Gezira J. of Agric. Sci. **3**(**1**): 117-122 (200**5**)

SHORT NOTE

Effect of Temperature on *in vitro* Survival of some *Bradyrhizobium* Strains

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Evaluating Rhizobium survival in inoculants exposed to high temperature may be considered the first stage in identifying potential inoculant strains that would withstand temperature stress on inoculated seeds in the field. High temperatures may adversely affect the survival of Rhizobium in packaged inoculants during storage and inoculation (Somasegaran *et al*., 1984), and the viability of rhizobia in inoculants may be lost in a few weeks at temperatures of 350C or higher (Smith، .(1987Low storage temperature, however, is not always better than room temperature, as some slow growing bradyrhizobia were found to survive better at 260C than at 40C (Vincent, 1982). Soil temperature is also an important environmental variable that affects general biological activity. Nodulation and N_2-fixation were observed under a wide range of temperatures with an optimum range between 20 and 30^0C. Elevated temperatures affect nodule initiation and development in temperate legumes, whereas, in tropical legumes it is mainly N_2 fixation efficiency that is affected (Somasegaran *et al.*, 1984). Temperature changes affect the competitive ability of *Rhizobium* strains and there are also specific temperature-sensitive Rhizobium legume combinations e.g. *R. Legumino- sarum biovar-trifolii* that forms nodules with *Trifolium subterranium* (Lewis-Henderson and Djordjevic, 1991).

Under prolonged exposure to high temperatures, 9 out of 10 *Rhizobium* strains tested could multiply at 28^0C and 37^0C, which was

found, to be lethal to all tested strains (Somasegaran *et al*., 1984). Soil temperature in Gezira soils may reach in the hot summer months and hence summer legumes and their symbiotic associations may be adversely affected.

The present study was conducted to investigate the tolerance and adaptability of local and introduced *Bradyrhizobium* strains to different temperature levels under laboratory conditions.

The experiment was conducted in the Microbiology and Biofertilizers laboratory of the Agricultural Research Corporation at Wad Medani. Two factors, i.e. temperature and *BradyrJ1izobium* strains were considered. The first factor comprised four levels of temperature, i.e. 25^0, 30^0, $40^0$and strains; USDA 3089, USDA 3385 and USDA 3386, were introduced from USA. The fourth, ENRRI 16A was supplied by the Environment and Natural Resource Research Institute (ENRRI) Khartoum, Sudan. The experiment included an uninoculated control treatment to determinate the basic optical density. The strains were grown in sterilized yeast extract mannitol broth media (Vincent, 1970), in conical flasks and incubated with shaking, then the optical density (growth) was measured at 4, 6 and 8 days. A completely randomized design with five replicates was used. Data were statistically analysed and treatment means were separated using Duncan's multiple range test.

After 4 days of incubation, the results showed significant ($P \leq 0.05$). differences among *Bradyrhizobium* strains. At 25^0C, USDA 3089 and USDA 3386 gave significantly higher growth over USDA 3385 and ENRRI16A, the latter being significantly higher than USDA 3385. Under 30^0C,USDA 3386 gave significantly higher growth over USDA 3089 and USDA 3385, however, there were no significant differences among the four strains at 40^0C. At 45^0C, USDA 3089 gave significantly more growth than USDA 3386 and ENRR116A. At 25^0C,30^0C and 40^0C there were no significant differences among the temperature treatments، whereas 45^0C significantly reduced all strains growth compared to other temperature levels (Table la).

After 6 days incubation, no significant differences were observed among the growth of the four *Bradyrhizobium* strains under 25^0C، whereas, under and 30^0C and 40^0C ENRRI 16A showed significantly higher growth over all other strains, while USDA 3089 and USDA 3385 were significantly higher than USDA 3386. When the temperature was raised to 45^0C ENRRI 16A gave a significantly higher growth over USDA 3386, while there were no significant differences among the other strains. Comparing temperature treatments, at 25^0C, the growth of *Bradyrhizobium* strains was significantly higher than that at either 30^0C or 40^0C. The latter two treatments, however, were not significantly different from each other, whereas 45^0Csignificantly reduced *Bradyrhi-zobium* growth compared to the other three treatments (Table lb).

After 8 days incubation, no significant differences were observed among the four *Bradyrhizobitum* strains under 25^0C and 45^0C, whereas under 30^0C, ENRRI 16A gave significantly ($P \leq O.05$) higher growth than USDA 3089 and USDA 3385. There were no significant differences among the other treatments. When temperature was increased to 40^0C USDA 3089 significantly gave the highest growth compared to USDA 3385and USDA 3386 There was no significant difference between ENRRI 16A and USDA 3385, which were significantly higher than USDA 3386. At 40^0C growth of *Bracdyhizobium* strains was significantly improved compared to 30^0C. No significant difference was observed between 25^0C and 30^0C whereas 45^0C significantly suppressed *Bradyrhizobitun* strains growth compared to the other treatments (Table 1 c).

In general, our results showed that the survival of *Bradyrhizobium* strains was sustained with increase in temperature up to 40^0C. When the temperature reached 45^0Cthe viability of *Bradyrhizobium* significantly declined. Significant differences were observed between *Bradyrhizobium* strains as affected by temperature. ENRRI 16A ,USDA 3385 and USDA 3089 were better adapted to high temperatures than USDA 3386. ENRRI 16A, being a local strain, may

be better adapted to high temperature and hence its overall performance at high temperatures was the best.

In conclusion, under laboratory conditions, *Bradyrhizobium* strains tested in this experiment could multiply at 25^0C, 30^0C and 40^0C but not at 45^0C which was found to be lethal to all strains tested. Furthermore‹ local strains are known to be better adapted to local conditions, especially the studied strains under field conditions remains to be tested.

Table l. The effect of temperature on the growth (optical density) of *Bradvrhizobium* strains after 4 days (l a), 6 days (l b) and 8 days (l c) of incubation.

Table 1. The effect of temperature on the growth (optical density) of *Bradyrhizobium* strains after 4 days (1 a), 6 days (1 b) and 8 days (1 c) of incubation.

(1 a)

Temperature / Strains	25^0C	30^0C	40^0C	45^0C	Mean
USDA 3089	0.58 a	0.48 b	0.44 a	0.17 a	0.42 ab
USDA 3385	0.38 c	0.51 b	0.53 a	0.15 ab	0.39 b
USDA 3386	0.57 a	0.57 a	0.48 a	0.12 b	0.44 a
ENRRI 16 A	0.49 b	0.53 ab	0.59 a	0.13 b	0.43 a
Control	0.02 d	0.02 c	0.02 c	0.02 c	0.02 d
Mean	0.40 a	0.42 a	0.41 a	0.12 b	

(1 b)

Temperature / Strains	25^0C	30^0C	40^0C	45^0C	Mean
USDA 3089	0.62 a	0.53 b	0.53 b	0.27 ab	0.49 b
USDA 3385	0.57 a	0.57 b	0.54 b	0.28 ab	0.49 b
USDA 3386	0.63 a	0.46 c	0.46 c	0.18 b	0.43 c
ENRRI 16 A	0.61 a	0.64 a	0.63 a	0.30 a	0.54 a
Control	0.02 b	0.02 d	0.02 d	0.02 c	0.02 d
Mean	0.49 a	0.44 b	0.44 b	0.21 c	

(1 c)

Temperature / Strains	25^{0}C	30^{0}C	40^{0}C	45^{0}C	Mean
USDA 3089	0.70 a	0.56 b	0.77 a	0.27 a	0.58 ab
USDA 3385	0.62 a	0.62 b	0.64 bc	0.27 a	0.54 bc
USDA 3386	0.67 a	0.64 ab	0.57 c	0.21 a	0.52 c
ENRRI 16 A	0.65 a	0.71 a	0.77 ab	0.26 a	0.60 a
Control	0.02 b	0.02 c	0.02 d	0.02 c	0.02 d
Means	0.53 ab	0.51 b	0.55 a	0.20 c	

Means followed by the same letter (s) in the same column are not significantly different at the 5% level according to Duncan's Multiple Range Test.

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