

## Symbiotic effectiveness of strains of *Rhizobium leguminosarum* biovar *phaseoli* isolated from soils of Rwanda\*

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### Abstract

We have isolated 48 strains of *Rhizobium leguminosarum* biovar *phaseoli* from nodules of *Phaseolus vulgaris* L. cultivated on 32 different soils at 22 various locations in Rwanda, Central Africa. The symbiotic effectiveness of the strains was appraised in the greenhouse by measuring shoots dry matter and total plant nitrogen content after six weeks of growth. Of the strains tested 19%, 58% and 23% were rated very effective, effective and ineffective, respectively. A very significant correlation ( $r = 0.96$ ,  $P < 0.01$ ) was observed between shoots dry matter and total N content. By using the total nitrogen balance method, it was estimated that in the presence of a very effective strain, up to 86% of the N present in the shoots comes from N<sub>2</sub> fixation. No significant correlations were observed between the symbiotic effectiveness of the strains and the pH of the soils from which they originated, the tolerance of the strains to acidity or their ability to produce organic acids. The nine very effective strains selected were highly competitive against two ineffective strains with the two *P. vulgaris* cultivars Rubona-5 and Kiryumukwe.

### Introduction

Nitrogen deficiency in many tropical soils is one of the major limiting factors in crops production, and because of their ability to fix atmospheric nitrogen, legumes should be an important component of tropical agro-systems (Gibson *et al.*, 1982). In many locations in Eastern Africa, field trials have indicated that it is possible to increase bean (*Phaseolus vulgaris* L.) yields by inoculation with selected strains of *Rhizobium leguminosarum* biovar *phaseoli* (Keya *et al.*, 1982). In Rwanda, bean production plays a major role in human nutrition because it is the most important source of protein; however, it did not show any response to

inoculation with some specific *Rhizobium* strains imported from Belgium, Holland, Mexico and Australia (Nyabyenda *et al.*, 1980). Variable responses to bean inoculation could be explained by limiting and environmental agronomic factors, such as acid soils and aluminium toxicity (Franco and Munns, 1982). Karanja and Wood (1988) isolated from Kenyan soils effective strains of *R. leguminosarum* bv. *phaseoli* tolerant to low pH and Al concentrations up to 20  $\mu$ M in pure cultures or in soils.

The purpose of the present study was to isolate strains of *R. leguminosarum* bv. *phaseoli* from soils in different locations in Rwanda with the aim of identifying very effective indigenous strains that could be used for the local production of an inoculum for bean.

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## Materials and methods

### Strains of *Rhizobium*

Forty-five strains of *R. leguminosarum* by *phaseoli* were isolated from nodules of bean (Vincent, 1970) cultivated on 32 different soils in 22 different locations in Rwanda (Table 1). Three were provided by l'Institut des Sciences Agronomiques du Rwanda (ISAR). The strains were maintained on yeast extract mannitol (YEM) agar and con-

Table 1. Location, pH and organic matter (O.M.) of the soils from which indigenous *Rhizobium leguminosarum* biovar *phaseoli* were isolated

Location	Strains	Soil properties	
		pH	% O.M.
Ngoma	1008	6.1	4.1
Nyabisindu	1402, 1406	6.1	2.4
Nyabisindu	1705	5.9	3.5
Nyabisindu	1805, 1810	6.1	3.3
Muganza	2002	6.3	3.9
Muganza	2206	6.1	5.4
Nyakizu	2505	5.7	6.2
Gishamvu	3305, 3408	6.0	5.1
Rusatira	4102	6.2	8.4
Kigembe	4405	6.9	3.6
Kuyira	4906	7.0	4.6
Nyamabuye	5403	6.3	3.3
Nyamabuye	5510	6.1	3.1
Nyamabuye	5605	5.5	3.3
Mukingi	6002	5.7	3.5
Mukingi	6108	6.0	2.1
Mukingi	6204, 6205	6.3	3.4
Kigoma	6701	6.5	2.9
Tare	HRw1	4.4	3.6
Ruhondo	HRw4	5.6	1.6
Ruhondo	HRw5	5.0	2.0
Ruhondo	HRw11, HRw12	7.2	3.5
Kinigi	HRw13	5.1	17.9
Kigombe	HRw19, HRw20	5.5	5.5
Kigombe	HRw21, HRw22, HRw23, HRw24	6.3	3.0
Nkuli	HRw25, HRw26	5.5	13.2
Nkuli	HRw30	6.5	2.8
Kayove	HRw32	4.4	5.6
Rukara	HRw40, HRw41	6.1	8.3
Buyoga	HRw43	4.7	2.5
Runda	HRw45	nd <sup>a</sup>	nd
Butamawa	HRw46	5.0	2.7
Runda	HRw47	nd	nd
Nyamabuye	HRw48	7.2	0.9
ISAR <sup>b</sup>	RGB1, RGB2, RGB3	nd	nd

<sup>a</sup> nd = not determined.

<sup>b</sup> ISAR = Institut des Sciences Agronomiques, Rwanda.

served at  $-80^{\circ}\text{C}$  by adding 0.2 mL of a solution of glycerol (50%) to 0.8 mL of a dense rhizobial culture in YEM.

### *Phaseolus vulgaris* cultivars

Seeds of Rubona-5, a dwarf cultivar, and Kiryumukwe, a semi-vine cultivar of *P. vulgaris* L. were kindly provided par l'Institut des Sciences Agronomiques du Rwanda (ISAR).

### Symbiotic effectiveness

Plastic pots (13 cm diameter) were sterilized with a 0.5% Oakite solution (Sanitizer no. 1, Oakite Products of Canada, Bramalea, Ontario) and were filled with an autoclaved mixture of 1 volume of sand and 2 volumes of vermiculite. Seeds of cultivar Rubona-5 were surface sterilized by soaking for 15 min in sodium hypochlorite (5.25%) after exposure to 95% ethanol (5 min), followed by several rinses in sterile water. Each pot received 4 surface-sterilized uniformly sized seeds and 200 mL of Hoagland's nutrient solution (Bordeleau *et al.*, 1977). Plants were thinned to 3 per pot 10 days after sowing. Seedlings were inoculated by adding 20 mL of the nutrient solution containing approximately  $10^9$  *Rhizobium* cells  $\text{mL}^{-1}$  to each pot. Control plants (uninoculated plants) received 20 mL sterile nutrient solution. Plants were harvested at anthesis at 40 days. Dry matter yields and plant total-N content were measured as previously described (Bordeleau *et al.*, 1977).

In the glasshouse, plants were grown under a 12 h light period ( $150\text{--}250\ \mu\text{E m}^{-2}\text{sec}^{-1}$ ) and a 12 h darkness at  $18\text{--}22^{\circ}\text{C}$ . A strain was arbitrarily rated very effective (VE) when the dry matter yield of the associated host was higher than the total mean of all strains plus the standard deviation, effective (E) when its yield was between that of the mean  $\pm$  the standard deviation and ineffective (I) when its yield was smaller than the mean minus the standard deviation. The experimental design was a randomized complete block with 4 replicates. The percent N derived from atmosphere (% Ndfa) was also approximately estimated according to Rennie (1984) with the following formula:

$$\% \text{ Ndfa} = \frac{\text{TPN inc} - \text{TPN cont}}{\text{TPN inc}} \times 100$$

where TPN inc is the total N content of plants inoculated with a *Rhizobium* strain and TPN cont is the total N content of the uninoculated control plants.

#### Soil analysis

The pH of the soils was measured in water (1:2), and soil organic matter was estimated by the modified Walkley and Black method (McKeague, 1978).

#### Acid production and tolerance to acidity

Strains were tested for acid production as described by Norris (1965), and the tolerance of the strains to acidity was measured as described by Bromfield and Jones (1980) and Howieson (1985).

#### Competition between VE and I strains

A competition study was performed with the 9 VE strains (HRw11, HRw12, HRw41, HRw46, RGB2, 1402, 4405, 4906, 6701) and with 2 of the I strains (HRw32 and 6002) by using 2 cultivars of *P. vulgaris*: Rubona-5 and Kiryumukwe. Plants were grown as described earlier but were inoculated with 20 mL of nutrient solution containing  $7 \times 10^9$  cells of a VE strain or a mixture in equal number of VE and I strains. A split-plot experimental design with 4 blocks (replications) was used. In each block, the main plots were the 2 cultivars of *P. vulgaris* and the subplots were the *R. leguminosarum* bv. *phaseoli* strains used individually or in mixture and an uninoculated control. The competitive ability (Ca) of a VE strain was estimated as described by Amarger (1981) as follows:

$$\text{Ca} = \frac{Y_m}{Y_i} \times 100$$

where  $Y_m$  is the dry matter yield of bean plant inoculated with a mixture of VE and I strains and  $Y_i$  is the dry matter yield with bean plant inoculated only with the VE strain.

## Results and discussion

### Symbiotic effectiveness

In the greenhouse, the shoot dry matter of the Rubona-5 cultivar and the plant total-N were significantly affected by the strains of *R. leguminosarum* bv. *phaseoli* added in the inoculum (Table 2). From the 48 strains tested, 9 were rated VE (19%), 28 E (58%), and 11 I (23%). The 11 I strains were isolated from 11 different soils in 10 different locations and the 9 VE strains were also isolated from 9 different soils in 8 different locations. The absence of VE or E strains in 34% of the soils tested (a total of 32 soils were studied) is an indication that bean plant will probably show a positive response to inoculation in Rwanda. With the I strain 3305, approximately 48% of the shoots total N derived from the atmosphere and with the VE strain 4906, it was estimated that up to 86% of the N present in shoots comes from  $\text{N}_2$  fixation, indicating that as with other cultivars of bean (Lalande *et al.*, 1986; Rennie and Kemp 1983a; 1983b), this process is also important in the cultivar Rubona-5. Very significant correlations were observed between the shoots dry matter weight and their total N content ( $r = 0.96$ , significant at  $P \leq 0.01$ ) or the percentage of nitrogen derived from fixation ( $r = 0.85$ , significant at  $P \leq 0.01$ ). Thus, as previously observed with lucerne (Bordeleau *et al.*, 1977) shoot dry matter yield of bean plant can be used as a tool to appraise the symbiotic  $\text{N}_2$  fixation efficiency of a strain of *R. leguminosarum* bv. *phaseoli*.

The lowest soil pH recorded was 4.4 and the highest was 7.2 (Table 1). No VE strains were isolated in soils having a pH lower than 5.0; however, I strains were isolated from soils having all pH values. Soil organic matter also varied from 1 to 18%. No significant correlations were found between the symbiotic effectiveness of the strains and the pH or the organic matter content of the soils from which they originated.

### Acid production and tolerance to acidity

When grown on Norris's medium (initial pH = 7.15), all the strains tested were acid producers, and no significant correlation was ob-

**Table 2.** Shoots dry matter yields and total N content of the cultivar Rubona-5 of *P. vulgaris* inoculated with strains of Rhizobium, and the percentage of N derived from the atmosphere (% Ndfa) in nodulated plants

Strains	Shoot dry weights (g pot <sup>-1</sup> )	Shoot total-N (mg pot <sup>-1</sup> )	% Ndfa	Symbiotic effectiveness	Final pH on Norris' medium
4906	4.11	189.75	86.25	VE <sup>a</sup>	5.50
1402	3.91	187.25	86.05	VE	5.44
6701	3.89	184.25	85.88	VE	5.42
RGB2	3.82	176.75	85.26	VE	5.29
HRw41	3.77	173.00	84.89	VE	5.46
4405	3.77	178.75	85.41	VE	5.70
HRw46	3.73	172.00	84.80	VE	5.20
HRw12	3.73	178.25	85.37	VE	5.10
HRw11	3.73	177.00	85.16	VE	5.30
HRw30	3.64	169.25	84.46	E	5.03
1406	3.62	155.00	83.18	E	5.33
1810	3.61	169.50	84.53	E	5.10
HRw20	3.59	169.25	84.58	E	5.20
6108	3.57	165.25	84.09	E	5.43
HRw45	3.56	172.25	84.68	E	5.76
5403	3.50	168.50	83.99	E	5.23
HRw22	3.50	160.50	83.67	E	5.06
HRw23	3.48	157.50	83.36	E	5.16
4102	3.45	157.00	83.33	E	5.20
HRw4	3.41	161.50	83.89	E	5.13
1008	3.40	152.00	82.89	E	5.12
1705	3.39	152.00	82.83	E	5.00
3408	3.38	148.00	82.29	E	5.25
HRw48	3.27	139.75	80.79	E	5.19
6204	3.18	142.00	81.64	E	5.16
RGB3	3.15	139.50	81.23	E	5.00
1805	2.99	125.00	78.87	E	5.03
5605	2.97	130.50	79.79	E	5.02
2002	2.77	121.50	78.22	E	5.20
HRw26	2.75	121.00	78.49	E	5.30
HRw19	2.65	108.75	75.99	E	5.00
2505	2.61	101.50	73.81	E	5.43
HRw21	2.57	105.75	75.13	E	5.12
HRw47	2.47	109.00	75.56	E	5.00
2206	2.45	105.25	74.69	E	5.03
HRw25	2.45	88.50	70.37	E	5.10
HRw43	2.34	65.50	59.73	E	6.16
HRw5	2.31	53.50	51.18	I	6.10
HRw24	2.29	88.50	69.87	I	5.03
6002	2.28	87.75	69.70	I	5.27
6205	2.24	89.00	70.34	I	5.10
HRw13	2.15	56.50	52.47	I	6.06
HRw1	2.02	58.75	55.31	I	6.20
RGB1	1.98	84.75	68.61	I	4.90
5510	1.98	60.50	56.13	I	5.36
HRw32	1.90	76.50	65.54	I	5.30
HRw40	1.79	79.25	66.12	I	5.00
3305	1.67	50.25	47.88	I	5.16
Control <sup>b</sup>	1.26	26.00	–	–	–
LSD 0.05	0.37	20.47	4.81	–	–

<sup>a</sup> VE = very effective; E = effective; I = ineffective.

Mean shoots dry weight = 3.02 g pot<sup>-1</sup> and standard deviation = 0.69.

<sup>b</sup> Uninoculated control.

served between the symbiotic effectiveness of the strains and acid production as observed with strains of *R. trifolii* (Jones and Burrows, 1969) or *R. meliloti* (Bordeleau and Antoun, 1978). In fact, the final pH of Norris's medium varied from 5.1 to 5.7 with the VE strains, from 5.0 to 6.1 with the E strains and from 4.9 to 6.2 with the I strains (Table 2).

The tolerance of the strains to low pH was not related to their symbiotic effectiveness (Table 3). For example, 8 VE, 26 E and 11 I strains were able to grow at pH 4.4, and 4 VE, 16 E and 8 I strains were able to grow at pH 4.2 when the culture medium was strongly buffered according to Howieson (1985).

*Competition between VE and I strains*

When an I strain was added in equal number in the inoculum with a VE strain, no adverse effect was observed on the shoot dry weights of the 2 cultivars Rubona-5 and Kiryumukwe of *P. vulgaris* or on the total-N content of the 2 cultivars (Table 4). This suggests that the 9 VE strains selected are highly competitive. In fact, the competitive ability of the VE strains varies from 84 to 113% (Table 5). However, as many environmental factors can affect the competition for nodulation of legumes (Dowling and Broughton, 1986), the VE strains selected in this study should be tested under field conditions.

**Conclusion**

The present study shows that soils of Rwanda contained very effective or ineffective strains of *R.*

*leguminosarum* biovar *phaseoli*. Because of the absence of very effective strains in many soils, *P. vulgaris* will probably show positive response to inoculation in several localities. The 9 very effective strains selected in this study exhibited high competitive abilities and can be favorably used in field inoculation assays.

Table 4. Shoots dry weights and total-N content of the 2 cultivars of *P. vulgaris* inoculated with very effective strains of *R. leguminosarum* biovar *phaseoli* alone or mixed in equal number with an ineffective strain

Treatments	Bean cultivars			
	Rubona-5		Kiryumukwe	
	Shoot dry weights (g pot <sup>-1</sup> )	Shoot total-N (mg pot <sup>-1</sup> )	Shoot dry weights (g pot <sup>-1</sup> )	Shoot total-N (mg pot <sup>-1</sup> )
1 <sup>a</sup>	3.40 ab <sup>b</sup>	147 abc	3.38 a	147 a
1 + 10	3.32 ab	122 bc	3.56 a	158 a
1 + 11	2.87 bc	109 c	3.46 a	157 a
2	3.29 ab	142 abc	3.34 a	140 a
2 + 10	3.51 ab	134 abc	3.77 a	155 a
2 + 11	3.22 ab	136 abc	3.53 a	151 a
3	3.25 ab	134 abc	3.36 a	149 a
3 + 10	3.50 ab	143 abc	3.45 a	155 a
3 + 11	3.29 ab	144 abc	3.51 a	165 a
4	3.41 ab	152 abc	3.36 a	156 a
4 + 10	3.49 ab	152 abc	3.54 a	161 a
4 + 11	3.32 ab	145 abc	3.40 a	153 a
5	3.37 ab	151 abc	3.55 a	163 a
5 + 10	3.79 a	160 ab	3.62 a	164 a
5 + 11	3.66 ab	166 a	3.83 a	174 a
6	3.22 ab	135 abc	3.50 a	161 a
6 + 10	3.33 ab	130 abc	3.39 a	149 a
6 + 11	3.39 ab	141 abc	3.45 a	155 a
7	3.68 ab	152 abc	3.32 a	148 a
7 + 10	3.38 ab	145 abc	3.44 a	157 a
7 + 11	3.40 ab	142 abc	3.50 a	162 a
8	3.26 ab	135 abc	3.42 a	157 a
8 + 10	3.29 ab	136 abc	3.50 a	156 a
8 + 11	3.55 ab	153 abc	3.40 a	153 a
9	3.07 ab	123 abc	3.57 a	157 a
9 + 10	3.46 ab	144 abc	3.63 a	171 a
9 + 11	3.29 ab	138 abc	3.39 a	153 a
10	2.20 cd	46 d	2.32 b	60 b
11	2.17 cd	44 d	2.30 b	53 b
Control <sup>c</sup>	1.62 d	24 d	1.78 b	23 b

<sup>a</sup> VE strains: 1 = HRw11, 2 = HRw12, 3 = HRw41, 4 = HRw46, 5 = RGB2, 6 = 1402, 7 = 4405, 8 = 4906, 9 = 6701; I strains: 10 = HRw32 and 11 = 6002.

<sup>b</sup> Numbers in the columns not sharing a common letter differ significantly ( $P \leq 0.05$ ) according to Tukey's test.

<sup>c</sup> Uninoculated control.

Table 3. Strains distribution as related to symbiotic effectiveness and tolerance to low pH of culture medium

Efficiency	Number of strains tested	No. of strains grown at pH values of		
		4.0	4.2	4.4
VE	9	0	4	8
E	28	0	16	26
I	11	0	8	11

Table 5. Competitive ability (Ca) of the 9 VE strains selected against 21 strains of *R. leguminosarum* biovar *phaseoli*

Strains in inoculum		Ca (%) <sup>a</sup>	
VE	I	Rubona-5	Kiryumukwe
HRw11	HRw32	104	105
HRw12	HRw32	107	103
HRw41	HRw32	108	103
HRw46	HRw32	102	105
RGB2	HRw32	113	102
1402	HRw32	103	97
4405	HRw32	92	104
4906	HRw32	101	102
6701	HRw32	113	102
HRw11	6002	84	106
HRw12	6002	98	106
HRw41	6002	101	105
HRw46	6002	97	101
RGB2	6002	109	108
1402	6002	105	99
4405	6002	92	105
4906	6002	109	99
6701	6002	107	95

<sup>a</sup> Competitive ability (for explanation, see materials and methods).

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### References

- Armarger N 1981 Selection of *Rhizobium* strains on their competitive ability for nodulation. *Soil Biol. Biochem.* 13, 481–486.
- Bordeleau L M and Antoun H 1978 Une méthode rapide de présélection des souches de *Rhizobium meliloti*. *Can. J. Plant Sci.* 58, 1125–1126.
- Bordeleau L M, Antoun H and Lachance R A 1977 Effets des souches de *Rhizobium meliloti* et des coupes successives de la luzerne (*Medicago sativa*) sur la fixation symbiotique d'azote. *Can. J. Plant Sci.* 57, 433–439.
- Bromfield E P S and Jones D G 1980 Studies on acid tolerance

- of *Rhizobium trifolii* in culture and soil. *J. Appl. Bacteriol.* 48, 253–264.
- Dowling D N and Broughton W J 1986 Competition for nodulation of legumes. *Annu. Rev. Microbiol.* 40, 131–157.
- Gibson A H, Dreyfus B L and Dommergues Y R 1982 Nitrogen fixation by legumes in the tropics. In *Microbiology of Tropical Soils and Plant Productivity*. Eds Y R Dommergues and H G Diem. pp. 37–73. Martinus Nijhoff/Dr W Junk Publishers, The Hague, The Netherlands.
- Howieson J G 1985 Use of an organic buffer for the selection of acid tolerant *Rhizobium meliloti* strains. *Plant and Soil* 88, 367–376.
- Franco A A and Munns D N 1982 Acidity and aluminium restraints on nodulation, nitrogen fixation and growth of *Phaseolus vulgaris*. *Soil Sci. Soc. Am. J.* 46, 296–301.
- Jones D G and Burrows A C 1969 Acid Production and symbiotic effectiveness in *Rhizobium trifolii*. *Soil Biol. Biochem.* 1, 57–61.
- Karanja N K and Wood M 1988 Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Infectiveness and tolerance of acidity and aluminium. *Plant and Soil* 112, 7–13.
- Keya S O, Balasundaram V R, SSALI H and Mugane C 1982 Multi-locational field responses of *Phaseolus vulgaris* in Eastern Africa. In *Biological Nitrogen Fixation Technology for Tropical Agriculture: Papers presented at a workshop held at CIAT, 9–13 March 1981*. Eds P H Graham and S C Harris. pp. 231–234. Centro Internacional de Agricultura Tropical, Cali, Columbia.
- Lalande R, Antoun H, Paré T and Joyal P 1986 Effets de l'inoculation avec des souches du *Rhizobium leguminosarum* biovar *phaseoli* sur le rendement et la teneur en azote du haricot (*Phaseolus vulgaris*). *Le Naturaliste Canadien* 113, 337–346.
- McKeague J A 1978 Manual of soil sampling and methods of analysis. Prepared by Can. Soil Survey Committee. *Can. Soc. Soil Sci.*, Ottawa, Ont. Canada.
- Norris D O 1965 Acid production by *Rhizobium*: A unifying concept. *Plant and Soil* 22, 143–166.
- Nyabyenda P, Sekanabanga C and Nyangurundi L 1980 Bean production in Rwanda. In *Regional Workshop on Potential for Field Beans in Eastern Africa, Lilongwe, Malawi, 1980*. pp. 91–121. Proceedings. Centro Internacional de Agricultura Tropical, 1981. Cali, Columbia.
- Rennie R J and Kemp G A 1983a N<sub>2</sub>-fixation in field beans quantified by <sup>15</sup>N isotope dilution. I. Effect of strains of *R. phaseoli*. *Agron. J.* 75, 640–644.
- Rennie R J and Kemp G A 1983b N<sub>2</sub>-fixation in field beans quantified by <sup>15</sup>N isotope dilution. II. Effect of cultivars of beans. *Agron. J.* 75, 645–649.
- Rennie R J 1984 Competition of N balance and <sup>15</sup>N isotope dilution to quantify N<sub>2</sub> fixation in fields grown legumes. *Agron. J.* 76, 785–790.
- Vincent J M 1970 A Manual for the Practical Study of Root-Nodule Bacteria. IBP. No. 15, Blackwell Scientific Publications. Oxford and Edinburgh, 164 p.