VARIABILITY IN INDIVIDUAL NODULE ACTIVITY OF SINGLE STRAINS OF *RHIZOBIUM ETLI* AND *R.TROPICI* IN SYMBIOSIS WITH *PHASEOLUS VULGARIS*[1]

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ABSTRACT - A green-house experiment in Leonard jars was conducted to study the variability of nitrogenase activity (acetylene reduction) of individual nodules of *Phaseolus* beans inoculated separately with 19 strains of *Rhizobium* tropici and 6 strains of *R. etli*. Both species showed a wide range of activities with a C.V.=80% for *R. tropici* and C.V.=70% for *R. etli*. These data indicate that *Rhizobium* species with reiterated nif genes may present similar variability of single nodule activity as *Rhizobium* species containing a single copy of nif gene and that the use of either *R. tropici* or *R. etli* may be used for inoculant production with precaution for loss of effectiveness.

Index terms: *Phaseolus vulgaris*, leonard jars, nitrogenase, acetylene

VARIABILIDADE NA ATIVIDADE DE NÓDULOS INDIVIDUAIS FORMADOS POR ESTIRPES DE *RHIZOBIUM ETLI* E *R. TROPICI* EM SIMBIOSE COM O FEIJOEIRO

RESUMO - Foi conduzido, em casa de vegetação, em vasos-de-leonard, um experimento para estudar a variabilidade da atividade da nitrogenase (redução de acetileno) de nódulos individuais de feijão (*Phaseolus vulgaris*), infectados separadamente com 19 estirpes de *Rhizobium tropici* e 6 estirpes de *R. etli*. As 2 espécies mostraram grande variabilidade nas atividades, com um C.V.=80% para *R. tropici* e C.V.=70% para *R. etli*. Estes dados indicam que espécies de *Rhizobium* com "gens nif" repetidos podem apresentar variabilidade similar na atividade de nódulos individuais em relação às espécies de *Rhizobium* que contêm uma única cópia dos "gens nif"; indicam, também, que a utilização tanto de *R. tropici* como *R. etli* na produção de inoculante deve ser criteriosa, em face da perda de efetividade.

Termos para indexação: *Phaseolus vulgaris*, vasos-de-leonard, nitrogenase, acetileno.

INTRODUCTION

Biological nitrogen fixation in *Phaseolus* beans has frequently been used as an example of poor response of a legume crop to inoculation. The presence of large population of rhizobia able to nodulate and fix nitrogen with this crop, its short cycle, the sensitivity of the host to environmental stresses and the genetic instability of the symbiont may be responsable, under different cropping systems, to the lack of response of *Phaseolus* bean to inoculation (Franco, 1977).

Rhizobia genetic instability in laboratory manipulation, resulting in loss or decreased ability of the symbiont to nodulate or fix nitrogen in symbiosis with the host, have been registered for a long time. These alterations may be a result of mutagenic agents such as acridines, UV-light, SDS, several types of radiation, etc. (Zurkowski et al., 1973, Mathis et al., 1985 and Barbur & Elkan, 1989). The alterations have also been observed to occur under stress of high temperature (Djordjevic et al., 1983, Weaver & Wright, 1987) or even spontaneously during the routine sub-cultivation (Weaver & Frederick, 1982). Franco (1974) observed large variation in colony morphology and symbiotic effectiveness of several *Bradyrhizobium* spp. strains grown and stored in yeast mannitol agar under oil at room temperature. Herridge & Roughley (1975) tested 17 stock cul-

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tures of *Bradyrhizobium* sp. CB-756, obtained from several culture collections in different institutions and found great variation among cultures. Some of them have lost effectiveness to less than half to that with the highest effectiveness. Freeze drying or maintaining the culture in yeast mannitol agar medium may yield variants (Roughley, 1976). Peres et al. (1984) have shown great internal variation in the effectiveness of N_2 fixation in several strains of *Bradyrhizobium japonicum* and Weaver & Wright (1987) in B. sp. (Vigna).

The genetic instability seems to be more frequent in fast than in slow growing rhizobia. The three species of *Rhizobium* (*R. leguminosarum* bv. *phaseoli*, *R. tropici* and *R. etli*) that forms effective symbiosis with *Phaseolus* bean are fast growing. In these strains, the genes controlling biological nitrogen fixation (nif genes) are located in plasmids (Psym), in contrast with *Bradyrhizobium* that have the nif genes located in the chromosomes (Rosenberg et al., 1981, Kondorosi et al., 1982, Martinez et al., 1990). *R. leguminosarum* bv. *phaseoli* and *R. etli* strains present multicopies of the nif genes and nodulate only *Phaseolus* bean (Martinez et al., 1987 and Segóvia et al., 1993), while *R. tropici*, which is also able to nodulate and fix nitrogen in symbiosis with *Leucaena leucocephala*, has only one copy of the nif genes (Martinez et al., 1991). The Psym represent the molecular base of variability and instability in symbiotic properties found in rhizobia able to form effective symbiosis with *Phaseolus* beans. *R. tropici* strains have been found to be more heat tolerant and genetic stable than *R. etli* or *R. leguminosarum* bv. *phaseoli* (Mercante, 1993). Even though *R. etli* has only recently been described and there are not many studies on this species we may consider that most of bean specific rhizobia isolated from the Americas may be classified within this species (Segóvia et al., 1993).

The measurement of nitrogenase activity by acetylene reduction has been questioned by Minchin et al. (1983). They observed a sharp decline in ethylene production after a 10-minute exposure of nodules to acetylene. This inhibitory effect has been found to be variable with symbiotic systems, plant age, light intensity, p02, low temperature, water lodging, water stress and by different ways of handling the material (Stralioto, 1990). In spite of those observations, Peres et al. (1984) have demonstrated that single nodule activities of several *Bradyrhizobium japonicum* strain were correlated with the effectiveness of their isolates with the host. Faria et al. (1984) have also observed in several legume trees good correlation between individual nodule activity and effectiveness of their isolates.

This study was aimed to compare, by measuring single nodule activity of 60 nodules of each strain, the genetic variability in the symbiotic effectiveness of 6 *R. etli* strains in comparison with 16 *R. tropici* strains.

MATERIALS AND METHODS

The experiment was conducted in a green-house using sterilized Leonard jars containing a 2:1 mixture sand: vermiculite (Vincent, 1970) and receiving 400 ml of nutrient solution without nitrogen (Norris, 1964). A complete randomized block design was used, with three repetitions, 6 strains of *Rhizobium etli* BR 365 (CNPAF 146), CPAC H 19 (Semia 476), CPAC H 30 (IPAGRO 1102), CPAC H 35 (IPAGRO 1378), CPAC H 23 (V23 RGS) and CPAC H 14; 13 strains of *R. tropici* BR 322 (CIAT 899), BR 10013 (Na 82), BR 817 (NGR 8), BR 818 (TAL 1145), BR 266 (Semia 492, CENA CO_5 II), BR 10014 (Car 22), BR 814 (DF 10), CPAC H 21 (UFP 491), CPAC H 20, CPAC H 36, CPAC H 38 (USA 1070), CPAC H 26 (IPAGRO 1020) and CFN 299. The rhizobia strains were tested in *Phaseolus vulgaris* L. cv. Negro Argel and at the same time in *Leucaena leucocephala* Witt cv. Peru to confirm to which species they belong. All strains, except BR 814, BR 817 and BR 818, were obtained originaly from *Phaseolus* bean nodules.

The inoculum was prepared by growing each strain in yeast mannitol broth (Vincent, 1970) to the final logarithimic phase, standardizing to 10^8 cells/ml and applying 2 ml of this suspension in each pot containing 2 plants.

The plants were harvested 28 days after emergence. Ten nodules of each plant of *Phaseolus* bean with fresh weight between 5 and 7 mg were detached from the root and placed into individual flasks, 10% of the air was replaced by acetylene, incubated for 10 minutes and the ethylene produced was measured as described by Peres et al. (1984).

In *Leucaena*, nodulation was considered positive when all plants of the three repetitions were nodulated.

RESULTS AND DISCUSSION

All 19 *Rhizobium* strains studied originated from the Americas were able to nodulate and fix nitrogen with *Phaseolus* bean, while 13 of them were also able to nodulate and fix nitrogen with *Leucaena*. As indicated by Segóvia et al. (1993), the first group may be classified as *R. etli* and the second group as *R. tropici* species. Even though only 3 strains were isolated from *Leucaena*, 13 of them were of the *R. tropici* species with a range of activity from 7 to 132 nmoles ethylene/h nodule^{-1}, similar to the range presented by the less promiscuous species *R. etli*, from 29 to 104 nmoles ethylene/h nodule^{-1} (Sá et al., 1993).

Peres et al. (1984) had observed a good correlation between the activity of the individual nodules and the efficiency of their isolates in symbiosis with the host. The 19 rhizobial strains used in this study were chosen among the most efficient strains for inoculation for *Phaseolus* bean. A large variation was found acetylene reduction of individual nodules within and amongst strains (Fig. 1, 2, 3, 4 and 5) with a range of coefficient of variation (C.V.) in each strain of 23 to 105 in *R. etli* and 32 to 165 in *R. tropici*. The mean C.V. for the 6 *R. etli* studied was 70% and for the 13 *R. tropici* was 80%. It is possible that amongst *R. tropici* there is a large range in effectiveness of the strains even though some of the strains are as/or more efficient than *R. etli* when in symbiosis with *Phaseolus* bean. The individual nodule activity of none of both *R. etli* or *R. tropici* fitted a normal distribution (Fig. 1, 2, 3, 4 and 5). When the activity of both species were pooled in the same figure (Fig. 6), the range of activity of individual nodules of both species was very similar, at the lowest (0-4 nmoles C_2H_4/h/nodule) up to the highest (100 nmoles 0-4 nmoles C_2H_4/h nodule^{-1}) activities.

Variability in symbiotic properties is a common characteristic in *Rhizobium* strains holding "nif gens" in plasmids (Flores et al., 1988). This fact can be more relevant among *R. etli* strains having more than one copy of "nif gens" in the plasmid (Psym), making them subject to a greater variabil-

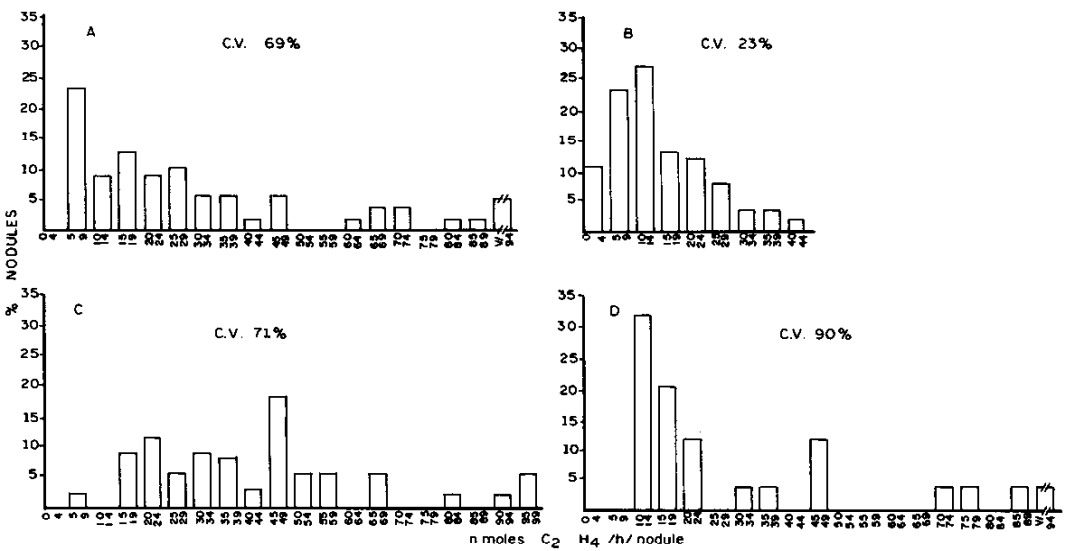

FIG. 1. Variability in nitrogenase activity (acetylene reduction) in individual bean nodules inoculated with *R. etli* strains: (A) CPAC H35, (B) CPAC H14, (C) CPAC H30 and (D) CPAC H23.

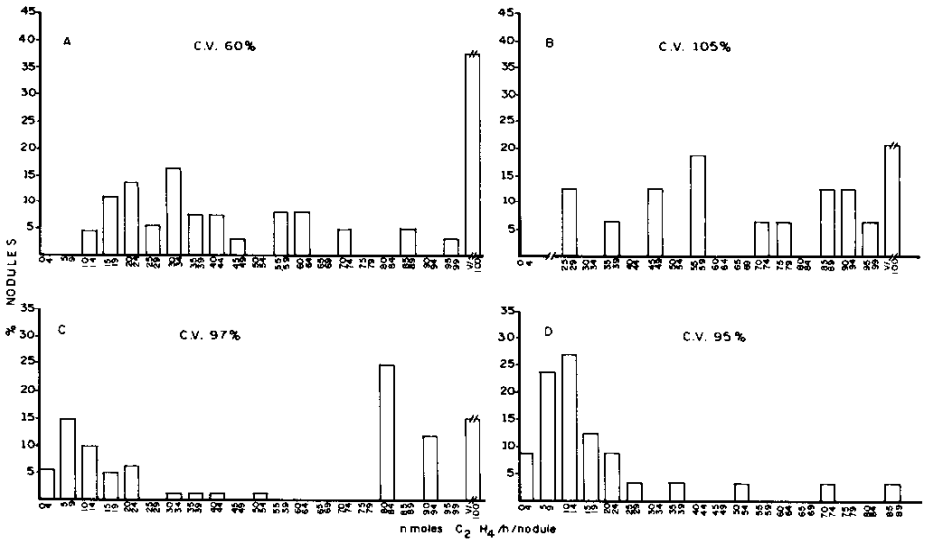

FIG. 2. Variability in nitrogenase activity (acetylene reduction) in individual bean nodules inoculated with *R. etli* strains: (A) CPAC H19, (B) Br 365 and *R. tropici* strains: (C) Br 10013, (D) CPAC H26.

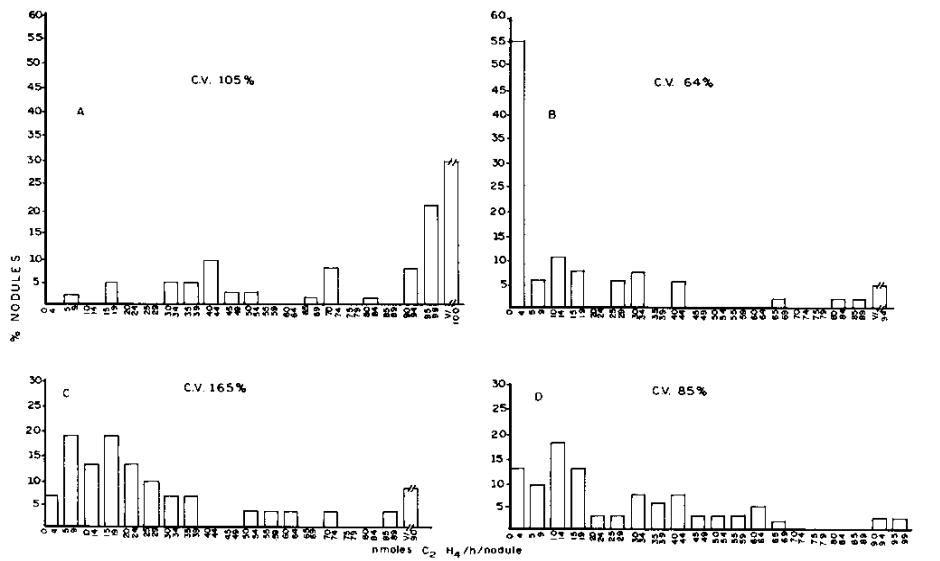

FIG. 3. Variability in nitrogenase activity (acetylene reduction) in individual bean nodules inoculated with *R. tropici* strains: (A) Br 322, (B) Br 814, (C) Br 266 and (D) Br 10014.

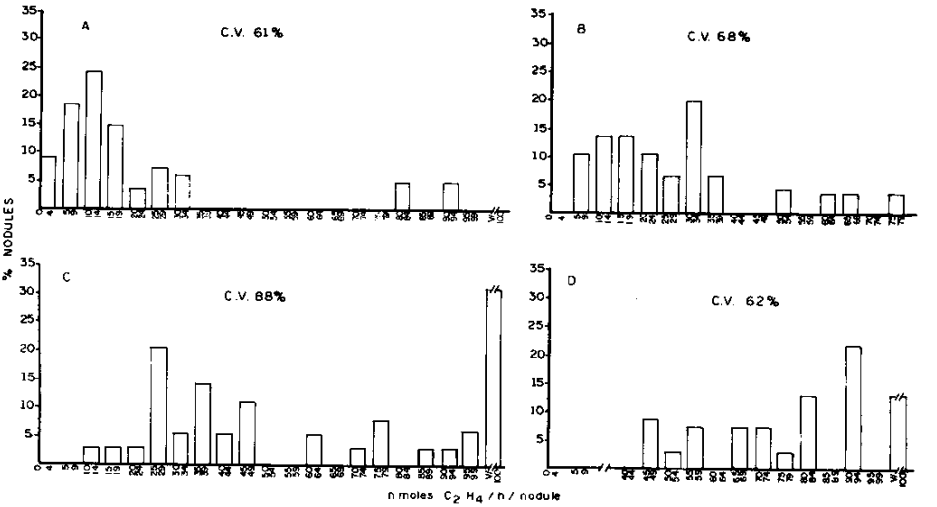

FIG. 4. Variability in nitrogenase activity (acetylene reduction) in individual bean nodules inoculated with *R. tropici* strains: (A) Br 817, (B) Br H36, (C) CPAC H20 and (D) CPAC H21.

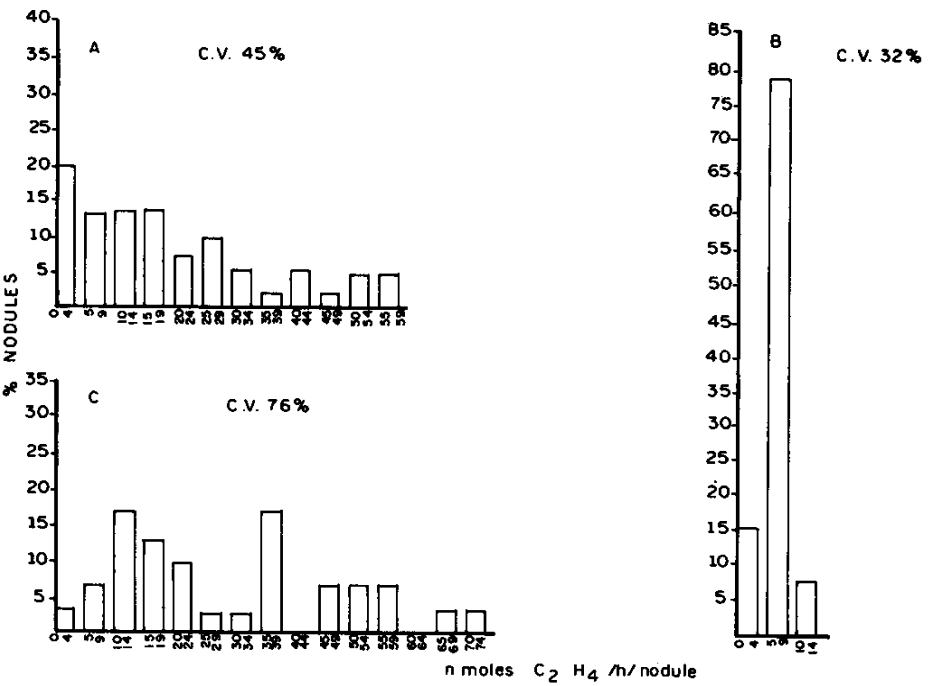

FIG. 5. Variability in nitrogenase activity (acetylene reduction) in individual bean nodules inoculated with *R. tropici* strains: (A) CPAC H38, (B) CFN 299 and (C) Br 818.

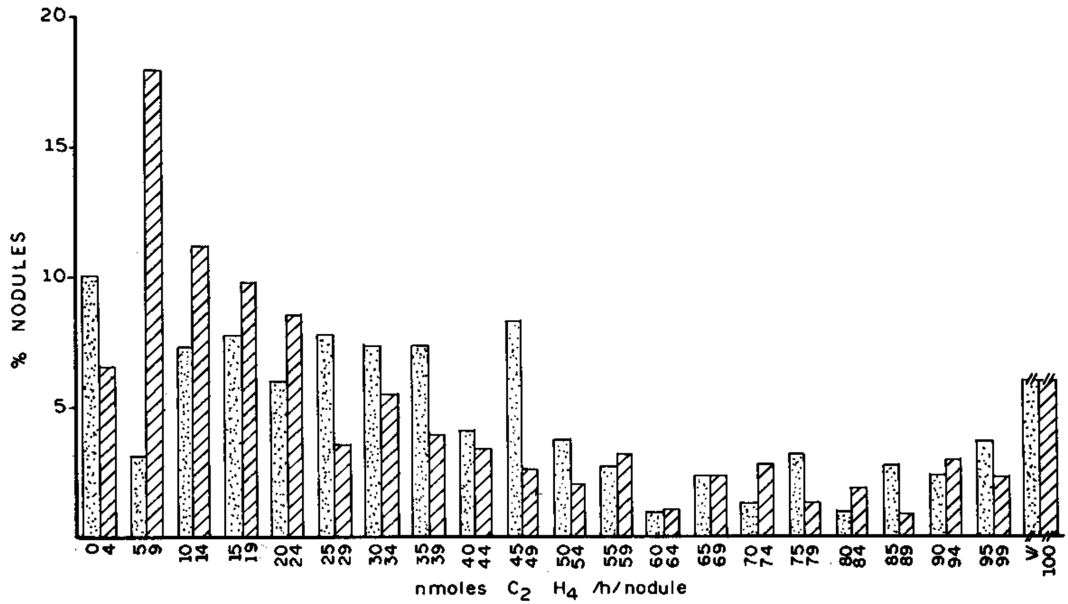

FIG. 6. Variability in nitrogenase activity (acetylene reduction) in 360 bean nodules (60 / strain) inoculated with *R. etli* strains (Br 365, CPAC H19, CPAC H30, CPAC H35, CPAC H23, CPAC H14) and 780 bean nodules (60 / strain) inoculated with *R. tropici* strains (Br 322, CPAC H21, CPAC H20, Br 10013, Br 817, Br 818, Br 266, Br 10014, CPAC H36, CPAC H38, CPAC H26, Br 814, CFN 299.

*** *R etli*, C.V. 70%** **_R. tropici_, C. V. 80%**

ity in relation to the *R. tropici* strains which present only one copy of nif gens and are considered more stable (Flores et al., 1988). They are also tolerant to stress factors such as acidity (Vargas & Graham, 1988) and high temperatures (Karanja & Wood, 1988, Romero & Rosenblueth, 1990). However, the strains of both species herein studied, showed high variability and at similar levels. This was also observed when both species were grown at their highest temperature they would grow (Sá et al., 1993).

The variability of individual *phaseolus* beans nodule activity and the genetic variability of the symbiont must be further studied. The data presented in this study indicate however that the use of either *R. tropici* or *R. etli* may be used for inoculant production with precaution for loss of effectiveness.

CONCLUSIONS

Strains of *Rhizobium etli* and *R. tropici* showed a wide range of nitrogenase activity (acetylene reduction). That indicates that *Rhizobium* species with reiterated nif genes may present similar variability of single nodule activity as *Rhizobium* species containing a single copy of nif gene and that the use of either *R. tropici* or *R. etli* may be used for inoculant productions with precaution for loss of effectiveness.

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