IMPROVEMENT OF THE NITROGEN FIXATION POTENCIAL OF COMMON BEAN IN LATIN AMERICA[1]

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SUMMARY

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legume crops in Latin America. Beans are a component of the traditional diet and an important source of protein, however limited availability allows only about one-half of the desirable per capita consumption. Often beans are produced in areas with infertile soils. Nitrogen and phosphorus are especially low, and where fertilizer is too expensive or in limited supply, this contributes to low yields. Increasing the N_2-fixation ability of improved cultivars to satisfy the nitrogen requirements provides an attractive method for increasing productivity, particularly when combined with disease resistance and stress tolerance.

Measurements of N fixed and various parameters of N_2 fixation have indicated that there is substantial genetic variability among cultivated germplasm, and suggest that improvement through selection is possible. The application of indirect measurements of N_2 fixation will continue to be important for breeding programs in Latin America. Direct measurements of N_2 fixation by ^{15}N isotope techniques are useful to estimate the level of improvement, and to develop improved selection criteria for the indirect estimation of N_2 fixation. However,

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isotope, techniques are too expensive to be used in small breeding programs. The inbred backcross line method of population development has proved to be very efficient introducing high N_2 fixation potential from exotic germplasm into desirable commercial bean types. It is recommended as a breeding method for the development of superior cultivars which are readily accepted by farmers and consumers.

RESUMEN

El frijol común (*Phaseolus vulgaris* L.) es una de las leguminosas de grano más importantes en América Latina. El frijol es un componente de la dieta tradicional y una fuente importante de proteínas. Sin embargo, las limitaciones en su abastecimiento solo permiten satisfacer cerca de la mitad del consumo per cápita deseado. Frecuentemente el frijol se cultiva en suelos infértiles, especialmente bajos en nitrógeno y fósforo, lo que en lugares donde el fertilizante es costoso o difícil de obtener contribuye a bajos rendimientos. Incrementando la habilidad de fijación de N_2 de cultivares mejorados se provee un método atractivo para aumentar la productividad del frijol, particularmente cuando se combina con resistencia a enfermedades y tolerancia a estrés.

Las medidas del N fijado y varios parámetros de la fijación de N_2 han indicado que existe una variabilidad genética substancial en el germoplasma cultivado, y sugiere que el mejoramiento a través de selección es posible. La aplicación de medidas indirectas de la fijación de N_2 continuará siendo importante para los programas de mejoramiento en América Latina. Las medidas directas de la fijación de N_2 mediante las técnicas de isótopos de ^{15}N son útiles para estimar el nivel de mejoramiento alcanzado, y en desarrollar criterios de selección para la estimación indirecta de la fijación de N_2. Sin embargo, las técnicas que usan isótopos son muy costosas para ser usadas en programas de mejoramiento pequeños.

El método lineal de retrocruza y autofecundación para desarrollar poblaciones ha probado ser muy eficiente en introducir un alto potencial de fijación de N_2 proveniente de germoplasma exótico en genotipos de frijol comercial deseables. Se recomienda como un método de mejoramiento para el desarrollo de cultivares superiores los cuales pueden ser inmediatamente aceptados por agricultores y consumidores.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important source of protein and calories in traditional diets of tropical and sub-tropical countries in Latin America. However, limited bean availability allows only about one-half of the desirable per-capita consumption in the Central American region (2,4).

Beans are grown in highly diversified cropping systems and often in low fertility soils, where nitrogen (N) and phosphorus are especially low (7,9,10,11). Infertile soils along with fertilizers which are expensive or in limited supply are contributing to low yields (10). Average yield for the Central American region for the 1971-80 decade was only 656 kg/ha, according to FAO estimates. In Honduras, about 58o/o of the beans are produced on farms smaller than 10 hectares (8); only about 13o/o of these farms are estimated to use some fertilizer (27).

Increasing the N_2 fixation ability of improved cultivars to satisfy their N requirements provides an attractive approach for raising bean productivity, particularly when combined with disease resistance and stress tolerance. Several reports have indicated that common bean plants can fix levels of nitrogen comparable to other grain legumes (10,23). This conflicts with previous information which suggested that beans were inferior in the ability to establish an efficient symbiosis and fix N_2 (13,29). Poor N_2-fixing cultivars and/or *Rhizobium phaseoli* strains along with adverse environmental conditions and poor procedures for estimating the amount of N_2 fixed in the field can account for the poor performance of beans reported earlier (10,21). The high levels of fixation obtained by some poorly adapted cultivars suggest the possibility of improving N_2 fixation by breeding improved cultivars that can support better N_2 fixation with either native rhizobia or superior inoculants (3,11,22,23,26). We are presenting in this paper some information on the performance of superior plants and methods used to select them. These resources are being utilized to select for enhanced N_2 fixation potential in common beans for Brazil and Honduras, and can be used in other Latin American countries as well.

BREEDING OBJECTIVES

The breeding objectives of these experiments are to identify common bean genotypes with enhanced N_2 fixation potential for further use either as cultivars or breeding parents, and to select superior recombinant genotypes for their increased N_2 fixation potential in combination with other desirable traits using efficient breeding methods (3). The criterion to select improved lines in these experiments was acetylene reduction (AR) activity (μM C_2H_2/plant/hr) on field-grown plants, an indirect measure of N_2 fixation. Gain from selection for the amount of N_2 fixed per plant, o/o of total N derived from fixation, and seed production resulting from fixation was estimated in a subsequent study using the ^{15}N-depleted $(NH_4)_2$ SO_4 method (DuBois et al., unpublished), the comparisons being to the standard cultivar used as the recurrent parent in each of two populations.

MEASUREMENT OF N_2 FIXATION IN THE FIELD

The accurate estimation of the amount of N_2 fixed by field-grown plants represents a major problem since these plants can also make use of N available from the soil and/or added fertilizer. Direct estimates of the total amount of N_2 fixed per plant and seed yield due to fixed N can be obtained by growing plants under controlled conditions on N-free media where the only source of N is from active fixation by nodulated plants (3,16,21); however, such conditions are very different from those found in actual bean production and usually lead to poor repeatibility of results in the field (16).

Moreover, for effective selection, large breeding populations must be grown under field conditions and the contributions of soil N and/or added fertilizer N must be determined. Accurate estimates of N_2 fixed by field-grown plants can be obtained using ^{15}N isotope methods (22,23,26,31). However, these methods often are outside the range of capability for most breeding programs because they are too expansive for a large number of estimates required, and the necessary equipment and expertise may be unavailable in modestly-supported programs. Never-the-less, the method can be applied on a limited scale to estimate the amount of improvement, and to develop better selection criteria for the indirect estimation of N_2 fixation.

Because of the restrictions of the direct methods, estimation of N_2 fixation and related traits by indirect methods is extremely important for host plant improvement in Latin America; but they must be accurate and should reflect actual amounts of N_2 fixed. Estimates of nodule mass by nodule number and weight, and visual rating, acetylene reduction, and plant growth (dry matter accumulation) and/or seed yield under low N soil conditions along with the use of non-fixing plants (non-nodulating lines and/or cereals) as checks for estimating the effects of soil N or added fertilizer N, are some of the indirect measurements available (14,15,24,25,28). Inadequate experimental procedures for estimating N_2 fixation, quantitative expression of traits that are affected greatly by the environment, and different patterns of plant growth and development are some of the factors affecting efficiency of the indirect methods.

IMPROVEMENT OF N_2 FIXATION IN COMMON BEANS

DEVELOPMENT OF BREEDING POPULATIONS

Genetically variable populations were produced by crossing four recurrent parents which were the well-adapted, commercially grown cultivars 'Sanilac', 'Porrillo Sintetico', 'Jamapa' and 'Ex Rico 23', with a high-fixing, high yielding cultivar 'Puebla 152', which served as donor parent in each cross. A breeding procedure similar to that suggested by Wehrhahn and Allard (30) for identifying major genes that control quantitative expression of complex metric traits was used to produce inbred backcross lines having genetic backgrounds similar to that of the recurrent parent, but which differ *inter se* for a given trait of interest (3,14,15).

The inbred backcross line method involves the following steps, as described by Bliss (3); crossing the recurrent parent with the donor parent to produce an F_1, backcrossing the F_1 to the recurrent parent, repeated backcrossing of a specific number (ca60) of plants to the recurrent parent until the desirable number of backcrosses are obtained, self-fertilizing each backcross to near homozygosity by a system of single seed descent, and multiplying each inbred backcross line to obtain enough seed for replicated trials. The main features of the inbred backcross line method are: 1) backcrossing produces similarities between the lines and the recurrent parent, with the resulting recombinant lines combining improved N_2 fixation with other desirable traits, such as seed and plant type, found in the recurrent parent, 2) since the inbred backcross lines each contain similar genetic backgrounds, genes affecting N_2 fixation and/or other complex traits are identified and quantified more easily, and that 3) the high level homozygosity (BC_2S_3, expected inbreeding coefficient 0.9687) allows extensive replication over time and in different environments. Even more, with replication it is then possible to a) increase the probability of identifying genotypically superior lines accurately, b) evaluate the same genotype for multiple traits expressed at different times during development, and c) use a destructive measurement technique for estimating N_2 fixation and related traits. Finally, near-homozygous lines can be used to substantiate the amount of gain from selection and for further studies of traits affecting N_2 fixation (3,14,15,24).

PERFORMANCE OF SELECTED LINES

Selection experiments were conducted on four populations of inbred backcross lines grown at the Hancock Experiment Station, Wisconsin, on a Plainfield loamy sand soil with low N content. Each population was evaluated using AR values as primary selection criterion; seed yield per plot was the secondary criterion, with plant type, earliness and seed type also being considered (14). The genetic analysis of these populations showed heritable differences for N_2 fixation parameters, traits supporting N_2 fixation, and seed yield. Transgressive segregation was evident and considerable variation for AR values and seed yield were found. These results suggested that the inbred backcross line method was effective in transferring additional variability for quantitative traits into agronomically acceptable cultivars (3,14,15). In a subsequent field study, nine lines from two inbred backcross populations, Population 21 ('Porrillo Sintetico' x 'Puebla 152') and Population 24 ('Sanilac' x 'Puebla 152'), the three parental lines and the non-nodulating soybean line 'Clay' were grown in 1984 at the Hancock, Wisconsin Experiment Station. The amount of N fixed per plant and the proportion of total N derived from fixation were estimated using the ^{15}N-depleted $(NH_4)_2SO_4$ method (DuBois et al., unpublished). In population 24 (Table 1) total N_2 fixed for the parental lines were 8 kg/ha for 'Sanilac' (recurrent parent) and

Table 1.- Seed yield, total N fixed, and accumulation of fixed N in seeds of parents and selected breeding lines. Estimation by ^{15}N labeling. Field grown, Wisconsin, 1984.

Parent or Line	Total N Fixed (kg/ha)	Seed N: Total (mg/pl)	Seed N: o/o DFA[a] (o/o)	o/o of fixed N in seeds (o/o)	Seed yield (kg/ha)
PUEBLA 152	92	1278	57	86	4150
24—17	63	1011	48	85	3355
24—21	23	728	24	78	2035
24—48	23	876	21	87	2495
24—55	21	775	22	84	2470
24—65	30	748	31	85	2755
SANILAC	8	631	12	88	1940

[a] Nitrogen derived from atmosphere by fixation.
From: DuBois et al., unpublished results.

92 kg/ha for 'Puebla 152' (donor parent); the progeny lines derived from the cross between these parents fixed between 21 to 63 kg/ha, an increase of from 3 to 8-fold over 'Sanilac'. Total N content in seeds and percentages of N derived from the atmosphere by fixation were also significantly higher in the progeny lines. Most of the fixed N (78-87o/o) was found to be allocated in seeds of mature plants suggesting that beans are very efficient in utilizing N from fixation for seed production, a conclusion similar to that of Westermann et al. (32). AR values at the R3 (50o/o bloom) stage were correlated positively with the amount of N fixed per plant and the percentage of N derived from fixation, suggesting that the AR assay is a reasonably good method for identifying superior common bean lines with enhanced N_2 fixation, if it is used properly (14,15).

THE INTERACTION WITH *R. phaseoli* STRAINS

Several studies have suggested that breeding would be more effective if strain selection were an integral part of the host breeding program (1,6,11,16,17). At present there appears to be two methods of doing this. The first is to produce a host plant *Rhizobium* superior combination by simultaneous selection of both symbionts (17). In this case, it is expected that the host plant will influence which strain in a mixture forms nodules, since this specificity in nodulation is broadly heritable and can be enhanced by plant selection (1,5,6,12). The second method of determining the effects of variable *Rhizobium* populations is to find genes or gene combinations which confer good phenotypic expression of N_2 fixation over a wide range of rhizobia strains (11,17). Few attempts have been made to substantiate this alternative, but these have shown a potential for generalisable effects (1,19,20,28). However, results of Mytton et al. (18) indicated low heritability of general effectiveness in lucerne with interactions effects being large enough to mask general genetic differences.

Table 2.- Symbiotic effectiveness of bean genotype/rhizobia strain combinations grown on 2 bean genotypes. Brazil, field 1985.

	UW 22-34		Rio Tibagi	
Treatment	ARA[a]	Nodule dry wt. (mg)	ARA	Nodule dry wt. (mg)
—N, —Inoculant	1.34	125	.51	66
+ N, —Inoculant	.45	18	.30	11
Strain 1	2.51	120	.37	55
Strain 2	.63	37	.35	54
Strain 3	1.47	204	.51	95
$\bar{X}$ 3 Strains	1.54	120	.41	68

[a] Acetylene reduction activity ($\mu M\ C_2H_2$/plant/hr).
From: Dazzo et al., unpublished results.

In common beans there is insufficient information to support the advantages of using each approach. Never-the-less, as an illustration of host superiority across different strains we are presenting some preliminary results obtained in Brazil (Dazzo et al., unpublished results) (Table 2). Lower nodule dry weight and AR activity was recorded by the rhizobia strains in combination with 'Rio Tibagi', a poor N_2 fixing cultivar; however, the line 'UW 22-34' showed a superior ability to establish effective symbiosis across strains, and enhanced the differences among strains. More studies are necessary to determine appropriate methods for selecting bean plants and rhizobia strains under field conditions.

IMPORTANCE OF N_2 FIXATION OF COMMON BEANS FOR LATIN AMERICA

The genetic potential for enhanced N_2 fixation, present in germplasm that is often poorly adapted to specific areas, can be introduced into well-adapted and commercially acceptable bean types using appropriate breeding and selection methods (3,11,14,15). Similar methods to those described here are being applied in our projects in Brazil and Honduras. Superior black seeded types for Brazil and small red-seeded types for Honduras with improved N_2 fixation are being identified in field trials conducted on experimental and farm sites. Line 'UW 22-34' has outyielded local cultivars in inoculated experimental trials in Brazil (Henson et al. unpublished results) (Table 3). In Honduras, superior genotypes have yielded over 3-fold more than local cultivars (Rosas et al., unpublished results) (Table 4);

Table 3.- Plant growth, N accumulation from fixation, and seed yield of a breeding line (UW 22-34) and 2 Brazilian lines. Field grown, Brazil 1984.

	21 DAE[a]		39 DAE		53 DAE		81 DAE	
Bean line	Shoot dry wt. (g/pl)	Total shoot N (mg/pl)	Shoot dry wt.	Total shoot N	Shoot dry wt.	Total shoot N	50 seed wt. (g)	Seed yield (kg/ha)
Rio Tibagi	.71	27	3.88	153	5.24	163	61	680
Negro Argel	1.10	41	5.33	204	6.64	215	85	748
UW 23-34	.73	26	4.39	157	7.46	232	88	1068

[a] Days after emergence.
From: Henson et al., unpublished results.

Table 4.- Seed yield of five genotypes and two local cultivars from field evaluations of small red-seeded bean germplasm. El Zamorano, Honduras, 1984A.

	Seed yield (g/plant)
Inoculated with R. phaseoli	
BAT 1220	17.3
Copan	16.4
BAT 1493	14.2
Rho 4832-223-1	15.3
RAB 58	15.2
Desarrural[a]	4.8
Zamorano[a]	6.0
Fertilized with 60 kg urea/ha	
Desarrural	11.1
Zamorano	11.2
Range (n = 42)	4.8 – 17.3
Mean	10.2 ± 3.2

[a] Local (check) cultivars.

From: Rosas et al., unpublished results

some selections are being crossed to high fixing donor parents. Along with better disease resistance and tolerance to stress, selection for enhanced N_2 fixation in common beans represents an attractive approach to increasing bean production in regions such as Central America (3,10).

Present average yield in Central America (slightly over 600 kg/ha) needs to be raised up to at least 1000 kg/ha in order to increase bean availability and reach desirable levels of comsumption (2,4).

Increasing bean productivity about 400 kg/ha will require a sizeable increase of N available to the plants; 400 kg/ha x 20o/o protein in grains = 80 kg/ha protein x 1/6.25 (N/protein)

= 12.8 kg/ha of N required by grains + 12.8 kg/ha of N required by other plant parts = 25.6 kg/ha of total N required to increase actual bean yields up to 1000 kg/ha. This requirement of 25.6 kg/ha of N could be derived from N_2 fixation or fertilizer application. The impact of increasing N_2 fixation potential of common beans in Central America will correspond to the following amounts of urea aplication to reach similar increase in productivity; 25.6 kg/ha N x 100/46(urea/N) = 55.6 kg/ha urea x .50 (assuming only 50o/o of applied N fertilizer is actually used by bean crop) = 111.2 kg/ha of Urea (23,26,31).

CONCLUSIONS

Enhancement of the N_2 fixation potential of common bean for Latin America can be realized using appropriate breeding and selection methods for the improvement of the host plant. The inbred backcross line method has proved to be very efficient in recombining higher levels of fixation with other desirable agronomic and commercial traits, and it can be easily applied to the development of bean cultivars which are readily accepted by farmers and consumers. The impact of improving N_2 fixation of common beans for Latin America is expected to be on supporting the higher N requirement of superior bean cultivars which combine high N_2 fixation with disease resistance, stress tolerance and yielding ability. Therefore, selection of beans for higher N_2 fixation must be an integral part of any bean improvement program. Supporting research activities similar to those described here will allow an increase in the levels of fixation of commercial beans and meet goals such as the one suggested for Central America.

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GUIA PARA EL DIAGNOSTICO Y CONTROL DE ENFERMEDADES DE PLANTAS
Vol. I

Mario Contreras y Octavio Ramírez
MIPH—EAP No. 35. 1986. 98 p.

Este volumen presenta un enfoque básico sobre los conceptos generales de enfermedades y los patógenos que las provocan. También hace una descripción de las categorías taxonómicas en que se agrupan los patógenos y se incluye información sobre la manera de obtener muestras y aislar los patógenos.

Además, contiene 25 hojas informativas ilustradas sobre igual número de enfermedades que afectan diferentes cultivos, estas hojas permiten el reconocimiento de los patógenos, sus síntomas y daños. También se explica el proceso de desarrollo de las enfermedades en diferentes condiciones ambientales, así como las medidas de prevención y control.

La información por cada enfermedad está publicada en hojas informativas independientes con su respectiva ilustración, que pueden ser retiradas o colocadas en carpetas con anillo, para un fácil manejo. Esto permitirá al usuario incorporar el segundo volumen de la Guía que estará disponible próximamente.