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Improving N_2-fixation in faba bean: *Rhizobium* inoculation and N nutrition

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SUMMARY - Efforts to increase yield and yield stability in faba bean should complement work to improve the sustainability of soil fertility in cropping systems. Optimizing dinitrogen fixation in the crop provides the opportunity to increase nitrogen inputs into systems which include this important legume. Efforts to increase the proportion of plant nitrogen derived from the faba bean-*Rhizobium* symbiosis must take into account effects of both host and microsymbiont, and their interaction. Breeding and selection of genotypes for increased N_2 fixation has been explored to only a limited extent, but holds considerable promise for decreasing utilization of soil N by the plant. In addition, increases in total crop N and yield through inoculation with superior selected *Rhizobium* strains in field studies indicate that N_2 fixation may be improved by manipulating the symbiosis through the rhizobia component. The merits and potential constraints of these two approaches, and possible combinations, are discussed.

RESUME - "Amélioration de la fixation de N_2 chez la fève: inoculation de Rhizobium *et nutrition azotée". Les efforts pour augmenter le rendement et sa stabilité dans la culture des fèves devraient complémenter les études qui visent à améliorer la fertilité durable du sol dans les systèmes agricoles. L'optimisation de la fixation de la molécule d'azote par la culture offre la possibilité d'augmenter les apports d'azote dans les systèmes où cette légumineuse est cultivée. Les efforts pour accroître la proportion d'azote végétal provenant de la symbiose fève-*Rhizobium *doivent tenir compte des effets de l'hôte, du microsymbiont et de leur interaction. L'amélioration et la sélection de génotypes pour une augmentation de la fixation de N_2 n'ont été étudiées que de façon limitée mais semblent prometteuses en vue d'une diminution de l'utilisation de l'azote du sol par la plante. En plus, l'augmentation de l'azote total de la culture et du rendement par inoculation de souches de* Rhizobium *améliorées dans les parcelles d'expérimentation montre qu'on peut améliorer la fixation de N_2 en manipulant la symbiose à travers les composants des rhizobia. Les avantages et les limitations potentielles de ces deux approches et leurs combinaisons possibles sont examinées.*

Introduction

Faba bean (*Vicia faba* L.) is generally grown in high rainfall or irrigated areas of the Mediterranean region, often in rotation with wheat. Efforts to increase yield and yield stability of faba bean should complement work to improve the sustainability of cropping systems, particularly in terms of soil fertility. The ability of the crop to fix atmospheric dinitrogen, as well as to assimilate soil N, provides the opportunity to balance nitrogen exports in grain with input from the atmosphere.

In order to maximize N_2 fixation in the *Vicia faba* legume-rhizobia symbiosis, both host and microsymbiont must be considered. The plant is commonly manipulated through breeding, and past successes of plant breeding imply that established methods can be used to increase the yield of biologically fixed nitrogen. However, manipulation of genetic patterns in the legume cannot be separated from the role played by *Rhizobium* genotypes. Three genetic components of variation in N_2 fixation must be considered: (a) general effects of host genotype, (b) general effects of *Rhizobium* strain, and (c) specific effects of host x strain interactions (Mytton *et al.*, 1977). Most soils where faba bean is grown contain high (10^2-10^4/g) numbers of rhizobia (Amarger, 1988), which vary considerably in their symbiotic capability (Sorwli and Mytton, 1986).

Manipulation of host legume

Two general theories have been proposed to improve the N_2-fixing performance in faba bean (Mytton, 1984, 1988). The first, to improve general symbiotic competence, should be applicable to both plant and *Rhizobium* genotype, but the most practical target may be the plant. Methods for establishing and maintaining new varieties as components of agricultural systems are known and successful; this is not true for *Rhizobium* populations in soil, especially where a diverse native population must be displaced.

The frequent correlation between the quantities of N_2 fixed and total N yield in the crop (Duc, 1986; Duc *et al.*, 1988; Fried *et al.*, 1983) indicates that limitations in dinitrogen fixation may be a function of photosynthate availability and/or translocation, or of interactions between fixed and soil nitrogen. Where there is no limit in the general efficiency of the native rhizobial population (Amarger, 1988), breeding for good cultivar x strain interactions will probably not be effective. In this case, plant selection for nitrogen yield in varying locations with moderate-low soil N should be a successful methodology for incorporating improved N_2 fixing ability.

Where rhizobial populations vary in effectiveness with location (Sorwli and Mytton, 1986), development of a broad-spectrum effectiveness would necessitate screening plants against a range of rhizobia, but definition of a representative rhizobial population may prove a problem. Mytton *et al.* (1977) adapted a method of joint regression analysis commonly used to interpret genotype-environment interaction in breeding programs. This allowed some interpretation of host-*Rhizobium* interactions and identified plant genotypes which had superior symbiotic capability with a limited range of *Rhizobium* strains.

In more recent investigations with faba bean, a low level ^{15}N-tracer technique was used to evaluate genetic variability of 10 locally-adapted cultivars with very diverse geographic origins for dinitrogen fixation in two different soils (Duc *et al.*, 1988). All genotypes were well nodulated by the indigenous *Rhizobium* populations, which were abundant in the two soils. The performance of cultivars varied widely, with percentages of plant nitrogen derived from fixation ranging from 40 to 77% and quantities of N_2 fixed between 16 and 171 kg N/ha (Table 1). These differences probably include effects of plant genotype, environment, the strain(s) of rhizobia selected for the hosts out of the native population and the host-strain interactions.

Mechanisms of host-*Rhizobium* strain selectivity do not necessarily mean selection of the most effective rhizobia available. Unknown, uncontrolled and probably very diverse *Rhizobium* populations could cause difficulties in breeding programs for improved N_2 fixation. A method of countering these variable bacteria population effects is to find plant genes or gene combinations which confer good phenotypic expression of dinitrogen fixation

Table 1. Characteristics of faba bean seed from two field locations, that relate to yield, quantity of biologically fixed dinitrogen and soil derived nitrogen (1982).

Name of genotypes	Location 1						Location 2					
	Protein		$\delta^{15}N$	N source			Protein		$\delta^{15}N$	N source		
	%	t/ha		Fixation		Soil kg/ha	%	t/ha		Fixation		Soil kg/ha
				%	kg/ha					%	kg/ha	
1. 370	30.7	1.07	14.9bc[a]	53	91	80	30.6	0.32	24.3c	53	27	24
2. 40	28.8	0.67	14.7bc	54	58	50	27.6	0.24	31.1d	40	16	22
3. PK	37.4	1.12	9.9ab	68	123	56	34.8	0.39	16.5ab	67	42	21
4. 127	34.4	1.09	7.1a	77	134	40	32.7	0.50	18.8b	63	51	29
5. Ascott	35.0	1.49	13.2bc	59	141	98	35.2	0.66	13.4a	73	77	29
6. 316	30.7	0.59	18.7c	42	40	54	29.0	0.23	24.9c	52	19	18
7. Deiniol	34.8	1.33	10.2ab	68	144	69	31.9	0.64	14.5a	71	72	30
8. 319	32.0	1.13	13.4bc	58	106	75	27.5	0.30	29.9d	42	21	27
9. 196	36.2	1.10	12.2ab	62	109	67	32.1	0.54	13.6a	62	62	24
10. FIAd23 x HG115	31.2	1.50	9.0ab	71	171	69	31.5	0.82	14.8a	71	93	38
Mean	33.1	1.10	12.3	61	112	66	31.3	0.46	20.2	61	48	26
LSD 0.05	3.9	0.14	3.4	11	30	20	1.4	0.10	2.5	5	10	9

[a]Duncan test at 5% level of probability

over a wide range of *Rhizobium* genotypes, or plant genes which will select the more efficient strains. A plant selection or breeding program for increased fixation, with selection in diverse representative agricultural sites, should yield good results if of sufficient duration.

Because dinitrogen fixation interacts with uptake of mineral N by the plant, investigations into the complementarity or concomitant activity of these two pathways and their consequence on yield will provide a plant ideotype for N nutrition, and thereby facilitate breeding for improved N_2 fixation.

Manipulation of rhizobia

The second method of improvement is to select for specific adaptation between host and rhizobia, which generally focusses on evaluation of strains to increase dinitrogen fixation in established cultivars.

Rhizobium populations conform to the expected in that differences in N_2 fixing ability are normally distributed (El Sherbeeny, 1977), and highly effective strains occurring at low frequencies may be at a selective disadvantage. It is partially on this premise that the practice of legume inoculation is based. Inoculation places a large number of pre-selected, highly effective rhizobia in the immediate vicinity of the growing root, so that early nodulation is dominated by the select bacteria. This practice, common in many developed countries, necessitates simultaneous selection of strains with plant genotypes, aimed at producing a co-adapted inoculation package designed for maximum N_2 fixation. A more potentially useful strain screening procedure would select strains highly effective in N_2 fixation over a wide range of plant genotypes.

Using this method at ICARDA, screening of 60 strains collected from widely varying locations against six cultivars has resulted in selection of five strains highly efficient in N_2 fixation with all the cultivars. Dinitrogen fixation was estimated by dry matter and total N accumulation of plants grown under conditions free from combined nitrogen (r = 0.93, p = 0.001 for d.m. and total N). A similar greenhouse factorial pot experiment by Mytton *et al.* (1977), with investigation of the effects of seven *Rhizobium* strains taken from the same location on seven *Vicia faba* cultivars in nitrogen-free conditions, showed that 73.8% of total variation in N_2 fixation was accounted for by host x strain interactions.

Strains selected in this manner were evaluated in a field experiment at ICARDA, with three superior strains used as inoculants on 14 diverse faba bean lines. Cultivars selected for the experiment included traditional landraces (Rebaya 40, BPL 1722), newly developed independent vascular supply (IVS 6) and determinate types (FLIP nos. 84-230, 84-239, 84-241), and commercial small- and large-seeded types (Lebanese local small, 'Giza 4', 'Giza 402', Syrian local large, 'Aquadulce', 'Seville giant', 'Reina blanca' and 'VD Policoro'). Inoculated treatments were compared with both uninoculated and nitrogen-fertilized (120 kg N/ha, application split as 60 kg/ha at planting and 60 kg/ha at mid-anthesis) treatments. The field contained 1.8 x 10^4 native faba bean rhizobia per gram of soil.

Response to inoculation above both uninoculated and N fertilized treatments across all cultivars was significant for total biological yield, grain yield and total nitrogen (Table 2). At the strain-cultivar interaction level large differences appeared. Strain 452, which gave significant

Table 2. Mean yields of 14 faba bean cultivars and 5 inoculation treatments, Tel Hadya 1987/88.

Treatment	Seed yield (t/ha)	Biological yield (t/ha)	% N	Total N (kg/ha)
INOCULATION TREATMENTS				
Uninoculated	2.95	8.01	2.58	206
Strain 420	3.20*	8.31	2.58	215
Strain 452	3.10	8.79*	2.64	231
Strain 481	2.82	7.79	2.57	200
120 kg N/ha	2.92	7.97	2.52	201
LSD .05	0.22	0.55	0.09	
CULTIVARS				
IVS 6	1.78	6.61	2.69	178
Rebaya 40	2.02	5.54	2.68	148
Giza 4	3.19	8.44	2.50	211
BPL 1722	2.88	7.12	2.39	170
Lebanese l.sm.	3.38	8.57	2.44	210
Giza 402	2.36	6.51	2.69	175
FLIP 84-241	2.56	7.88	2.49	196
FLIP 84-230	1.98	6.71	2.77	185
FLIP 84-239	2.69	8.74	2.51	219
Seville Giant	3.88	9.73	2.53	247
Syrian local lg.	4.10	11.26	2.66	300
Aquadulce	3.88	9.89	2.58	256
Reina Blanca	3.68	8.81	2.61	229
VD Policoro	3.55	8.64	2.57	222
LSD .05	0.36	0.75	0.15	
Overall mean	3.00	8.17	2.58	210
F TEST				
Strain	*	*	*	*
Cultivar	**	**	**	**
Interaction	**	**	*	**

*Significant at p = 0.05
**Significant at p = 0.01

yield increases for biological and N yields across the 14 cultivars, gave as much as 82 kg N/ha increase and 1.4 t/ha grain yield increase over uninoculated treatments (Fig. 1 and 2). This strain also, however, decreased N yield by 31 kg/ha and grain yield by 0.4 t/ha in cultivar 'VD Policoro' (Fig. 1 and 2). Other strains increased or decreased yields to a generally lesser extent. The greatest response to inoculation occurred in commercial, high-yielding cultivars, while yields of landraces and less-adapted lines changed little with treatments.

Nitrogen fertilization generally decreased yields in this experiment, while nearly all cultivars tested responded positively to at least one of the inoculant strains. Though the former implies that nitrogen is not limiting for yield in faba bean, it may be that the N application method with 60 kg N/ha at planting and 60 kg/ha at mid-anthesis could not fulfill N requirements for the crop, or that 120 kg N/ha plus soil N was not enough to provide for the nitrogen requirement of the crop, and fertilization may have decreased total N through interference with early fixation.

Response to selected strains suggests that N nutrition may be improved by manipulating the symbiosis through inoculation. Inoculation of three cultivars with a commercial multi-strain (Nitragin Co.) inoculant in Canada also increased grain yields in field experiments from 19 to 67%, in soils containing high populations of native faba bean rhizobia (Rennie and Dubetz, 1986). However, response to inoculation will depend to a large extent on effectiveness of the native rhizobial populations, and in some situations no response to selected strains will be observed (Amarger, 1988).

Measurement of N_2 fixation and N balance

Quantities of N_2 fixed in faba bean vary greatly, but it is generally agreed that of the grain legumes *Vicia faba* is among the highest in total N_2 fixed. Estimates of rates of fixation vary from 40 (Duc *et al.*, 1988) to 93% (Brun-

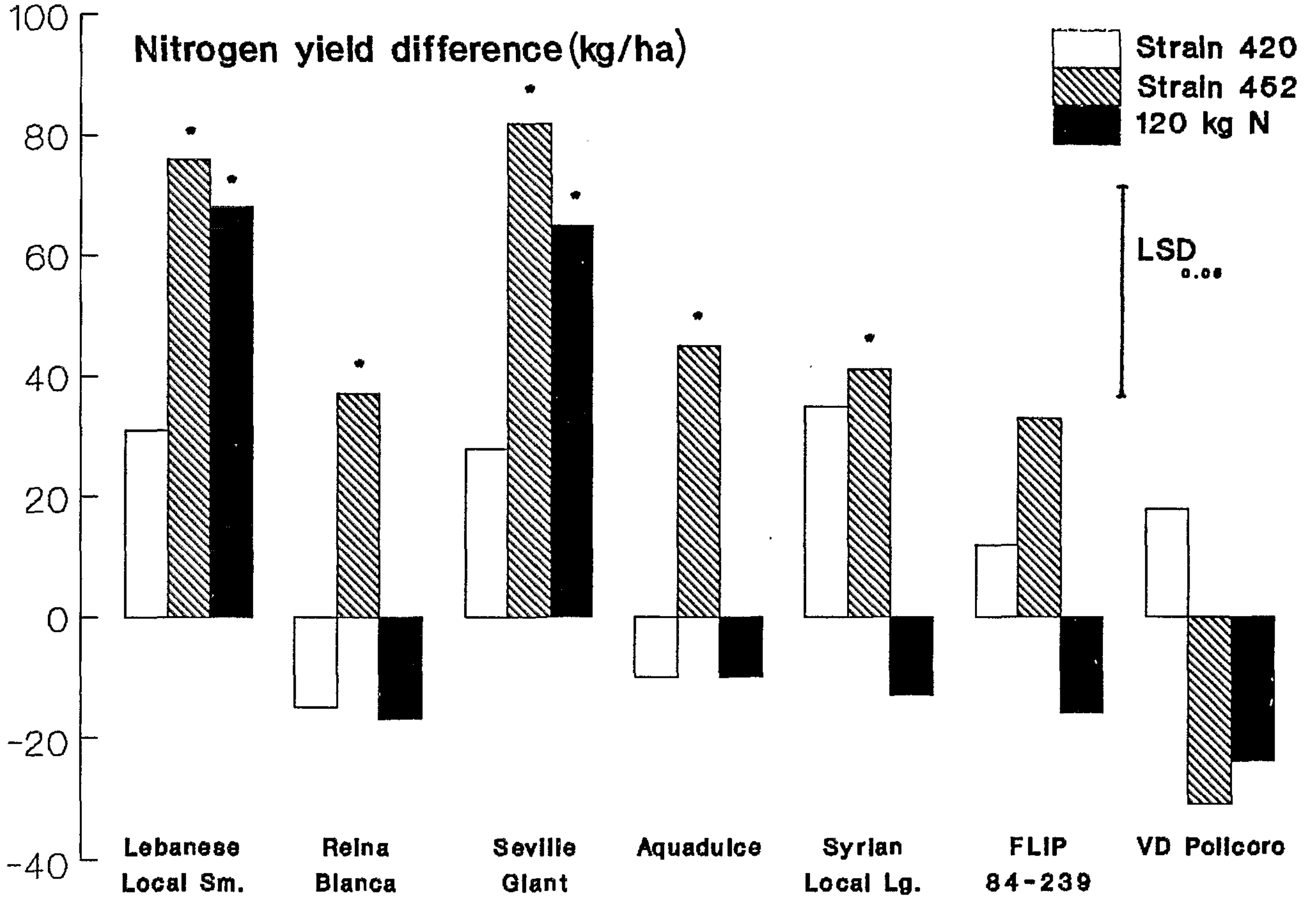

Fig. 1. Nitrogen yield difference from uninoculated control due to inoculation and nitrogen treatments in seven faba bean cultivars. Tel Hadya 1987-88.

ner and Zapata, 1984; Keatinge *et al.*, 1987) of crop N, and from 16 to 300 kg aboveground N per ha per crop.

The most pertinent, easily measurable parameter to determine effect of manipulation on the symbiosis, through breeding or inoculation, is N yield. Highly significant positive correlation has been found between total seed N (kg/ha) and quantity of fixed N in faba bean seed (Duc *et al.*, 1988), using a low level ^{15}N-tracer technique. However in using N yield to infer fixation rates, high levels of soil N, or differential ability of cultivars to obtain soil N may mask nitrogen fixing ability.

As illustration, experiments using ^{15}N to measure N_2 fixed in chickpea showed that inoculation significantly increased total crop N as well as N fixed in cultivar 'ILC 482' (Fig. 3). However, in line FLIP 83-98, neither total N nor yield were increased by inoculation, but the proportion of crop N derived from fixation was increased from 32 to 80%. In a crop rotation sequence, this would represent a nitrogen input into soil of more than 40 kg N/ha from inoculation. Though yield of the legume crop in this case was unaffected, an effect on the following cereal crop is possible. This phenomenon is currently under investigation in faba bean.

Clearly, the most accurate methodology to measure N_2 fixation is the ^{15}N dilution technique, though for breeding or general selection work it may be too expensive. Use of a low level of isotope enrichment of the soil has been demonstrated to be effective (Duc *et al.*, 1988), saving ^{15}N fertilizer costs, but the cost of sample analyses remains high. Choice of a reference plant may also affect determination of N_2 fixed, though investigators agree that because of their parallel growth and N uptake patterns, cereals such as oats (Duc *et al.*, 1988), barley (Rennie and Dubetz, 1986; Beck and Wery, 1989) and wheat (Brunner and Zapata, 1987; Smith *et al.*, 1987) are adequate reference crops for faba bean. Ideally, work to characterize the genetics of N_2 fixation in *Vicia faba* will lead to development of a non-nodulating line which could be used as a non-fixing reference crop to improve accuracy of ^{15}N methodology and allow greater use of the N difference method.

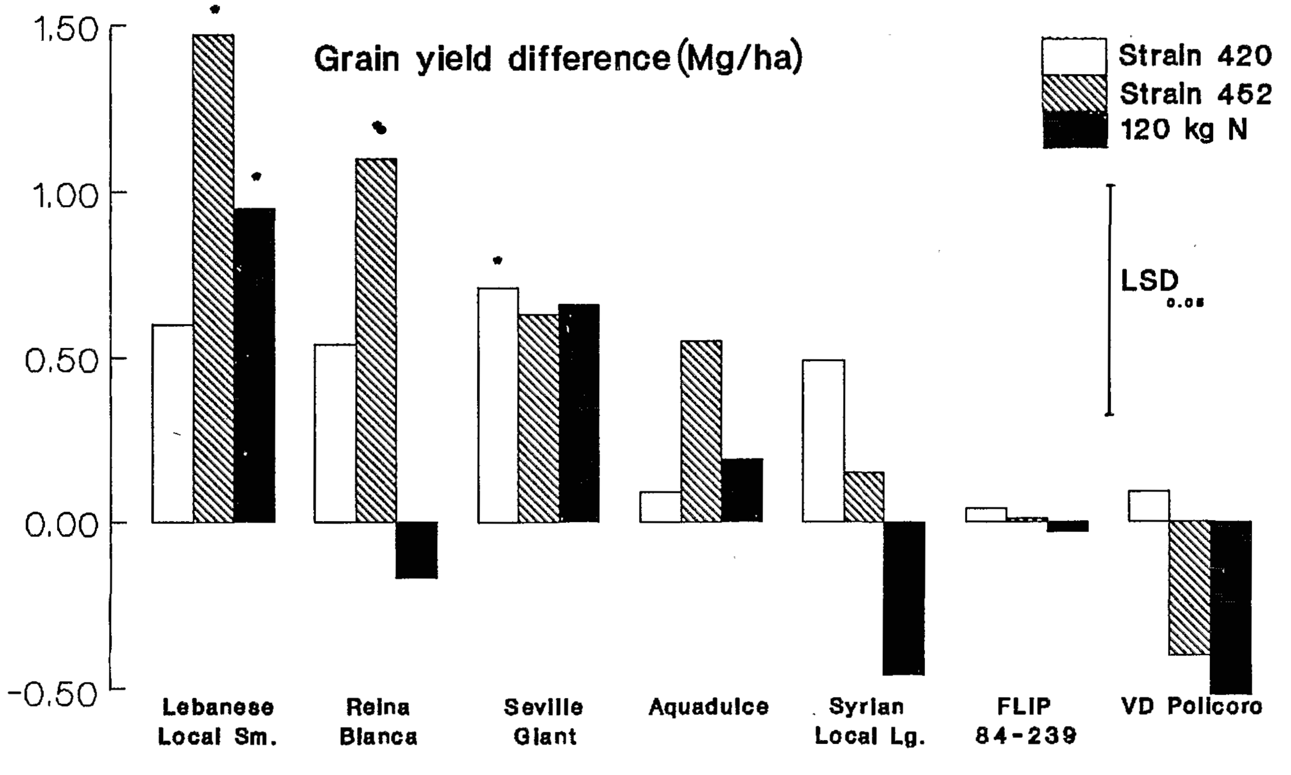

Fig. 2. Grain yield differences from uninoculated control due to inoculation and nitrogen treatments in seven faba bean cultivars. Tel Hadya 1987-88.

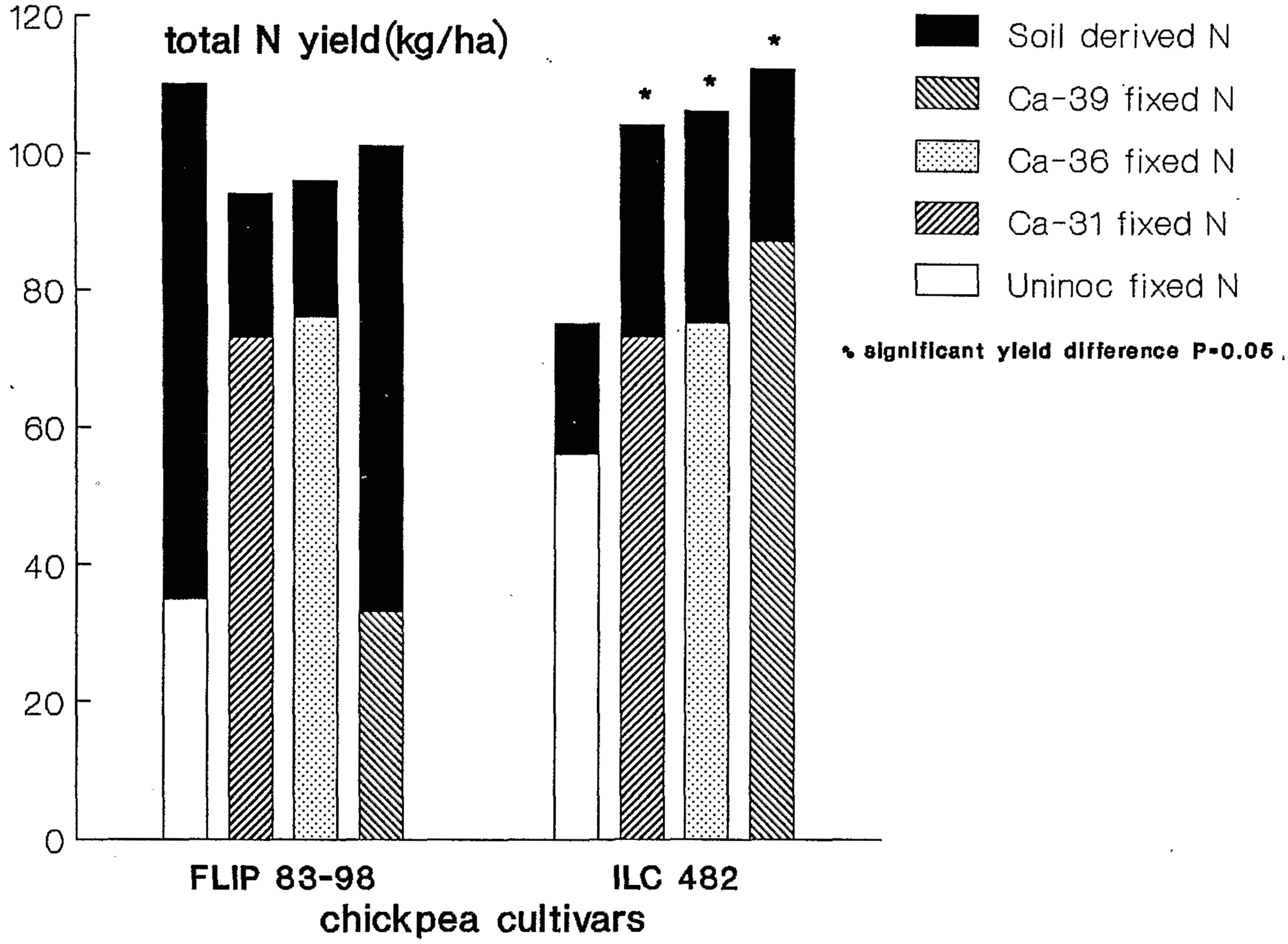

Fig. 3. Effect of *Rhizobium* strain treatment on plant nitrogen source in two chickpea cultivars, as determined by ^{15}N dilution technique. Tel Hadya, 1987-88.

Conclusion

The strength of current breeding programs for faba bean in the region favors this discipline in the long term to improve nitrogen inputs into farming systems through increased dinitrogen fixation. These programs have realized considerable success in improving the genetic yield potential of the crop (ICARDA, 1987, 1988), and recent data indicate that these improved cultivars show considerable scope for increased dinitrogen fixation. It is important that existing breeding efforts incorporate selection for increased N_2 fixation, taking into account the importance of cultivar x strain interactions. This might be best accomplished by early screening against a wide variety of *Rhizobium* strains under low N conditions for high total N production, or multilocation field screening of cultivars for high fixation using ^{15}N dilution methodology.

The large potential N yield response to inoculation in some situations makes this an attractive rapid method to increase nitrogen fixed in faba bean. A comprehensive strain selection program is, however, essential. Selected strains should be highly effective on all cultivars, competitive for nodulation with native strains, and able to survive in the soil through crop rotations. Also, when considering inoculation as a strategy to increase BNF in faba bean, it is important to understand the sensitivity of the technology. Appropriate inoculants are not difficult to produce, but thorough backstopping in quality control, distribution and extension education are essential to ensure that the inoculant produced is viable in the farmer's field.

References

AMARGER, N. (1988): Microbial aspects of faba bean culture. *In* Nitrogen Fixation by Legumes in Mediterranean Agriculture (Beck, D.P. and Materon, L.A., eds.). Martinus Nijhoff, The Hague, The Netherlands.

BRUNNER, H. and ZAPATA, F. (1984): Quantitative assessment of symbiotic nitrogen fixation in diverse mutant lines of field bean (*Vicia faba minor*). Plant and Soil *82*:407-413.

DUC, G. (1986): Search of genetic variability for nitrogen nutrition in *Vicia faba* L. Vortr. Pflanzenzüchtung *11*:158-162.

DUC, G., MARIOTTI, A. and AMARGER, N. (1988): Measurement of genetic variability for symbiotic dinitrogen fixation in field- grown faba bean (*Vicia faba* L.) using a low level ^{15}N-tracer technique. Plant and Soil *106*:269-276.

EL-SHERBEENY, M.H. (1977): Symbiotic variation in *Vicia faba* L. Ph.D. Thesis, University of Wales, Aberystwyth, U.K.

FRIED, M., DANSO, S.K.A. and ZAPATA, F. (1983): The methodology of measurement of N_2 fixation by non-legumes as inferred from field experiments with legumes. Can. J. Microb. *29*:1053-1062.

ICARDA (1987): Food Legume Improvement Program. Annual Report. ICARDA, Aleppo, Syria.

ICARDA (1988): Food Legume Improvement Program. Annual Report. ICARDA, Aleppo, Syria.

KEATINGE, J.D.H., CHAPANIAN, N. and SAXENA, M.C. (1988): Effect of improved management of legumes in a legume-cereal rotation on field estimates of crop nitrogen uptake and symbiotic nitrogen fixation in Northern Syria. J. Agric. Sci. Camb. *110*:651-659.

MYTTON, L.R. (1984): Developing a strategy to exploit quantitative variation in symbiotic nitrogen fixation. Plant and Soil *82*:329-335.

MYTTON, L.R. (1988): Host-*Rhizobium* relationships and their implications for legume breeding. *In* Nitrogen Fixation by Legumes in Mediterranean Agriculture (Beck, D.P. and Materon, L.A., eds.). Martinus Nijhoff, The Hague, The Netherlands.

MYTTON, L.R., EL-SHERBEENY, M.H. and LAWES, D.A. (1977): Symbiotic variability in *Vicia faba*. 3. Genetic effects of host plant, *Rhizobium* strain and of host x strain interaction. Euphytica *26*:785-791.

RENNIE, R.J. and DUBETZ, S. (1986): Nitrogen-15-determined nitrogen fixation in field-grown chickpea, lentil, faba bean and field pea. Agronomy J. *78*:654-660.

SMITH, S.C., BEZDICEK, D.F., TURCO, R.F. and CHENG, H.H. (1987): Seasonal N_2 fixation by cool-season pulses based on several ^{15}N methods. Plant and Soil *97*:3-13.

SORWLI, F.K. and MYTTON, L.R. (1986): Nitrogen fixing potential of *Vicia faba* rhizobia from different agricultural locations. Plant and Soil *92*:249-254.