Symbiotic Effectivity of Four *Phaseolus vulgaris* Genotypes after Inoculation with Different Strains of *Rhizobium* under Controlled Conditions

Bernhard Epping, Alexander P. Hansen, Bahman Djalali and Peter Martin

Institut für Pflanzenernährung (330), Universität Hohenheim, Fruwirthstraße 20, D-70593 Stuttgart, Bundesrepublik Deutschland

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Four varieties of *P. vulgaris* L. were tested for their symbiotic nitrogen fixation effectivity in combination with nine different strains of *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium tropici*. Plants were grown under controlled conditions and harvested 23 days after planting. Acetylene reducing activity, total N-content and dry weight of individual plant components were determined. Significant differences due to plant × bacterium interaction were assessed by ANOVA, especially for the total nodule mass per plant and the acetylene reducing activity per nodule dry weight. Data for acetylene reducing activity per plant correlated highly with the corresponding data for the total N-content. The comparison of the total N-content in symbiotically grown plants, lacking supply of mineral N, with plants luxuriously supplied with mineral N (relative N-accumulation rate) revealed high values (between 60% and 70% of maximal N-uptake) for some symbiotically active plant/bacterium combinations for this early developmental stage of the symbiosis in *P. vulgaris*. This indicates a substantial N_2 fixation potential for such symbioses.

Introduction

The symbiotic effectivity of a certain strain of *Rhizobium* with a given host plant cultivar is rarely predictable (Cresswell *et al.*, 1992). The lack of knowledge about genetically defined traits for the selection of plant/bacterium combinations with high effectivity for nitrogen fixation highlights the requirement to select suitable combinations of both symbiotic partners. This applies particularly for *P. vulgaris* since great variability of symbiotic effectivity has been reported for various plant/bacterium affiliations (Pacovsky *et al.*, 1984; Rennie and Kemp, 1983 a, 1983 b). Furthermore, breeding programs for the selection of bean genotypes superior in N_2 fixation are still hampered by low hereditity of the so far selected traits, mostly due to environmental effects (Bliss, 1993).

The *Phaseolus/Rhizobium* symbiosis has frequently been identified as an unreliable symbiotic system with regard to N_2 fixation. Various hypotheses have been brought forward to explain poor nitrogen fixation rates of this species. Fixation rates can be very sensitive to environmental factors (Hardarson *et al.*, 1993). Other cited explanations include a genetically determined poor nodulation capacity of *Rhizobium* strains (Chaverra and Graham, 1992) and short vegetative growth period of plants with resulting early carbohydrate starvation of nodules because of competition between nodules and developing fruits (Piha and Munns, 1987 a). Furthermore, there seems to be a genetic disposition of common bean favoring N-uptake instead of N_2 fixation (George and Singleton, 1992). In contrast, there are also data indicating that at least certain varieties of *P. vulgaris* can satisfy their N-demand exclusively through nitrogen fixation when inoculated with suitable *Rhizobium* strains (Piha and Munns, 1987 a, 1987 b). Consequently, results from different geographical areas and data obtained from other host/bacteria combinations cannot be generally applied.

Different approaches have so far been taken to improve the symbiotic performance of *P. vulgaris*. One obvious way is the selection of nodulation-improved lines (Pereira *et al.*, 1993). Previously, genetical manipulation of the nodulation character was attempted with EMS (ethylmethyl sulphonate) by Park and Buttery (1989). They were able to select supernodulating genotypes which potentially could be superior in N_2 fixation since such

Reprint requests to Dr. B. Epping.
Telefax: (0711) 4593295.

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genotypes nodulate abundantly. Other researchers chose investigations involving both symbiotic partners (Karanja and Wood, 1988a, 1988b).

The objective of the present study was to identify suitable host/rhizobia combinations between *Rhizobium* strains and four genotypes of *P. vulgaris* under controlled conditions. The study was undertaken as one part of a larger project aiming to induce and subsequently improve N_2 fixation of *P. vulgaris* in the highlands surrounding Chiang Mai in Northern Thailand, an area so far devoid of rhizobia compatible to this species. Plant genotypes were chosen to provide a wide range of genotypical differences and consequently included a "red kidney" type as well as white seeded varieties. The former characteristic was met by genotype Mokcham (a variety cultivated in Thailand for some time), while the latter property is represented by *cv.* Brilliant (a common variety in Germany) as well as OAC Rico and RBS 15 (provided by Drs. Buttery and Park, Research Station, Agriculture Canada Harrow, Ontario; Canada NOR 1G0). RBS 15, a supernodulating genotype isolated after EMS-mutagenesis of OAC Rico, has been shown to develop substantially more nodules compared to the parent cultivar (Buttery and Park, 1990; Park and Buttery, 1989). This genotype was included in the experiments in order to compare its symbiotic nitrogen fixation performance and its biomass production with the wild type OAC Rico and the other genotypes. Further this allowed an evaluation of any interaction between supernodulation and specific rhizobial strains.

Materials and Methods

Bacterial culture

Nine strains of *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium tropici* were used for inoculation. The strain CIAT 899 was supplied by CIAT, Cali, Columbia; UMR 1899 and 1165 were received from the University of Minnesota, U.S.A. UMR 1899 ist, according to Chaverra and Graham (1992), identical to CIAT 899. The strains WPBS 3644, 3622 and 3605 originate from the Welsh Plant Breeding Station, Aberystwyth, Wales, U.K. TAL 182 was obtained from NIFTAL (Hawaii, U.S.A.), and Ph 24, Ph 6 were purchased from Tokachi Nokio Ren, Hokkaido, Japan.

Individual strains of rhizobia were separately grown in yeast extract mannitol medium (Jordan, 1984) at 28 °C to late stationary phase. Prior to inoculation cultures were diluted with sterile YEM medium to obtain a concentration of 10^9 cells cm^{-3}.

Plant culture

An excess amount of seeds of each genotype was surface sterilized by imbibition in a solution of 1% NaOCl for 5 min. Subsequently seeds were thoroughly washed with sterilized water. Inoculation was performed by immersion of seeds in the corresponding bacteria solution for 50 min while gently shaking at 28 °C. Thereafter, seeds were sown under sterile conditions in dishes containing a 1:1 mixture (v/v) of coarse sand and vermiculite. Seedlings were raised in a growth cabinet under a constant temperature regime (24 °C) and a 16 h illumination period with a light intensity of 300 $\mu E\ m^{-2}\ s^{-1}$. Five days after germination, 11 plants of each plant/*Rhizobium* combination were selected and separately transferred to PVC tubes (40 cm length, 5 cm diameter) containing the same mixture of coarse sand and vermiculite. The bottom of the tubes was sealed with a nylon mesh of 0.5 mm pore diameter. Plants were irrigated daily with the following N-deficient nutrient solution: 1 mM $MgSO_4 \cdot 7H_2O$, 0.125 mM KH_2PO_4, 0.125 mM K_2HPO_4, 0.5 mM $CaCl_2$, 0.25 mM K_2SO_4, 50 µM Sequestren ^{138}Fe, 23 µM H_3BO_4, 0.91 µM $MnCl_2 \cdot 4H_2O$, 0.202 µM $ZnCl_2$, 73 nM $CuCl_2 \cdot 2H_2O$, 26 nM $Na_2MoO_4 \cdot 2H_2O$, 38 nM $CoCl_2 \cdot 6H_2O$.

As a plus N control plants were identically grown but remained uninoculated and received the above described nutrient solution supplemented with 3.75 mM NH_4NO_3.

Nitrogenase activity and plant analysis

Plant harvest for acetylene reducing assays, determination of dry weight and total N-content was performed 23 days after germination. At least 6 replicates of each plant/bacteria combination were examined. Nodulated roots were separated from the shoots, immediately placed in a 100 cm^3 vial, and closed with a suba seal. To achieve a concentration of 6% acetylene during the assay, 6 cm^3 of air were removed and 6 cm^3 of pure acetylene gas

injected by using syringes (Carroll *et al.*, 1987). Gas samples were withdrawn from the vial 5 and 10 min after the start of the incubation and analyzed for their ethylene content utilizing a gas chromatograph (HP 5890, Series II), equipped with a flame ionization detector (FID) and a Porapak-N column. Oven temperature was set constant at 130 °C. Calculations for nitrogenase activity were subsequently based on the time interval between collection of the gas samples. After the acetylene reducing assay nodules were separated from the roots and counted. Afterwards, shoots, roots and nodules were dried for 48 h at 80 °C and the dry weight was determined. The material of three to four plants was pooled, ground and the total N-content determined by Kjeldahl analysis (Peterson and Chesters, 1964). Total N-content of seeds was also measured and subtracted from the results for the whole plants so that the amount of nitrogen that had been fixed during the development of the symbioses could be computed.

Data were calculated on an Apple Macintosh LC computer using the program MS Excel Version 2.2.a. Statistical data processing involving analysis of variance (ANOVA) was carried out with the program StatView SE, Version 1.03 (Abacus Concepts, Inc.).

Results

Total nitrogenase activity, nitrogenase activity per nodule dry weight and the dry weight of nodules per plant for all the combinations examined are shown in Fig. 1. The graph demonstrates great variation in all three parameters due to the plant/bacteria combination. Highest values in total acetylene reducing activity per plant were obtained in most of the combinations with the bean cultivar Mokcham and in some of the combinations with cultivar Rico (Fig. 1 A). Generally lowest values were achieved with the cultivar RBS 15. In marked contrast to the very low results for total acetylene reducing activity shown by this cultivar are the relatively high values for the total dry weight of the nodules on this genotype (Fig. 1 C), which generally are comparable to those of the cultivar Mokcham. This led consequently to very low values for the nitrogenase activity per nodule dry weight on this genotype (Fig. 1 B).

The nitrogenase activity per nodule dry weight can be used as a parameter for the evaluation of

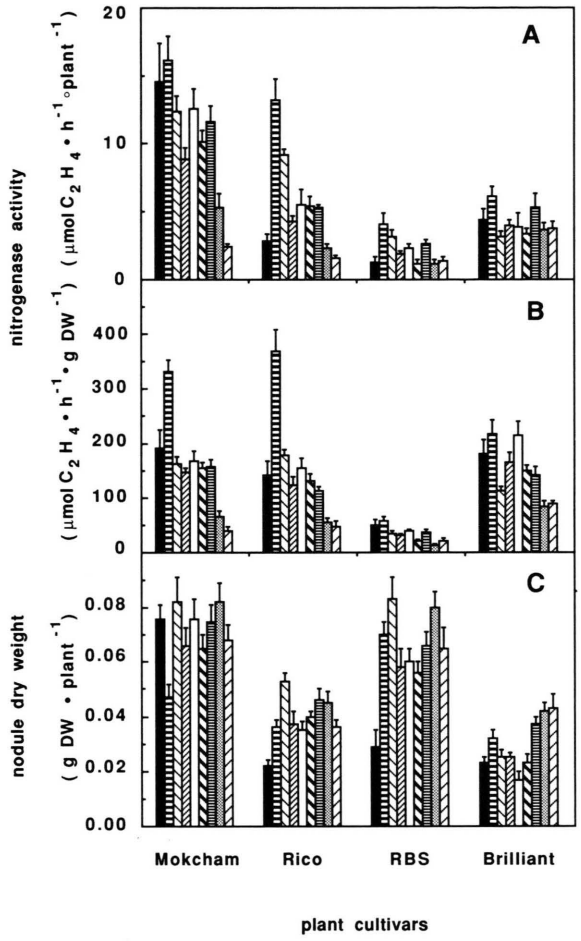

Fig. 1. Nitrogenase activity per plant (A), nitrogenase activity per nodule dry weight (B) and the dry weight of nodules per plant (C) of 23-day-old plants of *P. vulgaris* grown under controlled conditions in symbiosis with nine *Rhizobium* strains; the standard errors of the means are indicated by vertical bars, $n = 6-10$. The following strains were investigated: CIAT 899 (■), UMR 1899 (▤), UMR 1165 (▧), WPBS 3644 (▨), WPBS 3622 (□), WPBS 3605 (▧), TAL 182 (▤), Ph 24 (▨), Ph 6 (▨).

nodule effectivity in a symbiosis. In all bean cultivars highest values for this parameter were detected with the strain UMR 1899, whereas the strains Ph 6 and Ph 24 showed the lowest values for all cultivars. Significantly differing values (Scheffé test, $p < 0.05$, data not shown) were also obtained for the strains CIAT 899 and UMR 1899 on the bean cultivars Mokcham and Rico; these strains have previously been suggested to be identical (Aarons and Graham, 1991).

Total N-content of the plants was determined as a second parameter for the effectivity of the different symbioses. Total N-content provided an absolute measure for nitrogen fixation as the plants had been fully symbiotically grown without mineral nitrogen. These total N-data, corrected for the amount of N stored in the seeds, were correlated with the plant's nitrogenase activity values. The relationship was established independently for every single bean cultivar examined (Fig. 2). Although there is correlation between the two data sets (r = 0.68–0.90), the graphs also indicate that these two independent parameters for nitrogen fixation do not correspond well in all cases.

To get more insight into the N-status of the symbiotically grown plants, the shoot/root ratios of the percentage of dry weight and of the percentage of N-content were calculated for every plant cultivar/bacterial strain combination and for the N-fed plants (Table I). In both cases the shoot/root ratios for the uninoculated N-fed plants were – with only few exceptions – lower than those found for the symbiotically grown, indicating that the roots suffered more than the shoots from the nutrient demand of the new nodules. Generally the shoot/root ratio of the dry weight percentages was lower than the corresponding ratio for the N-percentages – confirming a generally greater N-demand of the shoots compared to the roots. Highest values in both cases were recorded within the supernodulating type RBS 15. The nodules in this cultivar revealed very high values for the percentages of the total dry weight (12.4–25.2%) and of the total N-content of the plants (19.8–34.8%). In the three other cultivars values for the total dry weight and total N-content percentages of this plant part ranged from 3.8% to 10%, and from 8.4% to 17.6% respectively.

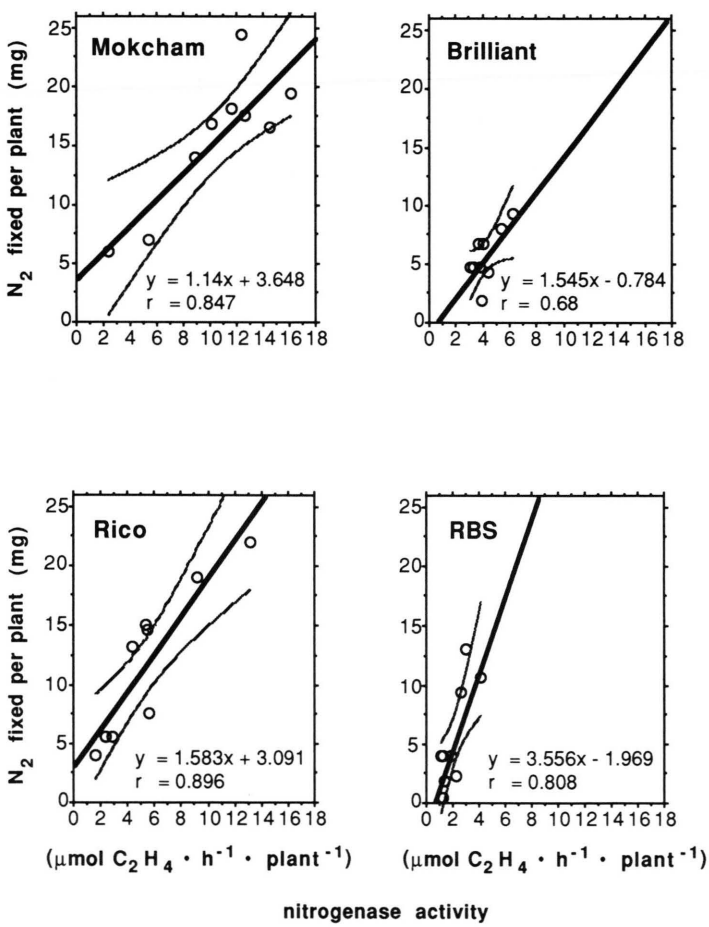

Fig. 2. Relationships between the nitrogenase activity on a per plant basis and the amount of N_2 fixed per plant of 23-day-old plants of *P. vulgaris* cultivated under controlled conditions in symbiosis with nine different *Rhizobium* strains. The limits of the 95% confidence intervals for the true mean of y are indicated.

Table I. Distribution of dry matter and N between shoots and roots of symbiotically grown plants of *P. vulgaris* expressed as shoot/root ratio.

Bean cultivar	Rhizobial strain/±N plants	Dry weight shoot/root ratio	N Shoot/ root ratio
Mokcham	CIAT 899	3.5	5.4
	UMR 1899	4.8	7.1
	UMR 1165	4.8	5.8
	WPBS 3644	3.0	5.4
	WPBS 3622	3.0	4.6
	WPBS 3605	3.2	5.1
	TAL 182	4.2	6.0
	Ph 24	3.1	4.1
	Ph 6	3.7	4.5
	+N	2.5	4.2
Rico	CIAT 899	2.6	4.7
	UMR 1899	3.8	6.0
	UMR 1165	4.3	6.1
	WPBS 3644	2.7	5.5
	WPBS 3622	3.2	6.4
	WPBS 3605	3.3	6.4
	TAL 182	4.2	5.5
	Ph 24	2.9	3.5
	Ph 6	2.4	3.7
	+N	2.1	4.1
RBS	CIAT 899	2.1	3.5
	UMR 1899	5.9	8.6
	UMR 1165	9.2	16.3
	WPBS 3644	4.9	10.5
	WPBS 3622	5.0	8.3
	WPBS 3605	5.0	10.3
	TAL 182	6.4	11.8
	Ph 24	4.9	8.3
	Ph 6	4.7	7.1
	+N	1.4	3.3
Brilliant	CIAT 899	3.3	5.3
	UMR 1899	3.0	4.4
	UMR 1165	3.8	6.4
	WPBS 3644	4.1	7.2
	WPBS 3622	2.3	12.0
	WPBS 3605	3.6	5.7
	TAL 182	4.4	7.3
	Ph 24	3.1	5.0
	Ph 6	3.1	3.9
	+N	2.2	3.8

The different performance of the supernodulating genotype is also revealed by the data shown in Fig. 3. The number of nodules per plant on RBS 15 was to some extent generally, and specifically for UMR 1899, UMR 1165 and TAL 182 elevated in comparison to the wild type Rico and the other two cultivars (Fig. 3 A); consequently values of the dry weight of the total nodule complement were much greater than in Rico (Fig. 1 B) but the dry weight of the individual nodule kept far behind the value for its counterpart on the wild type (Fig. 3 B).

The total N-content of N-fed plants was chosen as a point of reference for the effectivity of the different nitrogen fixing symbioses. These plants had been grown under luxurious N-supply in the nutrient solution enabling maximal N-uptake. The N-supply with the nutrient solution led to a considerable increase in the amount of total dry weight in all four cultivars examined (Fig. 4). It must be emphasized that even under these conditions the supernodulating type accumulated less dry weight and N-content than the wild type.

Total N-content of the symbiotically grown plants was expressed as percentage of the N-content of N-fed plants. The results are shown in Fig. 5. Every plant genotype as well as every bacteria genotype showed a broad range of relative N-accumulation over all of the examined combinations. Relative N-accumulation was never

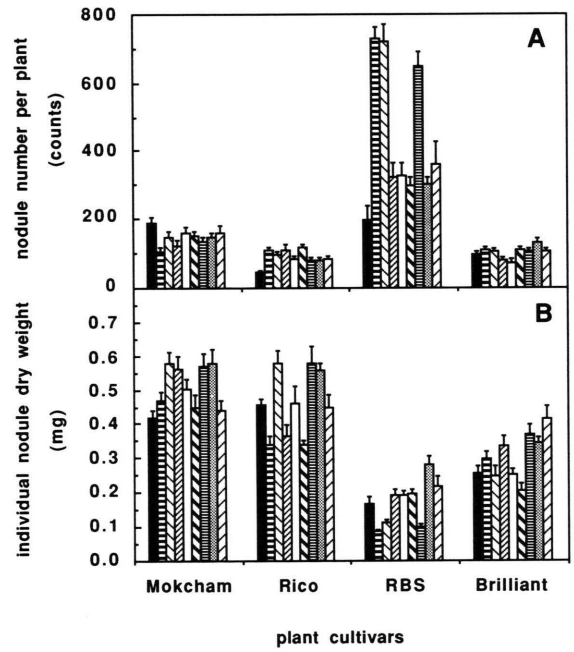

Fig. 3. Number of nodules per plant (A) and dry weight of individual nodule (B) of 23-day-old plants of *P. vulgaris* grown under controlled conditions in symbiosis with nine different *Rhizobium* strains. The standard errors of the means are indicated by vertical bars, n = 6–11. The following strains were investigated: CIAT 899 (■), UMR 1899 (▤), UMR 1165 (▧), WPBS 3644 (▨), WPBS 3622 (□), WPBS 3605 (◪), TAL 182 (▤), Ph 24 (▨), Ph 6 (▨).

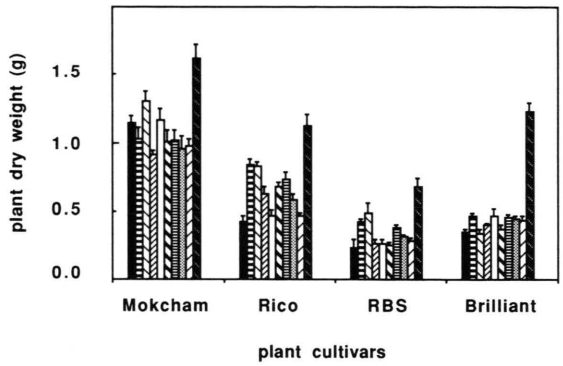

Fig. 4. Dry weight of 23-day-old plants of *P. vulgaris* grown under minus N conditions (symbiotically) or under conditions of N-supply with the nutrient solution. The standard errors of the means are indicated by bars, n = 6–11. The following strains were investigated: CIAT 899 (■), UMR 1899 (▤), UMR 1165 (▧), WPBS 3644 (▨), WPBS 3622 (□), WPBS 3605 (▩), TAL 182 (▤), Ph 24 (▩), Ph 6 (▨). There is a further column for the data of the treatment with 3.75 mM NH_4NO_3 (▩).

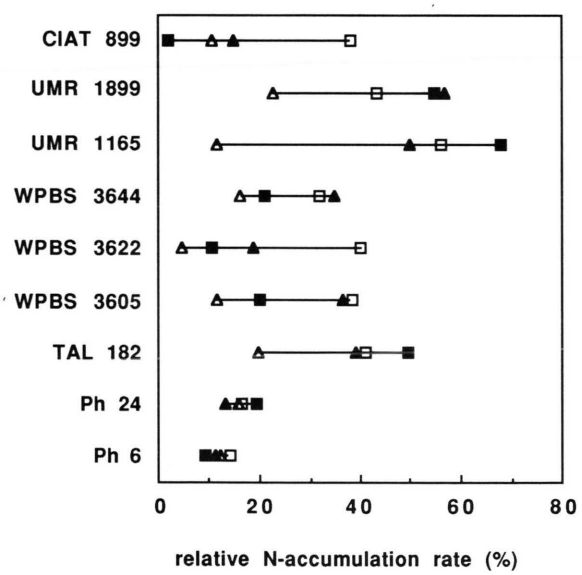

Fig. 5. Range of relative N-accumulation rates (total N in symbiotically grown plants/total N in plants grown under conditions of high mineral N supply) of 23-day-old plants of *P. vulgaris* grown under controlled conditions in symbiosis with nine different *Rhizobium* strains. The following plant genotypes were investigated: Mokcham (□), OAC Rico (▲), RBS 15 (■), Brilliant (△).

higher than 70% and values could be as low as almost zero (RBS 15 with CIAT 899). With regard to the different strains of rhizobia, it is clearly visible that several strains achieved only very poor rates of relative N-accumulation (*e.g.* Ph 24, Ph 6). As already determined by the acetylene reducing assays, different values were also obtained for CIAT 899 and UMR 1899.

The dependence of the nodulation effectivity and specific nitrogenase activity in the symbiosis between *P. vulgaris* and *Rhizobium* on plant genotype and bacteria genotype was verified by the results of the ANOVA (Table II). The plant cultivar × bacteria strain interaction was highly significant for both data sets (p = 0.0001).

Discussion

The reliability of data from acetylene reducing tests for the evaluation and comparison of different N_2 fixation effectivities has been a basis for controversy in the literature (Hansen *et al.*, 1987; Minchin *et al.*, 1983, 1986). Nevertheless, the method – when not used for quantitative estimates of N_2 fixation – can be useful for identifying effective plant-rhizobial combinations (Bliss, 1993). This is indicated by the high correlation coefficients shown in Fig. 2. Despite of general correlation, great care must be taken when the symbiotic effectivity of individual strains is compared, because different results can be achieved when the total N-content of plants is used as parameter for nitrogen fixation effectivity (see Fig. 5).

The different values for the slope of each regression line in Fig. 2 strengthen the view that correlation between data from total N-determinations and acetylene reducing assays exists, but can only be established for data from individual plant cultivars. Other authors did not find such a correlation, possibly due to the fact that they used combined data over several cultivars of *P. vulgaris* for their calculations (Pacovsky *et al.*, 1984).

Especially the data sets for RBS 15 reveal a different relationship when compared to those of the other three bean cultivars (Fig. 2): low data for the acetylene reducing activity correspond to values of total nitrogen in the plant that are remarkably higher than in the wild type Rico. The physiological reasons for this relative high N-accumulation rates and comparatively low values for the acetylene reducing activity in the mutant remain unclear; they might be discussed as a consequence of low carbon fixation, resulting from lower nutrient

Table II. Analysis of variance (ANOVA) for the data of the specific nitrogenase activity of symbiotically grown *P. vulgaris* and of the dry weight of nodules per plant.

Source	DF	SQ	MQ	F-Value	*p*-Value
Specific nitrogenase activity					
Plant (A)	3	831,066.4	277,022.1	89.9	0.0001
Bacterium (B)	8	1,002,079.9	125,260.0	40.6	0.0001
A × B	24	428,263.0	17,844.3	5.8	0.0001
Error	300	924,996.5	3,083.2		
Dry weight of nodules					
Plant (A)	3	0.92	0.031	106.5	0.0001
Bacterium (B)	8	0.019	0.002	8.4	0.0001
A × B	24	0.018	0.001	2.6	0.0001
Error	300	0.086	2.877×10^{-4}		

acquisition, due to a heavily affected root development (see below).

The data obtained for uninoculated plants grown under conditions with high N-supply indicate a general different growth behavior of the supernodulating type compared to the wild type. Even under these conditions, the mutant's growth rates kept far behind OAC Rico (Fig. 4). Simultaneously, the shoot/root ratios of the mutant were smaller than those for the wild type (Table I), indicating a generally altered nutrient distribution between the two plant organs. These findings are in contrast to the results of Buttery and Park (1990), who did not find altered growth characteristics between RBS 15 and OAC Rico in uninoculated plants grown with combined N. Our results indicate the possibility, that other genetical traits than only those concerning feedback regulation of nodule number may have been affected by mutagenesis and that it is not only supernodulation and its immediate consequences that reduce plant growth in symbiotically grown plants. Similar observations in other supernodulating types suggest the production of near-isogenic supernodulating lines by backcrossing (Micke, 1993).

When plants were inoculated and grown under minus N-conditions, the values for the shoot/root ratios augmented heavily in the mutant compared to the wild type (Table I). Obviously the mutant's root was more severely affected by the increased number of nodules and the resulting increased physiological demand than the wild type's. The higher shoot/root ratios of the mutant indicate an explanation for the delayed development of the mutant compared to the wild type under full symbiotical growth conditions: the smaller root may not be sufficient for the nutrient acquisition of the plants. Contrasting with the findings for the wild type, the nodule numbers found after inoculation with different rhizobial strains varied considerably on the supernodulating type (Fig. 3). This demonstrates an interaction between supernodulation and specific rhizobial strains.

The results of the ANOVA for the data of the specific nitrogenase activity and the nodule mass per plant (Table II) over all combinations confirm the necessity of screening for the most suitable symbiotic partners in each individual case. This is consistent with other data concerning the effectivity of legume root nodules (Chaverra and Graham, 1992; Mytton, 1975).

An unexpected result derived from the comparison between CIAT 899 and UMR 1899. The data obtained from the acetylene reducing assays (Fig. 1), and those obtained for the relative N-accumulation rates (Fig. 5), revealed different results for the two strains, which theoretically are identical (Aarons and Graham, 1991). It has been previously assumed that different storage conditions of strains might lead to mutational changes (Wood and Cooper, 1988). At least one of the strains may have experienced such changes during the cultivation in different laboratories.

The use of data for the total N-content of plants grown under conditions of high N-supply as reference point for the respective data of exclusively symbiotically grown plants led to the expression of relative N-accumulation rates. The observed

relative N-accumulation indicate high variation between different combinations with maximum rates up to 70%. This value is especially high compared with results of other authors, who found maximum rates of 39% with other bean genotypes in a similar experiment (Piha and Munns, 1987b). There are other data, indicating that several varieties of *P. vulgaris* can match their genetical N-demand at later stages of symbiotic development, when inoculated with suitable rhizobial strains (Graham and Halliday, 1977; Piha and Munns, 1987a). The high values of the nitrogenase activity per nodule dry weight and the relative N-accumulation rate during an early stage of symbiotic development shown by a few of the combinations in our study can be regarded as a prerequisite for symbioses that could match the full N-demand of *P. vulgaris* at least at later stages of development. According to these data one plant cultivar (*cv.* Brilliant) and two of the bacterial strains (Ph 24 and Ph 6) with general low symbiotic performance could be preselected.

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Aarons S. R. and Graham P. H. (1991), Response of *Rhizobium leguminosarum* bv. *phaseoli* to acidity. Plant Soil **134,** 145–151.

Bliss F. A. (1993), Breeding common bean for improved biological nitrogen fixation. Plant Soil **152,** 71–79.

Buttery B. R. and Park S. J. (1990), Effects of nitrogen, inoculation and grafting on expression of supernodulating in a mutant of *Phaseolus vulgaris* L. Can. J. Plant Sci. **70,** 375–381.

Carroll B. J., Hansen A. P., McNeil D. L. and Gresshoff P. M. (1987), Effect of oxygen supply on nitrogenase activity of nitrate- and dark-stressed soybean (*Glycine max.* [L.] Merr.) plants. Aust. J. Plant Physiol. **14,** 679–687.

Chaverra M. H. and Graham P. H. (1992), Cultivar variation in traits affecting early nodulation of common bean. Crop Sci. **32,** 1432–1436.

Cresswell A., Gordon A. J. and Mytton L. R. (1992), The physiology and biochemistry of cultivar-strain interactions in the white clover-*Rhizobium* symbiosis. Plant Soil **139,** 47–57.

George T. and Singleton P. W. (1992), Nitrogen assimilation traits and dinitrogen fixation in soybean and common bean. Agron. J. **84,** 1020–1028.

Graham P. H. and Halliday J. (1977), Inoculation and nitrogen fixation in the genus *Phaseolus.* In: Exploiting the Legume-*Rhizobium* Symbiosis in Tropical Agriculture (J. M. Vincent, A. S. Whitney and J. Bose, eds.), pp. 313–334, University of Hawaii.

Hansen A. P., Pate J. S. and Atkins C. A. (1987), Relationships between acetylene reduction, hydrogen evolution and nitrogen fixation in nodules of *Acacia* spp.: experimental background to assaying fixation by acetylene reduction under field conditions. J. Exp. Bot. **38,** 1–12.

Hardarson G., Bliss F. A., Cigales-Rivero M. R., Henson R. A., Kipe-Nolt J. A., Longeri L., Manrique A., Pena-Cabriales J. J., Pereira P. A. A., Sanabria C. A. and Tsai S. M. (1993), Genotypic variation in biological nitrogen fixation by common bean. Plant Soil **152,** 59–70.

Jordan D. C. (1984), Family III Rhizobiaceae Conn. In: Bergey's Manual of Systematic Bacteriology (N. R. Krieg and J. G. Holt, eds.), **Vol. 1,** pp. 234–256, Williams and Wilkins, Baltimore, London.

Karanja N. K. and Wood M. (1988a), Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Infectiveness and tolerance of acidity and aluminium. Plant Soil **112,** 7–13.

Karanja N. K. and Wood M. (1988b), Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Tolerance of high temperature and antibiotic resistance. Plant Soil **112,** 15–22.

Micke A. (1993), Mutation breeding of grain legumes. Plant Soil **152,** 81–85.

Minchin F. R., Sheehy J. E. and Witty J. F. (1986), Further errors in the acetylene reduction assay: Effects of plant disturbance. J. Exp. Bot. **37,** 1581–1591.

Minchin F. R., Witty J. F., Sheehy J. E. and Müller M. (1983), A major error in the acetylene reduction assay: Decreases in nodular nitrogenase activity under assay conditions. J. Exp. Bot. **34,** 641–649.

Mytton L. R. (1975), Plant genotype × *Rhizobium* interactions in white clover. Ann. Appl. Biol. **80,** 103–107.

Pacovsky R. S., Bayne H. G. and Bethlenfalvay G. J. (1984), Symbiotic interactions between strains of *Rhizobium phaseoli* and cultivars of *Phaseolus vulgaris* L. Crop Sci. **24,** 101–105.

Park S. J. and Buttery B. R. (1989), Identification and characterization of common bean (*Phaseolus vulgaris* L.) lines well nodulated in the presence of high nitrate. Plant Soil **119,** 237–244.

Pereira P. A. A., Miranda B. D., Attewell J. R., Kmiecik K. A. and Bliss F. (1993), Selection for increased nodule number in common bean (*Phaseolus vulgaris* L.). Plant Soil **148,** 203–209.

Peterson L. A. and Chesters G. (1964), A reliable total nitrogen determination on plant tissue accumulating nitrate nitrogen. Agron. J. **56,** 89–90.

Piha M. I. and Munns D. N. (1987a), Nitrogen fixation capacity of field-grown bean compared to other grain legumes. Agron. J. **79,** 690–696.

Piha M. I. and Munns D. N. (1987b), Nitrogen fixation potential of beans (*Phaseolus vulgaris* L.) compared with other grain legumes under controlled conditions. Plant Soil **98,** 169–182.

Rennie R. J. and Kemp G. A. (1983a), N_2 fixation in field beans quantified by ^{15}N isotope dilution. I. Effect of strains of *Rhizobium phaseoli.* Agron. J. **75,** 640–644.

Rennie R. J. and Kemp G. A. (1983b), N_2 fixation in field beans quantified by ^{15}N isotope dilution. II. Effect of cultivars of beans. Agron. J. **75,** 645–649.

Wood M. and Cooper J. E. (1988), Acidity, aluminium and multiplication of *Rhizobium trifolii:* Effects of temperature and carbon source. Soil Biol. Biochem. **20,** 89–93.