

# EFFECT OF EXCESSIVE TEMPERATURES ON RHIZOBIA GROWTH NODULATION AND NITROGEN FIXING ACTIVITY IN SYMBIOSIS WITH SIRATRO<sup>1</sup>

KUK-KI LEE<sup>2</sup> and JOHANNA DOBEREINER<sup>3</sup>

**ABSTRACT** - The heat tolerance of 17 strains of *Rhizobium* sp. was studied on agar plates, in liquid culture medium and in symbiotic associations with *Macroptilium atropurpureum*. To test this the plants were exposed during six hours per day and during incubation with C<sub>2</sub>H<sub>2</sub>, to temperatures ranging from 28 to 40° C. There were significant differences among strains in their tolerance to high temperatures, and five of them were able to grow well at 40°C. *Rhizobium* growth on agar plates or in liquid medium however did not correlate with symbiotic performance. One strain which showed fastest growth in liquid medium at 40°C showed almost linear decreasing nitrogenase activity with increasing temperatures from 28 to 40°C. Another strain which also showed good growth up to 40°C in culture medium showed unaffected nitrogenase up to this temperature. The temperature sensitive strains showed maximal nitrogenase activity at 32°C but all tested strains were able to nodulate and to fix some N<sub>2</sub> (reduce C<sub>2</sub>H<sub>2</sub>) at 40°C.

**Index terms:** symbiotic association, *Macroptilium atropurpureum*, nitrogen fixation, heat tolerant rhizobia, nodulation of Siratro.

## EFEITO DA TEMPERATURA EXCESSIVA NO CRESCIMENTO DE RIZÓBIOS NA NODULAÇÃO E NA FIXAÇÃO DE NITROGÊNIO EM SIRATRO

**RESUMO** - Foi estudada a tolerância, a temperaturas elevadas, de 17 estirpes de *Rhizobium* sp., em meio de cultura e na associação simbiótica com *Macroptilium atropurpureum*. Nestes experimentos, as plantas foram expostas a diferentes temperaturas (28 a 40°C), durante seis horas por dia e durante a incubação com C<sub>2</sub>H<sub>2</sub>. Observaram-se diferenças significativas entre estirpes, e cinco delas foram capazes de crescer à temperatura de 40°C. O crescimento do rizóbio em meio de cultura sólido (agar) ou líquido, entretanto, não correspondeu ao comportamento das diferentes estirpes em associação com a planta. Uma estirpe que mostrou crescimento mais rápido em meio líquido a 40°C apresentou atividade de nitrogenase decrescente com o aumento da temperatura de 28 para 40°C. Uma outra estirpe, que também cresceu até 40°C em meio de cultura, não teve sua nitrogenase afetada por temperaturas crescentes de 28 a 40°C. As estirpes mais sensíveis a temperaturas elevadas mostraram atividades de nitrogenase máxima em 32°C. Todas as estirpes de *Rhizobium* sp. testadas foram capazes de nodular e de fixar algum N<sub>2</sub> (reduzir C<sub>2</sub>H<sub>2</sub>) a 40°C.

**Termos para indexação:** associação simbiótica, *Macroptilium atropurpureum*, nitrogênio, nodulação em Siratro.

## INTRODUCTION

The necessity for leguminous plants in tropical agriculture has been reviewed and discussed by Doberiner (1977). In tropical areas, the soil temperature very often attains higher than 40°C.<sup>4</sup> Optimum temperatures for maximum yields of tropical legumes are usually considered to be around 30°C although for tropical grasses they can be as high as 40°C (Mott & Popenoe 1975). Therefore, it is necessary to study the

effects of high temperatures on the symbiotic performance of the various legumes which are grown in tropical areas and selection of heat tolerant rhizobia seems a promising research objective.

Lie (1974) reviewed the environmental effects on legumes and generalized that high temperatures retarded nodulation and nitrogen fixation. In *Pisum*, nodulation and nitrogen fixation were much lower at 32°C than at 25°C (Frings 1976). In alfalfa, the effect of excessive temperatures on nodulation and plant growth was more drastic when the nitrogen source was atmospheric nitrogen than when it was combined nitrogen (Munns et al. 1977). Soybeans inoculated with a Brazilian *Rhizobium* strain (Sm 1b) fixed more nitrogen when grown at 33°C than at 27°C

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<sup>2</sup> Eng° Agr°, Ph.D., Centro Nacional de Pesquisa de Arroz e Feijão (CNPAP) - EMBRAPA, km 4, BR 153, Caixa Postal 179, CEP 74000 - Goiânia, Goiás.

<sup>3</sup> Eng° Agr°, D.Sci., Serviço Nacional de Levantamento e Conservação de Solos (SNLCS) - EMBRAPA, Programa Fixação Biológica de Nitrogênio, km 47, CEP 23460, Seropédica, Rio de Janeiro.

<sup>4</sup> FONSECA, O.O.M. da. Personal communication, 1979.

while temperate *Rhizobium* strains did not fix any N<sub>2</sub> at 33°C (Dart et al. 1973). The soybean strains used by Linderman & Ham (1979) nodulated and fixed nitrogen best at 25°C while 30°C reduced especially the nitrogenase activity drastically. Also Dart & Day (1971) found that acetylene reducing activity of cowpea nodules was the highest at 40°C when the root system was incubated at 40°C only during the acetylene reduction assay period. *Rhizobium* strains isolated from tropical forest legumes grew better at 40°C than at 30°C.<sup>5</sup> These temperatures are in the range where *Rhizobium* plasmids have been cured resulting in the loss of nodulating ability (Zurkowski & Lorkiewicz 1979) and the possibility of genetic injury can not be discarded in tropical soils.

In this study, an attempt was made to investigate the effect of high temperatures on the multiplication of some tropical rhizobia *in vitro*, and on nodulation and nitrogen fixation in symbiosis with *Siratro*.

#### MATERIALS AND METHODS

The rhizobia used in this study and their origins are shown in Table 1. The cultures were streaked on yeast-mannitol agar with the following composition:

K<sub>2</sub>HPO<sub>4</sub>, 0.1 g; KH<sub>2</sub>PO<sub>4</sub>, 0.4 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g; NaCl, 0.1 g; mannitol, 10 g yeast extract, 0.4 g; 0.5 % bromothymol-blue in ethanol, 5 ml; agar, 15 g per 1,000 ml of water. The petri dishes were incubated at five different temperatures; 24, 28, 32, 36 and 40°C. Each treatment had three replications at each temperature level. One week after streaking, the growth of rhizobia was observed and recorded. Then, the strains: V3, V8, V20, Do4b, BR30, CB-1809, K29 and CJ-1 were used for further quantitative analyses of growth which was evaluated by optical density measurements. Three loops of *Rhizobium* were inoculated into 5 ml of liquid yeast-mannitol medium in 70 ml bottles (shallow layers to avoid anaerobic conditions). The cultures were inoculation, the optical density of the cultures was measured at 540 nm of wavelength.

Four strains (Do4b, BR30, K29 and CJ-1) were selected for a nodulation experiment at different temperature levels. The sterilized seeds of *Siratro* (*Macroptilium atropurpureum*) were germinated on agar. Two days after germination the seedlings were transferred to agar slant medium in test tubes with the following composition: CaCl<sub>2</sub>·H<sub>2</sub>O, 0.1 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.12 g; KH<sub>2</sub>PO<sub>4</sub>, 0.1 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 0.15 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.03 g; agar, 15 g; per 1,000 ml of water. Five days later, the plants were inoculated with 0.2 ml of liquid cultures of the various strains. Each treatment had four replicate tubes. The plants were exposed six hours per day to various temperatures (28, 32, 36 and 40°C) in the dark, remaining the rest of the time at 28°C in the

TABELA 1. Effect of high temperature on the growth of *Rhizobium* strains of various origin on agar plates.

Strains	Host-plant	Temperature level				
		24°C	28°C	32°C	36°C	40°C
V-02	<i>Vigna</i>	1	1	3	2	1
V-03	<i>Vigna</i>	1	2	3	2	1
V-08	<i>Vigna</i>	2	3	3	3	1
V-14	<i>Vigna</i>	1	3	2	2	1
V-20	<i>Vigna sinensis</i>	1	2	3	2	1
Do4b	<i>Dolichos lab-lab</i>	1	2	2	3	3
C101a	<i>Centrosema pubescens</i>	1	3	2	1	1
Pa1a	<i>Macroptilium atropurpureum</i>	1	3	3	2	1
K-29	<i>Pueraria javanica</i>	2	3	3	1	Tr
SMS-11	<i>Galactia striata</i>	1	2	3	1	1
I-1a	<i>Indigofera hirsuta</i>	2	3	3	2	2
CJ-1	<i>Crotalaria</i> sp	2	3	3	2	Tr
BR30	<i>Cajanus cajan</i>	2	3	3	3	3
Sm1b	<i>Glycine max</i>	1	3	3	3	2
CB-1809	<i>Glycine max</i>	1	3	3	3	3
965	<i>Glycine max</i>	2	3	3	3	1
29W	<i>Glycine max</i>	2	3	3	2	1
	3 Good	2 Medium	1 Poor	Tr Trace		

<sup>5</sup> SUBBA RAO, N.S. Personal communication, 1978.

light. On the fifteenth day after inoculation, the test tubes containing plants were prepared for acetylene reduction assays. At the end of the heated dark period the test tubes were sealed with suba seals, 10% of their gas phase was replaced by acetylene gas and the tubes were incubated for another hour at the four levels of temperature. Then, 0.5 ml samples of the gas phase were withdrawn by syringe and assayed for ethylene by gas chromatography. After wards the plants were removed, and plant and nodule dry weights and nodule numbers determined.

## RESULTS AND DISCUSSION

The growth of seventeen rhizobia strains at various temperatures was observed on the solid medium (Table 1). All strains but three showed maximum growth rates at 32°C. Some strains (V8, Do4b, BR30 and CB-1809) grew very well even at higher temperatures (36 or 40°C). The effect of high temperatures on *Rhizobium* growth was then confirmed in liquid medium where optical density measurements could be used. Very similar data as on the plats were obtained. The strain CB-1809 showed the fastest growth at 36°C (data are not given in Fig. 1), and Do4b and BR30 at 40°C. The growth of Do4b and BR30 was checked at 44°C, but there was none (data are not given in Fig. 1). The other strains grew best at 32°C. The results of the

strains, Do4b, BR30, K29 and CJ-1 are shown in Fig. 1 where they can be compared with those of the nodulation experiment.

At all levels of temperature, Siratro plants were nodulated, but the pattern of nodulation response to temperature level was quite different from that of growth of rhizobia *in vitro*. Strains BR30 and CJ-1 showed similar tendencies in response to temperatures in symbiotic performance as *in vitro*. The change in nitrogen fixation with temperature increases of Siratro nodulated by strain Do4b however was opposite to that of growth in liquid medium. Furthermore, the strain K29 did not seem to grow *in vitro* at 40°C, but nodulation and nitrogen fixing activity were observed. This strain showed very narrow temperature requirements for the highest nitrogenase activity (32°C). Response of nodulation and nitrogen fixation to excessive temperatures is therefore dependent on additional characteristics besides *Rhizobium* growth *in vitro*.

As mentioned in the introduction, it is generally assumed that high temperatures retard nodulation and nitrogen fixation in legumes (Lie 1974). The present results, however, indicate that the symbiosis of Siratro with certain *Rhizobium* strains can function and fix nitrogen when the plants were

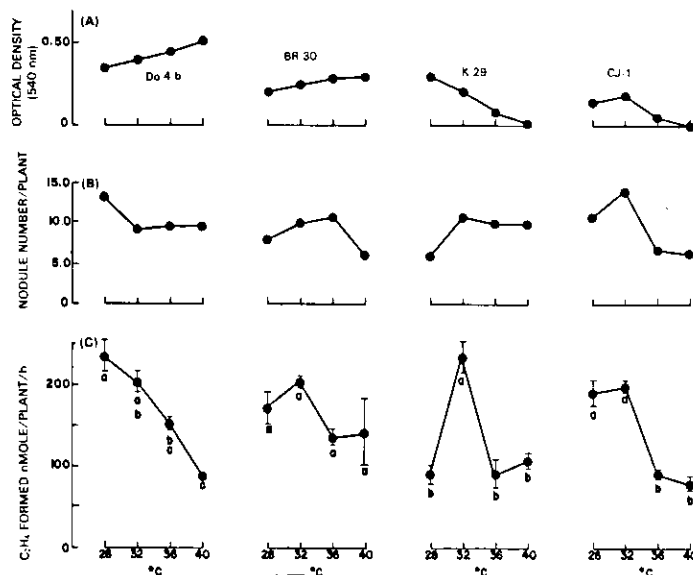


FIG. 1. Growth of rhizobia *in vitro* (A), number of nodules (B) and acetylene reducing activity in the symbiosis of Siratro-rhizobia (C). (A) was given temperature treatment during whole experimental period, while (B) and (C) were daily for six hours. In (C), the vertical lines show the standard error of the means and the points within a strain followed by the same letter are not statistically different at the 5% level.

exposed daily for six hours to 40°C. This indicates possibilities to select heat tolerant strains for the inoculation of tropical legumes.

The most important conclusion withdrawn from this study is that although daily heating at 40°C for six hours is generally considered to be critical for nodulation and nitrogen fixation, Siratro inoculated with tropical rhizobia as far as examined in this study could nodulate and fix nitrogen under such conditions.

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