Variability in nodulation of *Phaseolus vulgaris* L. with different rhizobial strains.

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Abstract

Most soils sown to common beans (*Phaseolus vulgaris* L.) contain indigenous *Rhizobium phaseoli* Dangeard, thus it is necessary to evaluate new strains of *R. phaseoli.* In 2004-2005 the effect of five strains of *R. phaseoli* on two cultivars of beans was investigated at Lincoln University. The cultivars Scylla and T-49 were inoculated with liquid cultures of strains RCR 3644, UK 2, H 20, PRF 81 and PhP 17 to determine their effect on nodulation, growth and yield. Nodulation was variable among strains. Over the sampling period T-49 consistently had higher nodule numbers/plant ranging from 4.74 at 21 days after inoculation (DAI) to 20.6 at 54 DAI. In Scylla the range was 1.42 at 21 DAI to 17.5 at 70 DAI.

Strain H 20 gave the highest number of nodules/plant in both bean cultivars and this was associated with the highest green pod yield/m^2 in Scylla. Scylla, with strain PRF 81 gave the highest overall shoot dry matter (DM) yield of 692 g/m^2. This gave a green pod DM yield of 202.3 g/m^2. In contrast, PRF 81 on T-49, which produced the second most nodules, gave a shoot DM yield of 521 g/m^2 and a green pod DM yield of 225.5 g/m^2. Strains H 20 and PRF 81 gave the best green pod yield in both bean cultivars. Scylla gave the highest shoot DM/m^2 with H 20 and PRF 81. In T-49, PRF 81 and PhP 17 produced shoot DM yields of 225.5 and 192.7 g/m^2 respectively. The results indicate that it should be possible to increase nodulation and yield of common beans in Canterbury by combining suitable bean cultivars with an appropriate strain of rhizobia.

Additional keywords: common bean, *Rhizobium,* nodulation, nodule number, shoot dry weight, green pod yield.

Introduction

A mutualistic symbiosis, of both agronomic and economic importance, occurs between legume plants and rhizobia; this involves the development of a specialised plant organ the root nodule (Brevin, 1991). The formation of a fully developed nitrogen (N)-fixing nodule is the result of many consecutive events involving recognition, root hair curling, infection thread formation, penetration of the root hair, induction of cortical cell division, and morphogenic changes coupled with bacteroid formation and release into plant host cells (Mylona *et al*., 1995). The process is the result of intensive communication between plant and bacterium and is often very specific, i.e. a given bacterial species can only nodulate one plant species. However, some rhizobia such as *Rhizobium* sp. NGR234 have a broad host range and can nodulate many different legume species. The phenomenon of narrow or broad host range is generally associated with the bacterial partner but may also hold true for some legumes (Michiels *et al.,* 1998).

The common bean plant (*Phaseolus vulgaris* L.) is known for its promiscuity and rhizobia isolates recovered from its nodules have shown considerable genetic diversity, suggesting that several different *Rhizobium* species can be associated with beans (Franco *et al*., 2001). Most soils in which common beans are grown contain indigenous populations of *Rhizobium phaseoli* (Graham, 1981) which compete for nodulation sites with the

Rhizobium strains added as inoculant. Frequent reports of poor nodulation and lack of response to inoculation is a common feature in the literature (Graham, 1981; Ramos and Boddey, 1987; Hardarson, 1993). Nodulation failure in some of these trials was attributed to the presence of large, but inefficient, populations of indigenous common bean rhizobia (Graham, 1981; Thies *et al*., 1991) and to environmental and plant genetic factors (Andrade and Hungria, 2002; Hardarson, 1993). The sensitivity of the common bean-rhizobia symbiosis to environmental stresses such as high temperatures and soil dryness, leading to low N_2 fixation efficiency has also been documented (Hungria and Vargas, 2000).

In New Zealand, commercial plantings of *Phaseolus* beans are not inoculated (George Hill personal communication). Responses to inoculation in experimental sowings have been variable, from no response to substantial response (Dapaah *et al*.. 1999). Similar results have been obtained in Brazil (Ramos and Boddey, 1987; Vargas *et al*., 2000) and in many other parts of the world (Graham and Temple, 1984). The growing realisation of coevolution between both *Rhizobium* strains and bean cultivars in their centres of diversification (Aguilar *et al*., 2004), highlights the need for continuous evaluation and selection to make maximum use of biological N fixation, to reduce or eliminate reliance on fertiliser N (White, 1989) and to better match micro and macro symbionts with conditions for enhancing yield potential.

This study assessed the combining ability for nodulation of two common bean cultivars (*Phaseolus vulgaris*) with elite rhizobial strains aimed at selecting a strain(s) of rhizobia to increase nodulation and yield under Canterbury, New Zealand, production conditions.

Materials and Methods

Site and design

This work is a continuation of work which was started in the 2003-2004 planting season, where two commercial inoculants CC 511 and RCR 3644 were evaluated on two common bean cultivars Scylla and T-49 for nodulation. Nodulation was not observed in the trial but excellent yields were obtained both at the green and dry bean stages of harvest (data not presented).

The current field experiment was located at the Horticultural Research Area, Lincoln University (43^{o} 39’ S, 172^{o} 28’ E, 11 m above sea level) Canterbury, New Zealand on a Wakanui silt loam soil (Cox, 1978) in 2004-2005. A completely randomised block designed with 4 replicates was used. Treatments consisted of 2 common bean (*Phaseolus vulgaris*) cultivars, (Scylla - a green dwarf bean and T-49 - a navy bean), and 6 *Rhizobium* strains including a control of no rhizobia, RCR 3644, UK 2, H 20, PRF 81 and PhP 17.

The field was previously in apples (*Malus* spp.), followed by oats (*Avena sativa*). The area was harrowed, rotavated and rolled before sowing. Plots were 2 m by 0.75 m, with 0.3 m between plots and 0.5 m between replicates. Experimental plots contained 5 rows, with 15 cm between rows and seed 10 cm apart within rows giving a population of 100 plants/plot (67 plants/m^2). Planting holes were prepared with a dibble board, 5 cm deep and seeds were sown by hand. After sowing, 2 ml of inoculant (3.4 x 10^9 colony forming units (cfu’s)/ml) was applied to the seed in each seed hole, lightly covered with soil and gently pressed. Irrigation was applied to eliminate any chance of water stress during plant growth.

Inoculum

Rhizobial isolates were grown on tryptone-yeast extract (TY) agar plates. Strains were streaked unto the agar and incubated at 28 ^{o}C for 18 hours. Then 100 µl of sterile water was pipette onto the agar plate and gently mixed with a sterile spreader. The mixed suspension was transferred by pipette into a 250 ml sterile conical flask containing tryptone-yeast broth. The tryptone-yeast broth/bacterial suspension mix was incubated at 150 rpm and 28 °C for 18 hours. At sowing, 2 ml of the incubated concentrated solution was used as the field inoculum. The strains used in the field experiment were selected based on signs of early nodulation at thinning (14 days after sowing) in a preliminary experiment.

Sampling

Samples were taken from the 3 centre rows of each plot, starting from a randomly selected end. The first two rows of plants were omitted to eliminate border effect and the sample size was approximately six plants (2 along the row and 3 across rows). A buffer of 3 plants was used between samplings. Samples were taken at 21 days after planting (DAP), when 50 % of the plants had at least 1 open flower, when over 50 % of the plants had at least 1 green pod 5 mm long, and at green bean harvest based on Heinz - Wattie's Australasia specifications. (When an average seed length of approximately 11 mm from the centre most seed in a sample of 25 randomly selected pods was attained).

Measurement of growth parameters

At each sample date plants were collected for determination of shoot and root dry weight and nodule number. Plant tops were dried immediately after harvest in a forced air dryer at 70 °C to constant weight. Roots were washed in tap water, nodules counted and they were then dried. Samples were ground to pass through a 1 mm sieve and the percent N was determined by the Kjeldahl N digestion methods.

The following characteristics were analysed statistically: nodules/plant, root dry weight, shoot dry weight and green pod yield. Weather data was recorded at the Broadfields Meteorological Station, 1.0 km from the experimental site. All statistical analyses were done using GenStat (GenStat Release 6.1 Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK).

Results

Climate

The 2004/2005 growing season was drier than usual. The monthly rainfall from January to March was 33.6, 18.6 and 36.6 mm respectively (Table 1). This was 47 % less than the long term average. In 2005, 89 mm of rain fell over the period compared to 142 mm of the long term mean. This made the 2005 season the driest in the last 3 years. The mean maximum daily temperature was 22.3 °C compared to 21.7 °C over the long term. The minimum long term temperature was 10.8 °C compared with 10.7 °C over the growing season

Nodulation response

Symbiotic characteristics of the *Rhizobium* strains used are shown in Table 2. Nodules produced were of various sizes, and pink to red in colour denoting the presence of leghaemoglobin. Nodules on uninocualted control plants, when present, were small and round. Nodules formed by the applied strains were globular in shape whether on Scylla or T-49. Strains H 20, PhP 17 and PRF 81 produced large globular clusters of nodules on both cultivars. Nodules produced by strain PhP 17 were located along the top third of the secondary roots, while with H 20 and PRF 81 nodules were concentrated around the junction of the main stem and the secondary roots and also along the secondary roots.

Nodules per plant

There were significant differences among *Rhizobium* strains in nodules/plant ($P < 0.05$) at 70 DAI. Generally there was an increase in nodules/plant as plant age increased. Each inoculation treatment attained its maximum number of nodules/plant at a different development stage (data not shown). Cultivar T-49 consistently produced more nodules/plant than Scylla at all harvests. In T-49, shoot development showed a similar pattern of increase from 21 DAI to 40 DAI (Figure 1). Shoot dry weight/plant decreased from 40 DAI and this corresponded with full bloom. It increased again when pods were rapidly developing (after 54 DAI) until green pod harvest at 70 DAI. The strain H 20 showed a rapid increase in nodules/plant until 40 DAI then gradually increased until 70 DAI. In the other treatments nodule number/plant increased gradually throughout the experiment.

Table 1. Weather data for the 2005 growing season and the long term averages at Lincoln University, Canterbury, New Zealand. (N/A = not applicable)

	January	February	March	Total
Rainfall (mm)				
2005 season	33.6	18.6	36.6	88.8
Long term mean	51.3	40.6	50.4	142.3
Max. daily temp. (^{o}C)				
2005 season	21.7	23.4	19.9	N/A
Long term mean	21.9	21.8	20.1	N/A
Min. daily temp. (^{o}C)				
2005 season	7.8	11.5	12.7	N/A
Long term mean	11.4	11.3	9.7	N/A

Table 2. **Symbiotic characteristics of rhizobial strains used in this study**.

Treatment	Nodule location	Nodule shape	No of nodules/plant	Shoot dry matter (g/plant)
Control (no inoculant)	Individually scattered on the secondary roots	Small and round	1	7.70
RCR 3644	Scattered along the secondary roots	Small and globular	1	7.78
UK 2	At the intersection of the main stem and the secondary roots	Large globular	9	8.14
H 20	At the junction of the main stem and the secondary roots	Globular clusters	54	7.82
PRF 81	Around the junction of the main stem and the secondary roots	Large globular clusters	24	9.75
PhP 17	Around top 1/3 of the secondary roots	Globular and scattered	6	8.60

Characteristics taken at 50 % flowering (40 days after inoculation)

On the cv. Scylla strain H 20 also gave a rapid increase in nodules/plant and this continued until 70 DAI (Figure 1). This strain was followed by PRF 81 which increased until the onset of flowering (40 DAI). It then declined sharply. The other strains gave a gradual increased in nodules/plant from 21 to 70 DAI. Scylla exhibited similar shoot dry weight development to T-49 until 40 DAI with all strains except UK 2 which showed a linear response from 21 DAI to 70 DAI. The other strains followed a pattern of increasing until 40 DAI, decreasing from 40 to 54 DAI and then increasing again after 54 DAI which corresponded with pod development as in T-49.

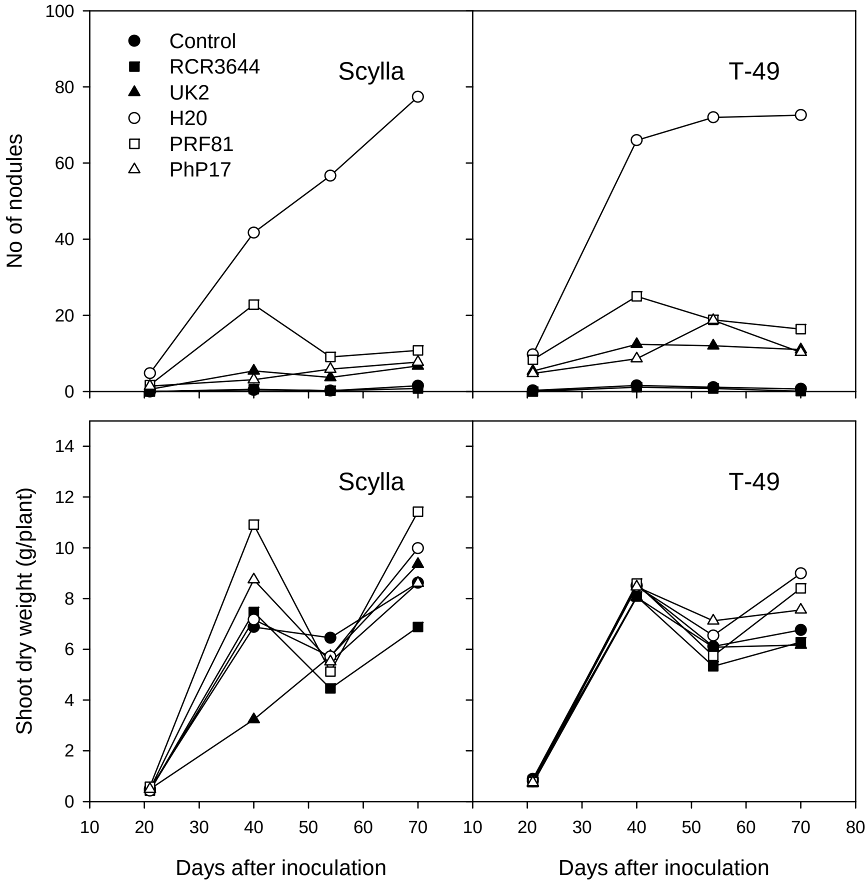

Figure 7. Variability in nodules/plant and shoot dry weight (g/plant) of cultivars Scylla and T49 at 21, 40, 54 and 70 days after inoculation with six inoculation treatments.

Table 3. Cultivar effect on nodulation (nodule number (NN), nodules/plant (NP) and shoot dry matter/plant (SDM) at various times after inoculation.

Harvest date	Cultivar	NN	NP	SDM (g/plant)
21 DAI	Scylla	7	1.4	0.49
	T-49	28	4.7	0.80
40 DAI	Scylla	67	12.4	8.23
	T-49	117	19.1	8.36
54 DAI	Scylla	69	12.4	5.50
	T-49	123	20.6	6.15
70 DAI	Scylla	102	17.5	9.14
	T-49	106	18.5	7.36

DAI: days after inoculation.

Table 4. Field yield parameters obtained at green pod harvest after inoculation of common bean cvs. Scylla and T-49 with different rhizobial strains.

	Scylla			T-49		
Rhizobium strains	NN	TDM/m^2 (g)	*Pod yield/m^2 (g)	NN	TDM/m^2 (g)	Pod yield/m^2 (g)
Control	8.6	526	152.0	4.2	424	158.1
RCR 3644	3.6	387	119.2	1.0	424	156.8
UK 2	36.3	520	178.4	72.6	419	152.5
H 20	463.8	658	252.4	396.4	527	196.1
PRF 81	52.8	692	202.3	94.6	521	225.5
PhP 17	46.2	572	173.7	64.3	541	192.7

NN: number of nodules; TDM: dry matter; Pod yield: dry matter yield of the green pods. *Green pod harvest (yield) was defined as when an average seed length of approximately 11 mm from the centre most seed in a sample of 25 pods was attained.

Biomass accumulation and yield

At 40 DAI (50 % flowering) H 20 produced the most nodules/plant (54), followed by PRF 81 (24) nodules, UK 2 (9), PhP 17 (6), with RCR 3644 and the control both producing 1. Although producing half the number of nodules of H 20, PRF 81 gave the highest shoot dry weight of 9.75 g/plant compared to the 7.82 g/plant with H 20. Strain PhP 17, with only 6 nodules/plant, gave a shoot dry weight 8.60 g/plant, while UK 2 produced a dry weight of 8.14 g/plant with 9 nodules/plant (Table 2).

Cultivar-strain interactions gave variable nodule responses (Table 3). At each harvest T-49 had the highest number of nodules, nodules/plant and shoot dry weight/plant. The exception was at 70 DAI when shoot dry weight was 7.36 g/plant for T-49, while in Scylla it was 9.14 g/plant. At this growth stage T-49 had more advanced pod development and senescence of lower leaves had begun. There was an increase in nodule occupancy over time in Scylla from 6.7 nodules/plant at 21 DAI to 101.9 nodules/plant at 70 DAI, while T-49 started at 27.9 at 21 DAI and increased to 105.5 at 70 DAI. Dry matter accumulation increased over time in both cultivars (Table 3). The cv. T-49 increased in weight (g/plant) by a ratio of 10:1 from 21 DAI to 40 DAI compared to a 16:1 ratio in Scylla. Although T-49 produced highest shoot biomass both cultivars grew at approximately 0.40 g/plant/day over the 19 day period. In comparison the daily shoot accumulation rate/day up to 70 DAI was 0.130 g and 0.105 g/plant for Scylla and T-49 respectively.

At green pod harvest, H 20 gave the most nodules compared to the other strains. The lowest value was from RCR 3644 for both bean cultivars. This strain is currently one of two recommended inoculum strain available in New Zealand for use on dwarf beans. The highest total dry matter (TDM) (g/m^2) (Table 4) was produced by Scylla and strain PRF 81 and with T-49 and strain PhP 17 respectively. The PRF 81-Scylla interaction had a harvest index (HI) of 0.29, while in the PhP 17-T-49 combination it was 0.36. Strain H 20 gave a TDM yield of 658.8 g/m^2 with Scylla and the highest HI at 0.38. Strain PRF 81 and T-49 had a HI of 0.43, while HIs of 0.37, 0.37, 0.36, 0.37 and 0.36 were obtained from the control, RCR 3644, UK 2, H 20 and PhP 17 with T-49 respectively.

A pod DM yield of 225.5 g/m^2 was obtained from the strain PRF 81-T-49 combination, followed by H 20 and the PhP 17-T49 combination with yields of 196.1 and 192.7 g/m^2 (Table 4). In comparison the H 20 and Scylla yielded 252 g/m^2 followed by 202.3 g/m^2 for PRF 81 and Scylla. On both bean cultivars the control treatment out yielded strain RCR 3644 in all measured parameters (Table 4). Strain UK 2, gave a pod yield of 178.4 g/m^2 with Scylla but produced the lowest pod yield with T-49 at 152.5 g/m^2.

Discussion

Effective nodulation is an essential feature of an efficient legume/*Rhizobium* symbiosis. Plants most susceptible to infection and capable of producing effective nodules should have a greater potential to fix more atmospheric N. This assumption often depends on other factors such as the environment, crop management, choice of micro and micro symbiont and the ability of the plant to support these levels of fixation (Pereira *et al*., 1993). The results of this experiment showed great variability in nodulation between the two cultivars and the *Rhizobium* strains used. This indicates apparent differences in compatibility between bean cultivars and rhizobial strains.

Phaseoleae are reported to be nodulated by both fast and slow growing bacterial symbionts (Dakora, 2000) and this could be responsible for some of the variation in nodules. The initial steps towards nodulation are initiated by a number of biochemical signals from both symbionts (Brevin, 1991). The acceptance of these signals is responsible for continued development and final nodulation. Some nodulation variability, as seen in this experiment could be the result of highly promiscuous behaviour of *Phaseolus vulgaris* (Graham, 1981; Dakora, 2000) and inadequate genetic compatibility between the cultivars used and strain applied (Milev and Genchev, 1997). Most nodules were situated in and around the tap root (Table 1), in a radius of 5 cm and at a soil depth of 3-10 cm. Nodules varied in size but were up to, and around, 5 mm in diameter which is consistent with values for *Phaseolus* nodules (Sprent, 2001).

In Brazil, Hungria *et al*., (2003) reported increased nodule occupancy with a number of strains which included H 20 and PRF 81 both used in this experiment. The strain PRF 81 is a recommended strain used in the Brazilian commercial inoculant industry (Hungria *et al.,* 2003). It gave good nodulation under Canterbury conditions, compared to strain RCR 3644 which is available as an inoculant strain for common beans in New Zealand. Strain RCR 3644 produced consistently poor results compared to the other strains used and was inferior to the control.

The ability of PRF 81 to increase bean yield under extremely dry conditions is well documented (Hungria *et al*., 2003). This could account for some of our results. The 2004-2005 growing season was one of the driest in the last five years. Inconsistencies in nodulation with this strain have been documented in field experiments in New Zealand when used with pinto beans (*P. vulgaris*) (Dapaah *et al*., 1995). This inconsistent response is probably associated with existing/indigenous and more competitive *Rhizobium* strains in the soil as some control plants were nodulated.

In New Zealand, *Phaseolus* beans are not usually inoculated, the two bean cultivars in this study are both the same botanical species but have been selected for pod production (Scylla) and seed production (T-49). A significant inoculation effect ($P < 0.05$) for inoculation did not necessarily give a significant shoot TDM or green pod yield. Shoot TDM/plant was consistently higher in Scylla irrespective of inoculation treatment highlighting the cultivars effect on shoot TDM/plant. Dobert and Blevins (1993) reported similar results in large seeded cultivars of lima bean exhibiting a type III growth habit, while some common bean cultivars from types I and II growth habits had nodule characteristics as good as type III cultivars (Milev and Genchev, 1997).

Green pod yield also varied on both cultivars and ranged from 152.5 - 225.5 g/m^2 on T-49 and from 119 - 202.3 g/m^2 on Scylla. Strains H 20, PRF 81, and PhP 17 increased green pod yield over the other inoculation treatments. Highly effective *Rhizobium/ Phaseolus* combinations have been shown to increase bean yield by 60 - 70 % above those obtained with N fertilised control plants (Rodriguez-Navarro *et al*., 1999). This work did show that inoculation improved yield over uninoculated control plants, which suggests that yield improvement can be obtained without N fertiliser provided suitable cultivar/strain combinations can be identified.

Conclusions

Both navy and dwarf beans can produce well without addition of N fertiliser in Canterbury. Rhizobium strains PRF 81, PhP17 and H 20 were identified as effective nodulating strains. Strain 3644 gives inconsistent results in bean nodulation in field trials in New Zealand. Bean yield increases can be obtained without N fertilisation provided suitable cultivar/strain combinations are identified. The continuous evaluation of cultivar/strain symbiosis is necessary to maximise the use of biological N fixation in New Zealand legume cropping systems.

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