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THE EFFECT OF HIGH TEMPERATURE ON *VIGNA RADIATA* NODULATION AND GROWTH WITH DIFFERENT BRADYRHIZOBIAL STRAINS

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HAFEEZ F. Y., ASAD S. and MALIK K. A. *The effect of high temperature on* Vigna radiata *nodulation and growth with different bradyrhizobial strains*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY **31**, 285–294, 1991.—A study was conducted to examine the effect of constant high temperatures and diurnally administered temperature regimes (day/night temperature was maintained at 30°C and increasing 2 hr temperature shocks of 36, 42 and 48°C were applied daily) on growth, nodulation and nitrogen fixation of mungbean (*Vigna radiata* L. Wilczek) plants and growth responses of five different cowpea bradyrhizobial strains. Mungbean genotype and bradyrhizobial strains responded differently to high temperatures. Mungbean plants survived at (1) diurnal regimens of high temperature and (2) constant root temperatures of 42 and 48°C (day and night), but germination at 48°C was reduced to 38%. The bradyrhizobial strains survived, grew and remained infective and effective after incubation at constant temperatures up to 42°C and diurnal regimen high temperatures. Two strains, Vm1 and Vr16, survived and multiplied at a constant temperature of 48°C. These strains were also effective on mungbean and siratro (*Macroptilium atropurpureum*) plants after incubation at 48°C for 5–10 days. Elevated temperatures (>36°C) depressed nodulation and nitrogen fixation. Constant high temperatures (42 and 48°C) at the seedling stage markedly affected nitrogenase activity (ARA).

Key words: Bradyrhizobium, N_2-fixation, temperature stress, *Vigna radiata*.

INTRODUCTION

Vigna radiata (mungbean) is an important grain legume for the dry, semi-arid regions of Pakistan. There is little natural nodulation on *V. radiata* in such environments although the indigenous cowpea bradyrhizobial population (2×10^4/g soil) is sufficient for effective nodulation.[1] Attempts are being made to identify the factors limiting the establishment and maintenance of mungbean–*Bradyrhizobium* symbiosis in these environments. Temperature, along with other environmental factors such as salinity[7,15] and drought,[9,20] influence *Rhizobium*/*Bradyrhizobium* legume symbiosis. The temperature record for soils planted with mungbean in Pakistan shows that the top 10 cm of the soil profile is frequently exposed to temperatures above 38°C during the summer and may reach up to 48°C (unpublished data). Detailed studies on temperature effects on nodulation and nitrogen fixation are confined to a relatively few tropical legumes.[2,4,5,13,14,22–24]

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Many studies have reported that the growth and survival of rhizobia/bradyrhizobia in soils are adversely affected by high soil temperatures.[11,12,17,19,21] A large number of mungbean cultivars/elite mutants were found to be poorly nodulated[8] despite the presence of large bradyrhizobial populations which seem to survive high temperatures but are unable to form an effective symbiotic relation with the host plant. In order to verify and elucidate the role of high root temperatures on growth, nodulation and nitrogen fixation of *V. radiata*, a series of experiments were performed, the results of which are presented below. In addition, the tolerance of five strains of cowpea *Bradyrhizobium* to high temperatures is also reported.

MATERIALS AND METHODS

The effect of high root temperatures on mungbean–*Bradyrhizobium* symbiosis was studied in Experiments-I and -II and the variability among bradyrhizobial strains in growth response to various temperatures was evaluated in Experiment-III.

Strains of Bradyrhizobium

Five cowpea bradyrhizobial strains, Vr16, Vr17, Vr19, Vm1 and USDA 3748, were used separately as inocula. Strains Vr16, Vr17 and Vr19 were isolated from mungbean and Vm1 from blackgram, cultivated in the fields at the Nuclear Institute for Agriculture & Biology (NIAB). Strain USDA 3748 was obtained from the Beltsville *Rhizobium* Culture Collection, Beltsville, MD, U.S.A. Vr16, Vr19 and Vm1 belong to the same serogroup; the remaining two strains are serologically distinct (unpublished data). Pure cultures of each strain were grown to a cell population of 1×10^9 cells/ml at 28 ± 2°C in yeast extract mannitol medium.[27]

Legume host

Seeds of *Vigna radiata* (L.) Wilczek (cv. NM 54), commonly known as mungbean or green gram, were obtained from the Mutation Breeding Division of NIAB, Faisalabad.

Experiment-I

V. radiata (cv. NM 54) was tested at five root temperatures with five *Bradyrhizobium* strains along with uninoculated (UC) and nitrogen controls (NC). Temperatures of rooting media were maintained continuously (day/night) at 24, 30, 36, 42 and 48°C. The latter temperature does not occur constantly in the mungbean growing area; this temperature was included to determine the upper tolerance limit of mungbean genotype and bradyrhizobial strains. A two-factor factorial experiment was conducted in a completely randomized design with three replicates for each treatment combination. Pots (25×45 cm) containing 400 g of prewashed sterilized sand were partially immersed in a waterbath and the rooting medium was thermostatically kept at a constant temperature. The temperature of sand inside the pots was monitored daily with a thermometer with a precision of ± 1.0°C. All treatments were fertilized with N-free Hoagland's nutrient solution except for the nitrogen control (NC) which received a weekly application of ammonium nitrate (6 mM/pot) 15 days after sowing. Seeds were surface sterilized with a 1% NaOCl solution. Four seeds were sown per pot and were thinned at emergence to a final stand density of two plants/pot. The pots were irrigated to field capacity with nutrient solution and tapwater alternately, as needed, until harvest.

Strain-inoculation treatments were applied at a rate of 0.5 ml of broth culture per seed. In set A, inoculum and temperature stress were applied at the time of sowing; in set B these were applied to seedlings 3 days after germination. Waterbaths containing plants were kept in a growth room with a 14-hr photoperiod, day/night air temperatures of $24 \pm$ °C and a photon flux density of 392 μmole m^{-2} sec^{-1} at seedling level. Plants were harvested 5 weeks after sowing and nodulation, acetylene reduction activity (ARA), dry weight and total N in plant tops were analyzed as described earlier.[7]

Experiment-II

In this study, day/night temperature was maintained at 30°C and increasing temperature shocks of 36, 42 and 48°C for 2 hr each were applied daily starting at 8 a.m. and ending at 2 p.m. local time. The desired root temperature was achieved

within 1 hr. The selection of these temperature shocks was based on observed soil temperatures during the summer when the crop was in the field (unpublished data). Experiments were carried out in sand as well as in soil. Strain inoculations were at 0.5 ml of broth culture per seed. In set A, inoculum and temperature stress were applied at the time of sowing, while in set B these were applied to seedlings 3 days after germination. Plants growing continuously at 30°C were taken as the control (set C). The other parameters were the same as described for Experiment-I. The data were subjected to an Analysis of Variance and upon obtaining a significant *F*-ratio, Duncan's Multiple Range Test was employed for multiple comparisons of paired means.

Experiment-III

Growth and survival of cowpea *Bradyrhizobium* strains at 24, 30, 36, 42 and 48°C were studied in (i) agar plates, (ii) broth cultures and (iii) soil. For continuous temperature stress, the strains were kept at the above temperatures for 10 days. For diurnally cycling high temperature treatments, strains were kept at 30°C and increasing temperature shocks of 36, 42 and 48°C for 2 hr each were given for 10 consecutive days. Survival was determined by placing the plates without colonies at 30°C for another 10 days. There were three replicates per experiment.

For agar plates, inoculation of each strain was done on yeast extract mannitol (YEM) agar. For liquid culture, YEM medium was used but without agar. Fifty milliliter cultures (log phase) were placed in 150 ml conical flasks which were kept in a controlled temperature waterbath set at 100 rpm. The soil experiment was performed on gamma irradiated soil which had been moistened to field capacity. Ten grams of soil were placed in 30 ml screw-capped universal vials. Inoculum treatments consisted of pure stock cultures which were washed clear of YEM broth by centrifugation with 0.85% saline and adjusted to a concentration of 1×10^7 cells/ml. Inoculation was made by adding 1 ml of this suspension to each vial. The vials were placed in plastic bags in order to avoid desiccation, and incubated at specified temperatures. Population levels of the *Bradyrhizobium* strains were measured at 0 and 10 days of incubation by the drop plate technique.[27] Serial dilutions were made with sterile 0.85% saline and placed on YEM agar at 28±2°C for plate counts. The results were expressed on the basis of gram dry weight of soil.

The ability of bradyrhizobial strains to nodulate mungbean and siratro plants after survival at 42 and 48°C and diurnal temperature regimes was checked in growth pouches.

RESULTS AND DISCUSSION

This study suggested the importance of high temperature in determining the effectiveness of mungbean–*Bradyrhizobium* symbiosis. At constant temperatures (24–42°C) seed germination was 100%, which was reduced to 38% with a delay of 4 days at 48°C. A 29% decrease in plant survival was noted when temperatures exceeded 36°C at sowing time (set A); a decrease of 86% in survival was noted for plants exposed to temperatures higher than 36°C after germination (set B). The effect of diurnal temperature regimes of 30–48°C on plant survival was 100 and 94% in soil and sand, respectively. A soil temperature regime of 24.2–38.8°C was optimum for germination of soybean.[26] The previously reported[13] most extreme temperature regime for growth of soybean plants was 41°C for 6 hr. In the present study, survival and growth of mungbean plants were observed in the highest temperature regime (48°C for 2 hr) as well as at a constant high temperature of 42°C. Therefore, the lethal temperature for mungbean plants depends not only on the temperature but also the duration of exposure and developmental stage of the plant.

The responses of nodulation, nitrogen fixation, dry matter yield and total nitrogen (N) concentration to constant high temperatures (Figs 1–3, Table 1) and diurnal high temperature changes (Tables 2, 3) in roots of mungbean were studied. The results revealed that maximum nodulation, nitrogen fixation, dry matter yield and total N concentration were observed with temperatures between 24 and 36°C. Soil temperatures of 42 and 48°C appeared to interfere with the development and functions of root nodules, which may constitute a significant constraint to early growth of mungbean. In contrast, soil temperatures between 30 and 35°C have been reported to inter-

Table 1. Effect of high temperature and inoculation on nodulation, ARA and growth of mungbean (Experiment-I)

Treatments	Nodule number /plant		Nodule dry wt (mg/plant)		μmole C_2H_4 g^{-1} dry nodule hr^{-1}		Shoot dry wt (g/plant)		Shoot N (mg/plant)	
	A	B	A	B	A	B	A	B	A	B
Temperature (°C)	Temperature means (average over strains)									
24	21.9B	15.1A	9.3A	7.8A	63.9C	17.1A	0.44A	0.30B	9.0B	6.0B
30	26.1A	14.0A	7.2B	9.6A	94.1B	8.9B	0.57A	0.53A	12.0A	9.9A
36	21.7B	14.5A	5.1C	7.1A	107.4A	3.2C	0.63A	0.33B	6.6C	6.2B
42	7.9C	1.6B	2.5D	0.6B	9.8D	2.1C	0.17B	0.11C	2.3D	1.6C
48	3.0D	1.1B	0.5E	0.1B	2.1E	1.1C	0.11B	0.02C	2.5D	0.9C
Bradyrhizobial strains	Strain means (average over temperatures)									
Vr16	12.1C	14.5A	6.6AB	8.0A	81.4B	7.7A	0.36A	0.30A	6.6A	5.8A
Vr17	12.5C	13.1A	4.9A	6.0A	121.1A	12.7A	0.36A	0.26A	6.8A	5.3A
Vr19	14.9BC	11.1A	7.8A	6.3A	64.2C	9.1A	0.42A	0.32A	7.9A	5.9A
Vm1	22.9A	16.1A	8.4A	8.7A	63.0C	9.2A	0.38A	0.38A	7.2A	5.3A
USDA3748	17.3B	9.9A	6.7AB	6.2A	58.0D	9.8A	0.39A	0.22A	7.6A	4.9A
LSD for interaction (temperature × strain)										
5%	12.9	NS	NS	NS	12.5	21.7	0.22	NS	4.5	NS
1%	NS	NS	NS	NS	16.7	NS	0.29	NS	6.1	NS

Means followed by same letter(s) in a column are not significantly different ($P > 0.05$) according to DMR test. Values are the average of 15 readings.

Set A: temperature stress and inoculation were applied at the time of sowing.

Set B: temperature stress and inoculation were applied after germination.

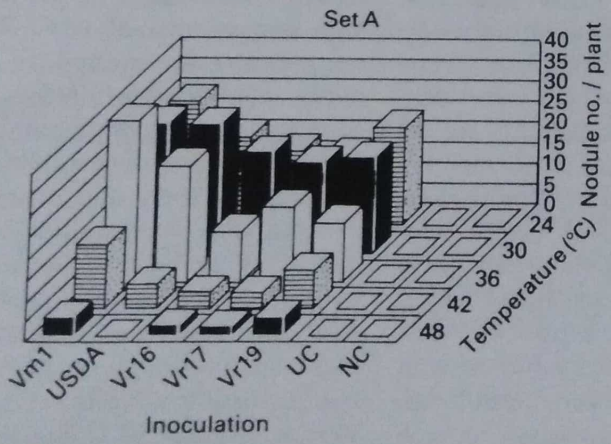

FIG. 1. Effect of high temperature and bradyrhizobial strains on number of nodules of mungbean. UC, uninoculated; NC, nitrogen control. Set A: temperature stress and inoculation were applied at the time of sowing.

fere with the development and function of root nodules in soybean.[16,18,19]

Significantly higher numbers of nodules were observed in Experiment-I (sets A, B) at 36°C with bradyrhizobial strain Vm1 (Fig. 1, Table 1). In set A, strain Vm1 differed significantly from the other four strains only for number of nodules (Table 1). Nitrogenase activity, as determined by ARA, was drastically reduced in set B as compared to set A (Fig. 2). In set A, a differential response of strains to various temperatures was observed for all traits except dry weight of nodules, whereas in set B there was no interaction between inoculation and temperature levels for all traits except ARA (Table 1). The adverse effect of high temperature stress on the growth and survival of the host plant was more pronounced at the seedling stage (set B) relative to

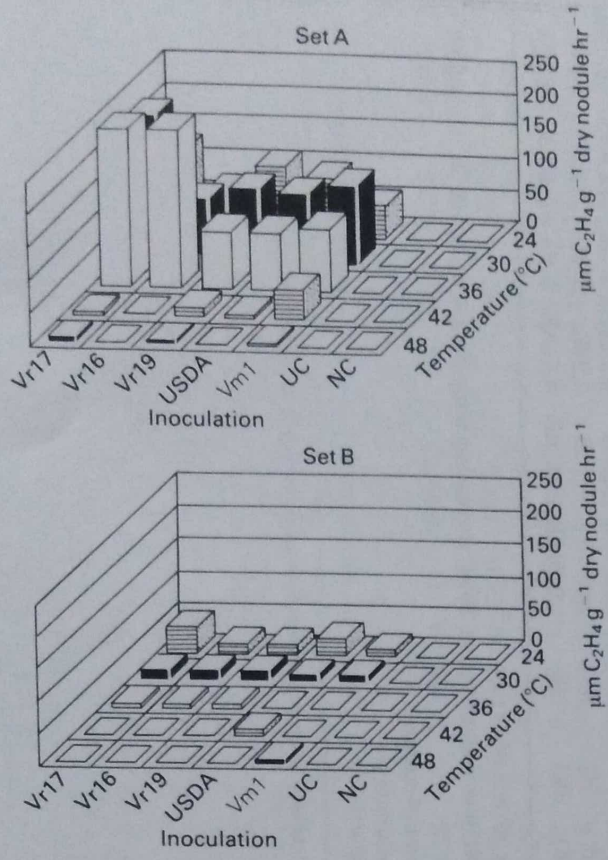

FIG. 2. Effect of temperature and bradyrhizobial strains on acetylene reduction activity; µmole C_2H_4 g^{-1} dry nodule hr^{-1} of mungbean. Set A: temperature stress and inoculation were applied at the time of sowing. Set B: temperature stress and inoculation were applied after germination.

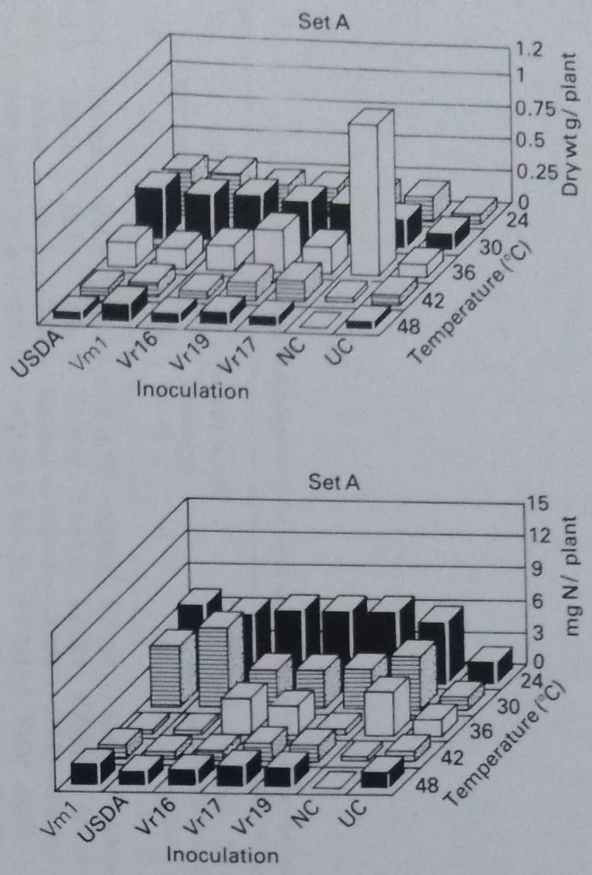

FIG. 3. Effect of high temperature and bradyrhizobial strains on shoot dry weight; g/plant and total nitrogen; mg N/plant. Set A: temperature stress and inoculation were applied at the time of sowing.

other treatments. The effectiveness of the nodule may depend on the timing of the temperature stress and the growth conditions of the mungbean plants.

A diversity of responses of cowpea *Bradyrhizobium* to constant high temperatures and diurnally administered high temperatures was observed among the five strains after 10 days of incubation in broth, agar plates and soil (Fig. 4; Tables 4, 5). The optimum temperature for these bradyrhizobial strains was 30–36°C except strain USDA 3748 which was sensitive to temperatures above and below 30°C. It is interesting to note that local isolates were more tolerant of elevated soil temperatures, i.e. 42°C. Two strains Vr17 and Vm1 showed 50% less growth in soil incubated at 42°C relative to their incubation at 36°C. Only two bradyrhizobial strains, Vm1 and Vr16, were able to survive and grow at 48°C on agar plates (Table 5). The growth rate of strain Vm1 increased between 36 and 42°C, indicating the optimum temperature for this thermotolerant strain was higher than that of other strains. Ability to grow well at 42°C was believed to be rare among rhizobia, even those of tropical origin.[3,6] Total loss in rhizobial viability was observed in chickpea, lentil and bean inoculation when they were exposed to an ambient temperature of 44°C,[25] however, a *R. phaseoli* strain survived and multiplied in YEM agar at 45–47°C.[10] A *B. japonicum* strain has also been reported to survive in liquid at 48.7°C but was

Table 2. Effect of high diurnal temperature regime and inoculation on nodulation and growth of mungbean in sand (Experiment-II)*

Strains	No of nodules/plant			Nodule dry wt (mg/plant)			μmole C_2H_4 g^{-1} dry nodule hr^{-1}			Shoot dry wt (g/plant)			Shoot N (mg/plant)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Vr16	30A	24AB	23A	15.5B	18.5AB	70.0A	78A	278B	101AB	0.33A	0.33A	0.54A	6.3A	6.6AB	9.0AB
Vr17	29A	27AB	20A	9.3B	8.2AB	60.0A	63A	186AB	190AB	0.24AB	0.26AB	0.52A	4.8BC	5.3BC	9.3AB
Vr19	12B	19B	20A	5.2B	14.8B	72.0A	21AB	97BC	352A	0.18B	0.22AB	0.50A	3.3BC	4.1BC	10.3A
Vm1	11B	33A	24A	3.2B	22.1A	64.0A	40AB	92BC	159AB	0.13B	0.27A	0.58A	1.6C	5.6BC	11.4A
USDA 3748	31A	22B	17A	37.9A	21.7AB	51.0A	29AB	94BC	308A	0.25AB	0.31A	0.52A	4.7BC	6.4AB	9.5AB
UC	2B	3C	2B	0.6B	5.3C	0.5B	2B	2C	11B	0.16B	0.13B	0.26B	2.7C	2.8C	4.9B
NC	0B	0C	0.6	0B	0C	0.7B	0B	0C	3B	0.26AB	0.21AB	0.44AB	9.9A	8.6A	13.7A

Means followed by the same letter are not significantly different ($P > 0.05$).

Values are the mean of three readings. * Day/night temperature was maintained at 30°C, increasing temperature shocks of 36, 42 and 48°C for 2 hr each were applied daily.

Set A: temperature stress and inoculation were applied at the time of sowing.

Set B: temperature stress and inoculation were applied after germination.

Set C: day/night temperature was maintained at 30°C (control).

UC: Uninoculated; NC: nitrogen control.

Table 3. *Effect of high diurnal temperature regime* and inoculation on nodulation and growth of mungbean in soil (Experiment-II)*

Strains	No. of nodules/plant			Nodule dry wt (mg/plant)			Shoot dry wt (g/plant)			Shoot N (mg/plant)		
	A	B	C	A	B	C	A	B	C	A	B	C
Vr16	8AB	5ABC	29A	2.0B	2.6B	12.5B	0.39A	0.13C	0.35CD	3.3BC	2.5C	8.4BC
Vr17	10AB	9A	28A	1.6B	3.9B	10.1B	0.18C	0.19BC	0.47BC	3.2BC	3.0C	11.3B
Vr19	9AB	8AB	20A	0.9B	4.4B	10.5B	0.15C	0.29AB	0.66B	2.5C	5.3AB	14.4B
Vm1	18A	10A	29A	1.8B	2.8B	12.8B	0.15C	0.23BC	0.58BC	2.8C	3.6BC	13.0B
USDA 3748	13AB	7ABC	21A	2.3B	3.5B	9.5B	0.17C	0.23BC	0.44BCD	2.8C	4.0BC	9.2BC
US	13AB	9A	27A	15.4A	12.7A	27.1A	0.30B	0.38A	0.94A	4.4AB	7.0A	22.0A
UC	0B	1BC	5B	0B	0B	1.7C	0.13C	0.13C	0.18D	2.6C	2.4C	4.4C
NC	0B	0B	0B	0B	0B	0C	0.17C	0.15C	0.37CD	5.8A	4.4BC	13.6B

Means followed by the same letter are not significantly different ($P > 0.05$).

Values are the mean of three readings. * Day/night temperature was maintained at 30°C, increasing temperature shocks of 36, 42 and 48°C of 2 hr each were applied daily.

Set A: temperature stress and inoculation were applied at the time of sowing.

Set B: temperature stress and inoculation were applied after germination.

Set C: day/night temperature was maintained at 30°C (control).

UC: Uninoculated; NC: nitrogen control; US: unsterilized soil.

Table 4. Effect of high diurnal temperature regime on growth and survival of bradyrhizobial strains*

Bradyrhizobial strains	% increase or decrease of bacterial population/initial population	
	YM broth	Soil
Vr16	0	0.25
Vr17	3.3	3.4
Vr19	1.0	−2.4
Vm1	8.6	0.4
USDA 3748	−10.0	+9.02

Values are the mean of three readings.
* Day/night temperature was maintained at 30°C, increasing temperature shocks of 36, 42 and 48°C for 2 hr each were applied daily.

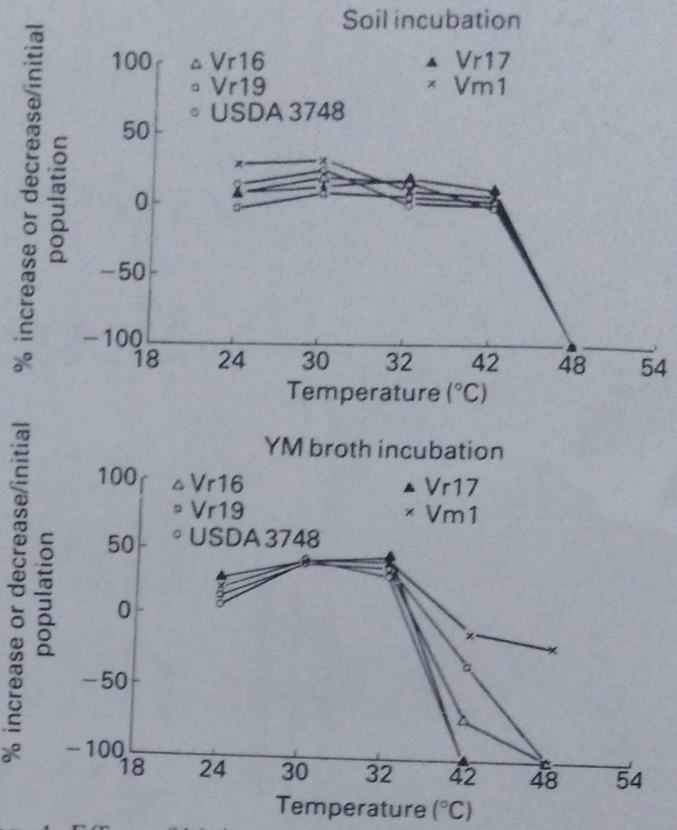

FIG. 4. Effect of high temperature on growth and survival of five *Bradyrhizobium* strains in soil and yeast mannitol broth.

ineffective.[17] Unlike previous findings,[10,17] the present study reports no loss of infectiveness and effectiveness of temperature-tolerant strains after incubation at continuously high temperatures (42 and 48°C) and diurnally high temperature regimes (Table 6).

A positive correlation between performance of *Bradyrhizobium* strains Vm1 and Vr17 as symbionts on mungbean and in pure cultures has been observed at constant high temperatures (Figs 1, 4; Table 4). These results are in agreement with other reports.[18,19] The present studies did not show a clear correlation between ability to survive and ability to nodulate mungbean plants under diurnally administered temperature stress in soil or in sand (Tables 2–5). Differences in the nodulation response of local bradyrhizobial strains on mungbean plants grown in soil with diurnal cycling high temperatures were non-significant although each strain had a different pattern of tolerance (Tables 3–5). No nitrogenase activity was observed in plants inoculated with bradyrhizobial strains except in unsterilized soil under

Table 5. Comparison of bradyrhizobial strains for growth and survival at high incubation temperatures on agar plates

Bradyrhizobial strains	Bacterial growth at (temperature, °C)						Bacterial survival at (temperature, °C)					
	24	30	36	42	48	* Temperature regime	24	30	36	42	48	* Temperature regime
Vr16	+	++	++	+	+	+	++	++	++	+	+	++
Vr17	++	++	++	+	−	+	++	++	++	+	−	+
Vr19	+	++	++	±	−	+	++	++	++	+	−	+
Vm1	+	++	++	++	+	+	++	++	++	++	+	++
USDA 3748	±	++	±	±	−	±	+	++	±	±	−	±

−, No growth; ±, inhibited growth; +, growth; ++, heavy growth; * day/night temperature was maintained at 30°C, increasing temperature shocks of 36, 42 and 48°C for 2 hr each were applied daily.

Table 6. Effectiveness of thermotolerant cowpea bradyrhizobial strains on mungbean and siratro

	42°C		48°C		*Temperature regime	
Strains	a	b	a	b	a	b
Vr16	+	+	+	ND	+	+
Vr17	+	+	ND	ND	+	+
Vr19	+	+	ND	ND	+	+
Vm1	+	+	+	ND	+	+
USDA 3748	+	+	ND	ND	+	+
Control	−	−	−	−	−	−

a, Incubated for 5 days; b, incubated for 10 days. ND, not determined; +, effective nodule; −, no nodule; *day/night temperature was maintained at 30°C, increasing temperature shocks of 36, 42 and 48°C for 2 hr each were applied daily.

temperature stress conditions (sets A, B). No significant differences were noted in the nitrogenase activity of the inoculated plants growing at 30°C in soil (set C). Significantly higher nodulation and nitrogenase activities were found only in unsterilized soil (US), indicating the presence of high temperature tolerant/efficient indigenous cowpea bradyrhizobial strains in the local soil samples. The nodulation and nitrogen fixation observed in sterilized soil experiments with inoculation of selected strains were comparable to those of mungbean plants growing in the field. In sand culture these bradyrhizobial strains formed nodules with various degrees of effectiveness on mungbean plants under diurnally administered temperature regime stresses. Nodulation, nitrogenase activity, dry matter and total nitrogen were severely affected by a temperature regime of 30–48°C relative to those of plants growing at 30°C, i.e. control (Table 2). The soybean genotypes had fewer and smaller nodules with *B. japonicum* strains in the field and in controlled conditions (sand).[28] In contrast the mungbean genotypes in our field condition experiments were poorly nodulated[8] by indigenous and inoculated bradyrhizobial strains but were well nodulated under controlled conditions in sterilized sand (inoculated) and unsterilized soil. The lack of or poor nodulation in our field environment may be due to local thermotolerant strains (they survive and they multiply at temperatures above 36°C) being less compatible than thermosensitive strains (they survive without multiplying at temperatures above 36°C) or the mungbean genotypes are resistant to indigenous populations under field conditions. The poor nodulation in sterilized soil in contrast to sand at higher temperatures is not fully understood and may be due to the accumulation of some inhibitory factors after steam sterilization of the soil.

The lethal temperature for *Bradyrhizobium* appears to depend on the strain, temperature and duration of exposure (Fig. 4; Tables 4, 5). Therefore, a careful selection of compatible thermotolerant microsymbiont and host genotype is required for maximum production of legumes in tropical and sub-tropical regions.

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REFERENCES

1. Asad S., Hafeez F. Y. and Malik K. A. (1989) Response of various legumes to *Bradyrhizobium*/*Rhizobium* inoculation in presence of indigenous population. Page 66, Poster abstract *in* 12*th North American Symbiotic Nitrogen Fixation Conference*, Ames, Iowa, U.S.A., 30 July–3 August 1989.
2. Bangall D. J. and King R. W. (1987) Temperature and irradiance effects on yield in cowpea (*Vigna unguiculata*). *Field Crops Res.* **16,** 217–229.
3. Bowen G. D. and Kennedy M. M. (1959) Effect of high soil temperature on *Rhizobium* ssp. Queensl. *J. Agric. Sci.* **16,** 177–197.
4. Dart P. J., Day J., Islam R. and Dobereiner J. (1976) Symbiosis in tropical grain legumes: some effects of temperature and the composition of root medium. Pages 361–383 *in* P. S. Nutman, ed. *Symbiotic nitrogen fixation in plants*. Cambridge University Press, Cambridge.
5. Day J. M., Roughley R. J., Eaglesham A. R. J., Dye M. and White S. P. (1978) Effect of high temperature on nodulation of cowpea (*Vigna unguiculata*). *Ann. appl. Biol.* **88,** 476–481.
6. Eaglesham A. R. J., Stowers M. D., Maina M. L., Goldman B. J., Sinclair M. J. and Aya-

NABA A. (1987) Physiological and biochemical aspects of diversity of *Bradyrhizobium* sp. (*Vigna*) from three West African soils. *Soil Biol. Biochem.* **19,** 575–581.

7. HAFEEZ F. Y., ASLAM Z. and MALIK K. A. (1988) Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek. *Pl. Soil* **106,** 3–8.
8. HAFEEZ F. Y., IDRIS M. and MALIK K. A. (1986–1987) Screening with nuclear and other techniques for high yielding and disease resistant varieties of mungbean, blackgram and chickpea with improved N_2 fixing capability. Annual progress report of IAEA Research Contract No. 4513/FA.
9. HARTEL P. G. and ALEXANDER M. (1984) Temperature and desiccation tolerance of cowpea rhizobia. *Can. J. Microbiol.* **30,** 820–823.
10. KARANJA N. K. and WOOD M. (1988) Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: tolerance of high temperature and antibiotic resistance. *Pl. Soil* **112,** 15–22.
11. KENNEDY A. C. and WOLLUM A. G. II (1988) Enumeration of *Bradyrhizobium japonicum* subjected to high temperature: comparison of plate count, most probable number and fluorescent antibody techniques. *Soil Biol. Biochem.* **20,** 933–938.
12. KLUSON R. A., KENWORTHY W. J. and WEBER D. F. (1986) Soil temperature effects on competitiveness and growth of *Rhizobium japonicum* and *Rhizobium* induced chlorosis of soybean. *Pl. Soil* **95,** 201–207.
13. LA FAVRE A. K. and EAGLESHAM A. R. J. (1986) The effects of high temperatures on soybean nodulation and growth with different strains of bradyrhizobia. *Can. J. Microbiol.* **32,** 22–27.
14. LA FAVRE A. K. and EAGLESHAM A. R. J. (1987) Effects of high temperatures and starter nitrogen on the growth and nodulation of soybean. *Crop Sci.* **27,** 742–745.
15. LAUTER D. J., MUNNS D. N. and CLARKIN K. L. (1981) Salt response of chickpea as influenced by nitrogen supply. *Agron. J.* **73,** 961–966.
16. LINDEMANN W. C. and HAM G. E. (1979) Soybean plant growth, nodulation and nitrogen fixation as affected by root temperature. *Soil Sci. Soc. Am. J.* **43,** 1134–1137.
17. MUNEVAR F. and WOLLUM A. G. II (1981) Growth of *Rhizobium japonicum* strains at temperatures above 27°C. *Appl. Envir. Microbiol.* **42,** 272–276.
18. MUNEVAR F. and WOLLUM A. G. II (1981) Effect of high root temperature and *Rhizobium* strain on nodulation, nitrogen fixation and growth of soybean. *Soil Sci. Soc. Am. J.* **45,** 1113–1120.
19. MUNEVAR F. and WOLLUM A. G. II (1982) Response of soybean plants to high temperature as affected by plant cultivar and *Rhizobium* strain. *Agron. J.* **74,** 138–142.
20. OSA-AFIANA L. O. and ALEXANDER M. (1982) Clays and the survival of *Rhizobium* in soil during desiccation. *Soil Sci. Soc. Am. J.* **46,** 285–288.
21. OSA-AFIANA L. O. and ALEXANDER M. (1982) Differences among cowpea rhizobia in tolerance to high temperature and desiccation in soil. *Appl. Envir. Microbiol.* **43,** 435–439.
22. PHILPOTTS H. R. (1967) The effect of soil temperature on nodulation of cowpea (*Vigna sinensis*). *Aust. J. exp. Agric. Anim. Husb.* **7,** 372–376.
23. PIHA M. I. and MUNNS D. N. (1987) Sensitivity of common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature. *Pl. Soil* **98,** 183–194.
24. RANGA RAO V. (1977) Effect of root temperature on the infection processes and nodulation in *Lotus* and *Stylosanthes*. *J. exp. Bot.* **28,** 241–259.
25. SOMASEGARAN P., REYES V. G. and HOBEN H. J. (1984) The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage, and distribution. *Can. J. Microbiol.* **30,** 23–30.
26. TYAGI S. K. and TRIPATHI R. P. (1983) Effect of temperature on soybean germination. *Pl. Soil* **74,** 273–280.
27. VINCENT J. M. (1970) *A manual for the practical study of the root nodule bacteria*. Blackwell Scientific, Oxford, U.K.
28. WEISER G. C., SKIPPER H. D. and WOLLUM A. G. II (1990) Exclusion of inefficient *Bradyrhizobium japonicum* serogroups by soybean genotypes. *Pl. Soil* **121,** 99–105.