Ethyl-methane sulphonate (EMS) induced nodulation mutants of common bean (*Phaseolus vulgaris* L.) lacking effective nodules

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**Abstract**

Seed of white bean (*Phaseolus vulgaris* L. cv. OAC Rico) was treated with 0.04 M ethyl-methane sulphonate. Screening of the M\textsubscript{2} progeny from 175 M\textsubscript{1} lines in the presence of 1 mM nitrate revealed two nodulation mutants. One line was essentially non-nodulating in several tests, but small white nodules were observed occasionally in other tests with 16 separate *Rhizobium* strains. The other line formed tiny, creamy white, non-functional (ineffective) and tumor-like pseudo-nodules. The M\textsubscript{3} and M\textsubscript{4} progenies were fertile and bred true.

**Introduction**

Common bean is an important food legume and utilizes inorganic and symbiotically fixed nitrogen like many other grain legumes. Genetic variations in nodulation and nitrogen (N\textsubscript{2}) fixation are known, and altered nodulation characteristics have been reported from chemical mutagenesis in common bean, including nitrate-tolerant super-nodulating (Park and Buttery, 1988) and non-nodulating (Davis et al., 1988) mutants. Ineffectively nodulated mutants, characterized by small tumor-like nodules incapable of fixing nitrogen have been reported in peas (Duc and Messager, 1989) and chickpea (Davis, 1988), but not previously in common bean. Non-nodulation and ineffective nodulation characteristics can sometimes be circumvented by high concentration of bradyrhizobial inoculant as shown in soybean (Mathews et al., 1987) or as in chickpea, by using different Rhizobium strains (Davis et al., 1986). Non-nodulating and ineffectively nodulating genotypes are useful as reference plants for measuring N\textsubscript{2} fixation in plants (Rennie and Kemp, 1983; Williams et al., 1977), in understanding Rhizobium infection and nodulation processes, and in screening for genetic variability within Rhizobium strains.

The present study reports two types of non-fixing nodulation mutants of white (navy) bean mutagenized by ethyl-methane sulfonate (EMS) and response of the nodulation mutants to different Rhizobium strains.

**Materials and methods**

Mutagenesis of white bean *Phaseolus vulgaris* L. cv. OAC Rico was initiated by subjecting about 4500 water-soaked seeds to 0.04 M EMS in 1985. M\textsubscript{1} seeds were grown in the greenhouse, the plants were selfed and M\textsubscript{2} seeds were harvested individually from 175 M\textsubscript{1} plants as they matured. These seeds were used in a previous study (Park and Buttery, 1988) and also in this study.

Selection and screening was carried out in a glasshouse in March-April and in September-October. Conditions were not closely controlled. The daily temperature range was generally between 16 to 25°C but occasionally reached 30°C.
Average hours of sunshine were 6 to 7, and light within the greenhouse was 85 to 90% of full daylight.

To screen for non-nodulating and non-fixing mutants in the M2 population in the greenhouse in 1987, 12 seeds from each of 75 M2 lines were sown in 12-L plastic pots filled with a mixture of vermiculite and perlite (1:2 v/v). Seeds inoculated with *Rhizobium leguminosarum* bv. *phaseoli*, strain TAL 182, applied as turbid yeast-mannitol broth culture (ca. 10^7 cells mL^-1; 1 mL per seed) at seed level before covering the seed with the potting medium. Pots were watered for a week until seedlings emerged, and then 330 mL of a nitrogen-free nutrient solution containing 1.2 mM KH_2SO_4, 1.0 mM MgSO_4, 1.5 mM K_2SO_4, 0.08 mM Fe citrate, plus a balanced micro-nutrient supplement was supplied daily in the initial screening. After 4 weeks, when nodulation was expected to be near maximum, plants were carefully removed from the potting medium and visually rated as follows for nodulation: non-nodulating (score 0) to profusely/supernodulating plants (score 5). Putative non-nodulating/ineffectively nodulating plants (chlorotic unless supplied with inorganic N) were replanted and given 5 mM nitrate to increase plant growth and to boost seed production.

In 1988, 95 additional M2 lines were screened in the same manner as in 1987, except that 1 mM nitrate was added to the nutrient solution to increase seed production because plants were otherwise too chlorotic and weak. Subsequent progeny tests with M3 and M4 plants were carried out in 1 mL nitrate medium in the same manner.

The mutants and non-mutagenized OAC Rico (control) were inoculated with 16 different strains of *R. leguminosarum* bv. *phaseoli*, TAL 182, Kim 5s, USDA 2667, USDA 2668, USDA 2669, USDA 2670, USDA 2671, USDA 2673 and USDA 2675, CIAT 676, CFN 3, CFN 42, CFN 227, H_2C, RCR 3622, and CE3 to determine strain specificity of the host plants. The inoculants were applied as a turbid yeast-mannitol broth culture (ca. 10^7 cells mL^-1; 1 mL per seed) at seed level before covering the seed with the potting medium of a heat-sterilized mixture of vermiculite and perlite (v/p) (1:2 by volume). For each strain, three plants were tested in 10-cm diameter pots in the absence of nitrogen. Host plants were grouped in completely randomized arrangement within each *Rhizobium* strain to avoid contamination.

**Results and discussion**

Putative non-nodulating/ineffectively nodulating mutant plants were identified, some of which either did not produce seed or did not breed true. Progeny tests with M3 plants of two M2 families confirmed two nodulation mutants, R69 and R99. R69 produced sparsely nodulated tiny pale white nodules, and R99 produced few tiny pale nodules or no nodules (Fig. 1). R69 and R99 are presumed to be incapable of fixing nitrogen because in the absence of nitrate they both form small yellow shoots that die after 7–8 weeks without setting seed. In the same test, the wild type parent OAC Rico produced large pinkish nodules with four-to five-fold higher nodulation scores than that of the mutants (Table 1).

In the subsequent progeny test with M4 plants, R69 produced a similar number of nodules to OAC Rico (Table 1), but nodules were extremely small and pale yellow, and therefore the dry weight of the nodules was not recorded. The tiny nodules of R69 appeared to be non-functional (ineffective) in N_2 fixation as evidenced by chlorotic shoot growth. On the other hand, R99 produced almost no nodules. Shoot and root growth of the two mutant types was much depressed in comparison with the wild-type cultivar (Table 1).

Inoculation of the mutants and OAC Rico by the 16 strains of *R. leguminosarum* bv. *phaseoli* showed some strain x host interaction. R69 nodulated as much as the wild type OAC Rico with all 16 strains but nodules were tiny, pale white and appeared to be non-fixing. R99 showed varying degrees of nodulation ranging from non-nodulation with seven strains (Kim 5s, USDA 2671, 2673 and 2675, CFN42, and RCR 3622) to several tiny pale nodules with the rest of the strains tested. These results suggest that there may be strain-specificity to the mutant R99, which requires further testing.

The non-functional R69 was similar to the ineffective type (nod^+ fix^-) in pea (Duc and