	11.35cde	19.25f
		40.0	0.3g	0.75f	13.01bcd	3.59h
HUR 137	ND 2	12.5	4.5ab	13.85b	12.93bc	151.33b
		25.0	1.7de	3.12e	12.96bc	29.25c
		40.0	0.6fg	0.92f	14.78ab	13.76g
VL 63	Uninoculated	12.5	0.0	0.0	3.85f	0.0
		25.0	0.0	0.0	7.93f	0.0
		40.0	0.0	0.0	10.91de	0.0
VL 63	ND 1	12.5	4.6a	15.38a	13.62ab	176.62a
		25.0	2.1d	6.58d	13.85ab	37.01d
		40.0	1.2ef	2.15e	15.25a	13.35g
VL 63	ND 2	12.5	3.6c	8.79c	9.62e	110.87hi
		25.0	0.7fg	0.92f	10.20e	3.26ij
		40.0	0.2g	0.06f	11.02cde	1.79j
SEM			0.2205	0.4118	0.6348	0.5561

Means of five replicate pots. Values within columns followed by at least one common letter are not significant at 5% level of significance (Duncan's multiple range test)

Table 4. Effect of N levels and *Rhizobium* strains on N content of different plant parts 35 days after sowing, and on grain yield and seed N content of *Phaseolus vulgaris* genotypes in sterilized saline-sodic soil

Genotype	*Rhizobium* spp. strain	Fertilizer N (mg kg^{-1} soil)	N content (mg $plant^{-1}$)				Grain yield (g pot^{-1})
			Roots	Stems	Leaves	Seeds	
HUR 137	Uninoculated	12.5	6.5i	4.5k	9.5l	65.5g	6.1j
		25.0	7.5fg	5.3k	15.2j	97.6f	9.7hi
		40.0	10.8c	8.2j	19.4i	115.2de	12.4efg
HUR 137	ND 1	12.5	7.3gh	25.9cd	39.3c	125.5ef	12.5def
		25.0	7.6fg	18.2fg	30.5e	119.3cde	10.3ghi
		40.0	9.5d	17.7g	25.9g	136.5b	14.5bcd
HUR 137	ND 2	12.5	9.7d	38.6b	43.5b	130.9bc	13.6cde
		25.0	9.9d	21.8f	31.6e	119.2cde	9.2fgh
		40.0	10.6c	19.1fg	27.0fg	138.8b	15.7b
VL 63	Uninoculated	12.5	7.9f	5.1k	8.6m	64.9g	5.5j
		25.0	8.7e	6.0k	13.3k	96.6f	8.8i
		40.0	14.3a	8.2j	18.5j	129.7bcd	11.2fgh
VL 63	ND 1	12.5	10.6c	30.4a	48.2a	140.4ab	16.3b
		25.0	10.7c	24.2d	35.5d	122.2cd	10.9fgh
		40.0	11.8b	21.5e	28.7f	152.7a	18.4a
VL 63	ND 2	12.5	6.6i	24.6c	36.9d	137.3b	14.9bc
		25.0	6.8hi	14.2h	23.2h	119.1cde	10.1hi
		40.0	7.2gh	10.5i	16.5j	144.2ab	16.3b
SEM			0.1817	0.5630	0.6248	1.3458	0.6412

See footnotes to Table 3

Table 5. Effect of N levels and *Rhizobium* spp. strains on nodulation, plant dry weight, and nitrogenase activity of *Phaseolus vulgaris* genotypes in sterilized normal plains soil

Genotype	*Rhizobium* spp. strain	Fertilizer N (mg kg^{-1} soil)	Nodules (no. $plant^{-1}$)	Dry weight of nodules (mg $plant^{-1}$)	Mean dry weight of three plants (g)	Nitrogenase activity (μmol C_2H_4 h^{-1} g^{-1} nodules)
PDR 14	Uninoculated	12.5	0.0	0.0	5.3w	0.0
		25.0	0.0	0.0	11.2u	0.0
		40.0	0.0	0.0	15.7rs	0.0
PDR 14	USDA 2689	12.5	6.3cd	16.6c	19.71mn	149.38de
		25.0	2.8fg	5.7h	20.2jklm	31.25f
		40.0	1.7hijkl	2.1jkl	22.5ghi	6.56i
PDR 14	USDA 2674	12.5	5.4de	13.7d	17.8nopq	168.32cd
		25.0	1.9ghijk	2.5j	19.5klmn	26.20gh
		40.0	1.2jklm	1.9jkl	21.6hij	4.51lmn
PDR 14	ND 5	12.5	6.5bc	17.9c	20.3jkl	166.29cde
		25.0	2.3fghi	5.8h	23.9efg	29.52g
		40.0	1.4ijklm	2.3jk	25.6cd	3.75mno
HUR 15	Uninoculated	12.0	0.0	0.0	6.2w	0.0
		25.0	0.0	0.0	12.9t	0.0
		40.0	0.0	0.0	17.3opqr	0.0
HUR 15	USDA 2689	12.5	7.3ab	21.6a	26.6c	198.39b
		25.0	3.2f	7.3g	29.2b	41.56ef
		40.0	2.1ghij	4.1i	31.7a	19.63hi
HUR 15	USDA 2674	12.5	6.5bc	17.9b	21.2ijk	183.92c
		25.0	2.5fgh	4.2i	22.9fghi	26.55ghi
		40.0	1.8ghijkl	1.9jkl	24.6def	13.97ijk
HUR 15	ND 5	12.5	6.2cd	15.8c	18.91mno	178,38cd
		25.0	1.0klm	1.7jkl	19.2st	3.95mno
		40.0	0.5m	0.91	21.6hij	1.21pqr
HUR 138	Uninoculated	12.5	0.0	0.0	4.8w	0.0
		25.0	0.0	0.0	9.3v	0.0
		40.0	0.0	0.0	13.2t	0.0
HUR 138	USDA 2689	12.5	4.8e	12.5e	16.9pqr	123.72def
		25.0	1.2jklm	2.6j	19.6mnopq	6.21klm
		40.0	0.6m	1.0kl	21.6hij	2.50opq
HUR 138	USDA 2674	12.5	7.6a	19.7a	21.7hij	206.85a
		25.0	2.5fgh	3.9i	23.6fgh	27.62fg
		40.0	1.7hijkl	1.7jkl	25.2cde	7.55hij
HUR 138	ND 5	12.5	4.6e	10.9f	14.9s	113.88def
		25.0	1.2jklm	1.3jkl	16.6gr	9.35ijk
		40.0	0.81m	0.9e	18.51mnop	2.06nop
SEM			0.3197	0.3995	0.5717	1.3952

See footnotes to Table 3

interaction among strains, genotypes, and N levels was significant for N accumulation and grain yield. The combination of HUR 138 and USDA 2674 with 40.0, 25.0, or 12.5 mg N kg^{-1} soil gave the highest grain yields (25.2, 19.5, and 23.2 g pot^{-1}) and seed N contents (160.7, 132.9, and 156.6 mg $plant^{-1}$ seeds), respectively, followed by PDR 14×USDA 2689 at all N levels.

Discussion

Salt-tolerant *P. vulgaris* genotypes and *Rhizobium* spp. strains were isolated and selected for their ability to effectively grow and fix N under salt-stress conditions in saline-sodic soil. The effect of various salts on the growth of the different strains was variable, and it appears that like most of the *Rhizobium* strains, *Rhizobium* spp. strains of *P. vulgaris* are sensitive to higher concentrations of salts (Rai 1987). Genetic determinants in ND 2 and ND 1 may have been responsible for the better salt tolerance of these strains, resulting in more growth, greater populations, and effective symbiotic N_2 fixation with salt-tolerant genotypes in saline-sodic soil (Tables 1, 2, 3). The mechanism of salt-tolerance in *P. vulgaris* genotypes is not known; their longer roots that penetrate deeper into the soil (unpublished data) may be an escape mechanism, as salts frequently rise upwards in soil because of capillarity in the winter season. Other physiological mechanisms cannot be ruled out, however. The genetic mechanism of salt-tolerance seems to help the bacterium to thrive, compete, and perform better in symbiosis with salt-tolerant genotypes at lower levels of applied N in saline-sodic soil (Table 3). Further, the two selected strains (ND 1 and ND 2), which showed equal salt tolerance produced variable patterns of nodulation and other yield characteristics (Table 3, 4), perhaps due to the activities of host genes and/or to applied N levels (Rai and Singh 1979).

Table 6. Effect of interaction among N levels, *Rhizobium* spp. strains, and genotypes of *Phaseolus vulgaris* on N content of plant parts 35 days after sowing, and grain yield and seed N content in sterilized normal plains soil

Genotype	*Rhizobium* spp. strain	Fertilizer N (mg kg^{-} soil)	N content (mg plant^{-1})			Grain yield (g pot^{-1})	Seed N (mg plant^{-1})
			Roots	Stems	Leaves		
PDR 14	Uninoculated	12.5	6.3pgr	5.5p	10.3q	7.5r	64.2st
		25.0	8.5hijkl	6.8op	17.4p	11.9pq	93.8q
		40.0	14.3a	10.2mn	25.6no	20.7ghij	136.7j
PDR 14	USDA 2689	12.5	7.5klmnop	32.6cd	46.2bcd	22.7bcdefg	143.5fg
		25.0	9.7fgh	20.6hijk	33.5ijk	17.7klmn	129.5klm
		40.0	12.2cd	15.91m	30.6jklm	23.9bcde	154.9bc
PDR 14	USDA 2674	12.5	6.9mnopqr	29.5de	43.5cde	18.3ijklm	141.2ghi
		25.0	8.5hijkl	20.8ghij	31.9jklm	16.9lmn	125.5no
		40.0	12.7bc	17.0jkl	28.7klmno	20.2ghijk	147.8e
PDR 14	ND 5	12.5	7.9jklmno	38.8a	47.5dc	21.8defgh	138.9ij
		25.0	8.8ghijk	20.5ijk	35.7ghi	17.7jklmn	123.5op
		40.0	10.2ef	15.81m	24.30	24.5abcd	156.2b
HUR 15	Uninoculated	12.5	7.5klmnop	6.60p	11.6q	10.8q	54.7t
		25.0	9.9fg	8.2op	20.2p	13.6op	90.5qr
		40.0	13.2abc	10.5no	27.5mno	21.0efghi	137.8ij
HUR 15	USDA 2689	12.5	6.5opqr	36.5abc	50.3a	24.8abc	142.5gh
		25.0	7.3lmnopq	26.2ef	43.5cde	19.5hijkl	130.2kl
		40.0	9.5fghi	19.2ijkl	32.6jkl	26.9a	151.5cd
HUR 15	USDA 2674	12.5	6.9mnopqr	34.6abc	48.9b	22.6bcdefg	139.5hij
		25.0	8.5jklmn	24.5fgh	36.8fgh	18.3ijklm	126.2mno
		40.0	9.2ghij	17.7ijkl	30.9jklm	24.8abc	152.9bc
HUR 15	ND 5	12.5	7.0mnopqr	33.9bc	46.6bc	21.6defgh	142.6gh
		25.0	9.2ghij	20.2ijk	33.4ijk	17.0lmn	127.21mn
		40.0	11.5de	18.5ijkl	28.21mno	23.9hijkl	154.5bc
HUR 138	Uninoculated	12.5	6.6opqr	5.2p	11.5q	6.8r	60.8s
		25.0	8.2ijklm	6.8op	19.3p	10.2q	87.6r
		40.0	13.7ab	9.8no	29.4klmn	18.5ijklm	131.9k
HUR 138	USDA 2689	12.5	6.9mnopqr	32.7cd	47.9bc	21.6defgh	147.9e
		25.0	8.6ghijkl	26.3ef	41.8defg	16.5mn	121.6p
		40.0	9.8fgh	16.9kl	31.6jklm	23.2cdefgh	149.2de
HUR 138	USDA 2674	12.5	4.9s	38.8ab	53.2a	23.7bcde	156.6b
		25.0	5.8rs	24.4fgh	41.5efg	19.5hijkl	132.9k
		40.0	7.2mnopqr	21.2ghi	34.6hij	25.2ab	160.7a
HUR 138	ND 5	12.5	5.8grs	31.2f	49.5ab	20.5efgh	146.3ef
		25.0	9.7nopqr	25.0fg	40.2efg	15.2no	123.9nop
		40.0	7.9jklmno	21.1ijkl	30.6jklm	23.6bcdef	154.2bc
SEM			0.4202	1.261	1.452	0.8025	1.182

See footnotes to Table 3

The differential interactions observed between genotypes and strains as affected by various levels of applied N were quantitative rather than qualitative, and each variable considered in this investigation may be controlled by a polygenic system (Tables 5, 6). Selected cultivars benefitted from inoculation with the efficient and effective *Rhizobium* spp. strains of USDA 2689, USDA 2674, and ND 5 and also responded significantly to applied N. Thus the grain yield of plants supplied with 40.0 mg N kg^{-1} soil was greater than the yield of inoculated plants supplied with 12.5 mg N kg^{-1} soil only.

The present findings show that although mineral N stimulated plant growth, it decreased or inhibited N_2 fixation as estimated by nitrogenase activity or by the total N content of the plant, indicating that N_2 fixation is not affected by synergistic effects of mineral N. Interactions among strains, genotypes, and N levels caused variations in nodulation, nitrogenase activity, the N content of plant parts, grain yields and seed N levels in sterilized normal soil (Tables 5, 6). However, the highest N level (40.0 mg N kg^{-1} soil) promoted the vegetative growth of plants, resulting in greater plant dry weights and seed yields. The effects of applied N on the N content of different plant parts and seeds clearly indicate that N accumulations were greater in roots than in stems or leaves, whereas the N_2 fixing system promoted greater N accumulations in stems and leaves than in the root system; similar observations have been reported by Westermann et al. (1985) in beans and Neves et al. (1985) in soybeans.

Furthermore, in the uninoculated treatments, not only the plant cultivars but also the bacterial strains affected the remobilization of N from vegetative parts (Table 6), and similar results have been reported by Zeiher et al. (1982). It is also evident from the present results (Tables 5, 6) that an efficient symbiotic system may be beneficial for seed production using both a low level of applied N (12.5 mg N kg^{-1} soil) and N_2 fixation. Although the best cultivar/strain combinations used in the present

study responded to low fertilizer N levels in saline-sodic and normal soils and significant seed yields were obtained, there was no further increase in grain yield with the higher levels of fertilizer N over the symbiotic system. Therefore, it is important to select plant genotypes and *Rhizobium* spp. strains for salt-affected and normal soils in order to produce high seed yields from N_2 fixation, as the use of N fertilizer is not economically viable for the farming system of a developing country like India. This suggests that any breeding programme for *P. vulgaris* should include, as a high priority, selection for high N_2 fixation levels in salt-affected and normal soils.

Acknowledgment. I am grateful to Shri Sunil Kumar Tyagi for statistical analysis of the data and Dr. B.S. Srivastava (C.D.R.I., Lucknow), for providing some laboratory facilities.

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