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Breeding common bean for improved biological nitrogen fixation

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Abstract

Common bean (*Phaseolus vulgaris* L.), which is an important food crop in the Americas, Africa and Asia, usually is thought to fix only small amounts of atmospheric nitrogen. However, field data indicate considerable genetic variability for total N_2 fixation and traits associated with fixation. Studies have shown that selection to increase N_2 fixation will be successful if: (1) discriminating traits (selection criteria) are measured precisely, (2) variability in germplasm is heritable, (3) selected parents are also agronomically suitable, (4) units of selection facilitate quantification of selection criteria, and (5) a breeding procedure that allows maximum genetic gain for N_2 fixation and recombination with essential agronomic traits is chosen. Breeding lines capable of fixing enough atmospheric N_2 to support seed yields of 1000–2000 kg ha^{-1} have been identified and new cultivars with high N_2 fixation potential are being released.

Introduction

Common bean (*Phaseolus vulgaris* L.), in symbiosis with *Rhizobium leguminosarum* biovar *phaseoli* has been considered a poor N_2-fixing grain legume. Both the total amount of nitrogen (N) derived from the atmosphere (total Ndfa) and the proportion of the plant N from the atmosphere (% Ndfa) in commonly-grown cultivars often are insufficient for producing economically attractive seed yields (e.g. 1,000–2,000 kg ha^{-1}). In contrast to the belief that beans are invariably poor N_2 fixers, there is evidence for genotypic variability for traits associated with N_2 fixation potential (e.g. Graham and Rosas, 1977; Park and Buttery, 1988, 1989); and for amount of N_2 fixed (e.g., McFerson, 1983; Rennie and Kemp, 1983; Pereira et al., 1989). Furthermore, selection has produced breeding lines able to fix high levels of N_2 (McFerson, 1983; Miranda and Bliss, 1991; St. Clair et al., 1988; St. Clair and Bliss, 1991), and the improved breeding lines are being tested for commercial use (Bliss et al., 1989). In field experiments some bean lines have been shown to fix enough N_2 to support seed yields of 1,000–2,000 kg ha^{-1}, when effective rhizobial populations are present either naturally or from inoculation, and there are no other major yield limiting factors.

Because of traditionally low returns, common bean often is produced on marginal soils. Other mineral elements besides N may be limiting, high temperatures and insufficient moisture often prevail, and production practices are less than optimal. The limitations to N_2 fixation imposed by the plant genotype can now be alleviated through breeding and selection of improved cultivars; therefore a ‘production package’ that is suitable for the small farmer using minimal purchased inputs should be designed and implemented. This package should include; an adapted cultivar capable of high N_2 fixation, assurance of an effective and efficient rhizobial population through proper inoculation, a source of high quality, disease-free seed, and management practices that are acceptable to the farmer for the prevailing agroecological system.

Effective selection for increased N_2 fixation

Bliss (1991) pointed out that effective selection for increased N_2 fixation in grain legumes depends on the following factors: (1) choice of traits as selection criteria that can be measured precisely and economically, while providing for discrimination between superior and inferior selection units; (2) variability in legume germplasm and heritability of differences for either plant N derived from the atmosphere (Ndfa) or traits indicative of fixation potential; (3) identification of genetically diverse parents that are also suitable agronomically; (4) choice of selection units (i.e., individual plants or families) that facilitate precise quantification of selection criteria and allow production of progeny from selected plants; and (5) use of a breeding procedure (e.g. mass selection, family selection) that provides maximum genetic gain per year for increased N_2 fixation and recombination with essential agronomic traits. If improved N_2 fixation potential is to be realized, selection must be practiced under soil N conditions that allow discrimination between high- and low-fixing lines. Field trials should be carried out without application of large amounts of N fertilizer to facilitate selection and simulate farming conditions where low purchased inputs are common. The practice of "on-farm" testing in environments that reflect real-life conditions should be expanded.

Methods of estimating N_2 fixation and choice of traits as selection criteria

Several methods can be used to estimate N_2 fixation potential and evaluate traits related to N_2 fixation. The acetylene reduction (AR) assay has been used extensively to estimate the potential for N_2 fixation at a specific time point (Burris and Wilson, 1957; McFerson, 1983; Turner and Gibson, 1980). Although AR has been useful for identifying effective plant-rhizobial combinations and the potential of plant genotypes for N_2 fixation, it should not be used for quantitative estimates of N_2 fixation (see other chapters in this book). Furthermore, since most AR assays utilize mainly the crown roots and central root core, fixation by lateral root nodules may be underestimated. Studies by Wolyn et al. (1989) in common bean and Hardarson et al. (1989) in soybean have shown that fixation by lateral root nodules contribute substantially to total N_2 fixation. Contrary to suggestions, that in grain legumes N_2 fixation declines at the onset of seed-fill, N_2 fixation in some common bean lines continues actively during seed fill, with much of the total N accumulation occurring after the R3 stage (Table 1) (Attewell and Bliss, 1985; Peña-Cabriales et al., 1993). Other estimates of N_2 fixation potential can be based on nodulation traits such as nodule mass, nodule number, nodulation score, etc. (Rosas and Bliss, 1986). Plant biomass, total plant N and grain yield are useful indicators of N_2 fixation for plants grown on minimal or no-N media.

The application of ^{15}N isotopic-labelled fertilizer to actively fixing legumes and a non-fixing standard crop allows quantitative estimation of the amounts and proportions of plant N coming from the soil (Ndfs), fertilizer (Ndff) and the atmosphere (Ndfa). The accuracy of this method for determining Ndfa depends on proper use of a suitable non-fixing reference crop (see other chapters in this book). Non-nodulating isolines of soybean have been used satisfactorily where they are well-adapted to the prevailing daylength. The availability of a non-nodulating, adapted bean line would be quite useful, but to date, none has been developed. Several other crops (e.g. barley, wheat, maize, sorghum) have been used with mixed results (see other chapters in this book). However it should though be mentioned that relative screening of common bean lines can be done without a reference crop.

The total N difference method can be used to estimate N_2 fixation when plants are grown on low-N soil and when either minimal or no N was applied. Either a non-fixing reference crop or the bean line with the least total accumulated N can be used as the minimal-fixing reference (Miranda and Bliss, 1991). The Kjeldahl method or infrared spectrophotometry calibrated against Kjeldahl N (Mundel and Schaalji, 1988) can be

Table 1. Total nitrogen accumulation in plants of progeny lines and parents of population 24. Field, Wisc., 1984

Line	Total plant N at R1 (mg plant^{-1})	R3	R7	R3/R7
24-40	36	289	552	0.52
24-55	32	322	668	0.48
24-21	50	393	1045	0.38
24-17	49	172	1068	0.16
24-5	54	466	1149	0.41
24-12	57	198	1388	0.14
24-4	57	282	1396	0.20
24-19	37	265	1471	0.18
Sanilac	45	207	591	0.35
Puebla 152	68	646	1429	0.45

Source: Attewell and Bliss, 1985.

used to measure total N in the different plant tissues.

Various selection criteria can be used effectively, as long as it recognized that the primary trait for improvement is the amount and proportion of plant N derived from fixation. Therefore, selection criteria other than Ndfa must be viewed as indirect selection, and Ndfa as a correlated response. The selection method chosen often will reflect the capabilities of the experimenter to conduct the work effectively. However, a method chosen for simplicity, ease of application and low cost, but which produces little genetic gain for the trait of interest (i.e. N_2 fixation) is of limited value and should be avoided. Ultimately the seed yield resulting from the N present in the plant is important.

Variability for N_2 fixation and related traits

There is substantial genotypic variation for N_2 fixation and traits related to fixation in common bean germplasm (see other chapters in this book). Although there are few published estimates of heritability for these traits, usually environmental and genotype × environment effects are large, resulting in low to moderate heritability, especially when measured on a single plant basis. Therefore, greater selection efficiency can be achieved if selection is based on family mean rather than individual value as the unit of selection. The evaluation of families rather than individual plant allows replication for reducing the effects of non-genetic (environmental) factors that obscure genotypic value and for improving the precision of selection.

Most methods of estimating N_2 fixation, i.e., acetylene reduction, ^{15}N isotopic labelling, total plant N, are plant destructive. Therefore, both destructive plant sampling for analysis and saving the seed of single plant selections are not possible. The evaluation of families allows sampling of a portion of the family for estimation of N_2 fixation and other traits if desired, then saving seed from the remaining plants in the selected lines (Wynne et al., 1987).

In common bean, production of inbred families following hybridization of an adapted cultivar with a high N_2-fixing parent can be accomplished using either single seed descent (SSD) or the inbred backcross line (IBL) method in which one or two backcrosses is made to the adapted cultivar following the initial cross to the high fixing donor line. After backcrossing, selfing for one or two generations using SSD is practiced to produce near-homozygous IBL's for replicated testing (McFerson et al., 1982).

Breeding methods

Several factors influence the choice of breeding methods most suitable for improving N_2 fixation in common bean. Usually, there are distinct regional preferences for the commonly-grown cultivars, which relate to cropping system, day length, plant type and seed characteristics. In addition to being grown in many diverse areas, there are numerous consumer preferences based on seed shape and color, cooking quality, plant type, adaptability, etc. No single cultivar enjoys exclusive, wide-spread production; instead many different bean cultivars are grown worldwide or even in one region of a single country. Therefore, to meet these varied demands, breeding for improved N_2 fixation must be incorporated into a bean improvement program as a standard practice and be given a priority equivalent to other objectives such as yield improvement, suitable plant type, disease and insect resistance, adaptability, etc.

Practically speaking, the breeding method used must allow for easy recovery of a desirable genotype(s) when N_2 fixation is being improved. Recovery of elite genotypes may be enhanced by intercrossing parents with similar phenotypes, but often the lines with high N_2-fixation potential (e.g. Puebla 152) are unlike the standard cultivar being improved (e.g. cv. Sanilac). If the phenotypes of the parents are similar, modified pedigree selection among inbred lines developed by SDD or recurrent mass selection among families produced by intermating the phenotypically similar parents can be used effectively. If the donor line having increased N_2 fixation is quite different from the standard cultivar, backcrossing is an effective means of recovering the recurrent parent genotype while maintaining adequate variability for the trait being improved. The production of inbred backcross lines combines the desirable features of backcrossing and the production of inbred lines using SSD (Fig. 1). Since N_2 fixation is quantitatively expressed, single plant heritability is low, and methods for assaying N_2 fixation are plant destructive, the inbred backcross line (IBL) method facilitates breeding for improved N_2 fixation by allowing for family selection based on replication.

Family selection also allows easier evaluation of experimental units for multiple traits since portions of the same replicate (e.g. row plot) or different replicates of the same family can be evaluated for different traits. The improvement of any trait in common bean should allow also for simultaneous evaluation of yield to ensure either that yield is improved or at least maintained, because yields of most current cultivars are somewhat low and improvement usually is of a high priority in breeding programs. The symbiotic nature of the host-rhizobial association requires that evaluation of the effectiveness of either natural populations or inoculant of *R. phaseoli* be done simultaneously with plant genotype improvement. The breeding method should allow easy evaluation of host-inoculant combinations in multiple locations and years. Use of family mean performance is well suited for these multiple evaluations.

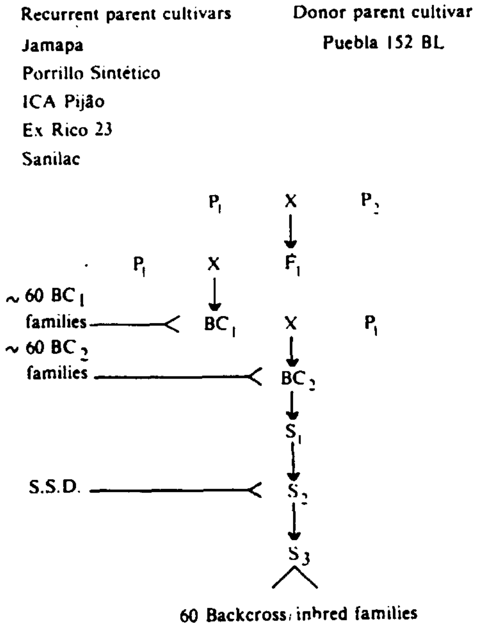

Fig. 1. The backcross inbred method of population development. Source: McFerson et al., 1982.

Realized plant improvement from selection

There are only a few published reports where the results of hybridizing selected parents, selecting the best families in variable offspring populations and evaluating the selections under field conditions have been studied. In those cases, the results support the predictions that genetic improvement through breeding for N_2 fixation in common bean is feasible. There are no empirical data to suggest that increasing N_2 fixation of adapted bean lines is accompanied by unfavorable effects on other desirable traits (e.g., yield) if suitable breeding methods that allow evaluation of multiple traits are used.

Graham (1981) and Graham and Rosas (1977) reported that, based on conclusions from acetylene reduction assays, plants with a larger photosynthetic area fix more N_2 than those with less capability. That finding supported the conclusion that type IV (indeterminate, climbing) plants fixed more N_2 than type III, and that type I plants with determinate bush habit were invariably poor N_2 fixers. While the latter often is true, there are notable exceptions, especially within snapbean cultivars. In trials on low-N, sandy soils in Wisconsin, the cv. Lake Shasta was found to fix up to 70–80 $\mathrm{kg\,ha^{-1}}$ during the 60–70 day growing period based on difference method estimates using non-nod soybeans as the non-fixing crop (F.A. Bliss and K.A. Kmiecik, unpublished data). The results of Graham and Rosas (1977) also led people to conclude that since many existing type I lines are poor N_2 fixers and that indeterminate types (i.e., types II, III and IV) with greater photosynthetic capacity were capable of fixing more N_2, breeding for improved N_2 fixation in type I and II bean plants would be of little value. This has not been the case, since photosynthetic capacity is only one of several (many) plant factors affecting N_2 fixation.

The Mexican landrace cultivar Puebla 152 (black-seeded) which has a type III growth habit has been identified as a superior high N_2 fixing line based on AR assays. Puebla 152 has been used widely as a parent in crosses with type I and II cultivars. Superior selections from among inbred backcross progeny lines from crosses with Sanilac (type I, white-seeded), Porrillo Sintetico (type II, black-seeded), Jamapa (type II, black-seeded), ICA Pijao (type II, black-seeded) and Ex Rico 23 (type II, white-seeded) have been identified (McFerson, 1983). Several traits have been evaluated including total plant N, total and % Ndfa in the plant, total and % Ndfa in the seed, and seed yield. Various methods have been used to estimate N_2 fixation, including ^{15}N isotopic estimation using labelled fertilizers, the difference method with non-nod soybean as the non-fixing standard, and indirect measures based on total plant and seed N, and nodulation traits such as nodule weight mass, number and score. In all segregating populations, recombinant lines have been recovered with increased N_2 fixation or fixation potential and acceptable seed yield, and other agronomic traits similar to or exceeding the standard parent. Sometimes, as with other traits, further improvement is necessary because important traits (e.g. disease resistance) may be lacking.

The white navy bean cv. Sanilac was grown widely in the U.S., especially in Michigan and other north central states, until recently. Although possessing many desirable traits, it produces modest yields and is a poor N_2-fixing plant. The population of inbred backcross lines resulting from the cross of Sanilac × Puebla 152 has been studied extensively (McFerson et al., 1983; and McFerson, 1983, St. Clair et al., 1988). Promising selections based on AR assay, seed yield, plant type and days to harvest were made in field trials on low-N sandy soils. St. Claire et al. (1988) reported that some BC_2S_3 lines with agronomic traits similar to Sanilac (e.g., 24–21, 24–55) had more total Ndfa and % Ndfa than the recurrent parent but less than the donor parent, Puebla 152 (Table 2). Among S_2 families produced from intercrosses among the best BC_2S_4 lines there were superior families for high N_2 fixation that also had satisfactory seed yield, seed traits, early maturing and other good agronomic traits (St. Clair and Bliss, 1991). The total Ndfa and % Ndfa of the best families exceeded that of the recurrent parent Sanilac and were similar to Puebla 152 (Table 3). Indeterminate plant type was not an essential trait for expressing high N_2 fixation, although a larger leaf area presumably can contribute more photosynthate for plant growth, N_2 fixation and seed yield.

ICA Pijao is an outstanding type II black bean cultivar bred for subtropical conditions (i.e. CIAT, 1986). However, depending on the situation, it has been found to fix variable amounts of N_2. A population of BC_2S_2 lines derived from the cross of ICA Pijao × Puebla 152 showed substantial segregation for numerous important traits, including seed yield, plant type, maturity, N_2 fixation and growth under low phosphorous levels (Pereira and Bliss, 1989). Lines resulting

Table 2. Estimates of N_2 fixation, N yield per plant, and % Ndfa for common bean inbred backcross lines and parents at the R9 growth stage. Hancock, WI, 1985. Line mean values within each ^{15}N experiment (En = ^{15}N-enriched, Dp = ^{15}N-depleted)

Line	N yield		N_2 fixed		Ndfa (%)	
	En	Dp	En	Dp	En	Dp
	(mg N/plant)					
21-16	986	1158	584	450	59.2	38.4
21-38	1074	919	649	337	60.8	36.8
21-43	985	1076	510	471	52.0	43.1
21-58	1022	1029	634	302	54.2	29.2
Porrillo Sintetico	1163	1230	643	550	54.3	44.9
Puebla 152	1053	1392	661	688	62.5	49.3
Sanilac	629	848	72	19	9.8	3.1
24-17	1010	1136	507	442	48.0	39.1
24-21	933	1041	391	222	40.6	21.2
24-48	784	866	329	136	40.9	16.3
24-55	741	931	296	288	37.5	31.3
24-65	933	1077	417	242	44.8	24.3
LSD (0.05)	268	243	224	167	14.4	12.9
r_s†	0.85**		0.61*		0.74**	

*, ** Significance at the 0.05 and 0.01 levels, respectively.
† r_S = Spearman rank correlation coefficients for line ranks between En and Dp experiments.
Source: St. Clair et al., 1988.

from this cross (i.e., Reg. No. GP-71-PI520603; GP-72-PI520604; GP-73-PI520605; GP-74-PI520606; GP-75-PI520607) which were selected and evaluated (e.g., CIAT, 1987) in the U.S. and Brazil have been released as improved germplasm having improved N_2 fixation ability (Bliss et al., 1989). The selected line WBR 22-34 was compared to the widely-grown standard cv. Rio Tibagi and to Negro Argel which has been found to have better nodulation. WBR 22–34 fixed more total Ndfa and % Ndfa and was higher yielding than these two Brazilian black bean cultivars.

The five released lines (Bliss et al., 1989) as well as other breeding lines selected at CNPAF/EMBRAPA for high N_2 fixation and important agronomic traits (e.g. Honduras 35) are being evaluated in Brazilian state trials. Although moderate to high yields usually are reported under low-N soil conditions, their utility as recommended cultivars will depend also on other traits such as several disease reactions, i.e., bean rust, bean common mosaic virus and anthracnose. For regions where these diseases are limiting factors to production, breeding is underway to combine resistance and high N_2 fixing ability into adapted breeding lines (Pereira, P.A.A., CNPAF/EMBRAPA, personal communication).

Because common beans of many different seed types are grown in Brazil (e.g., carioca, mulatinho, rosinha, etc.), the incorporation of genes for increased N_2 fixation into white navy and black-seeded cultivars is viewed as a model system that can be extended to other materials. The concept of selection for improved N_2 fixation has been incorporated into the objectives of the bean improvement program at CNPAF/EMBRAPA in a manner similar to that for other important traits, i.e., selection for yield, disease resistance, etc. (Pereira, P.A.A.; person communication). Currently, priority is given to identifying high N_2 fixing parents, selecting for high N_2 fixation in segregating populations and evaluating promising selections made for high yield, resistance and good adaptability for N_2 fixation as well.

Miranda and Bliss (1991) reported genotypic variability for total seed N in populations of F_3 lines resulting from crosses of different breeding lines and cultivars to a single testor chosen for N_2 fixation ability. Family mean selection among plants grown on a low-N soil resulted in lines

Table 3. Mean values of five traits for 15 F, families (i.e., 10% of the population) selected for high N_2 fixation, the four parental IB lines, the recurrent parent 'Sanilac' and the donor parent 'Puebla 152', Hancock, WI, 1985

Entries	At mid-podfill (R7)				At maturity (R9)
	Ndfa (%)	N_2 fixed (mg/plant)	Shoot N (mg/plant)	Shoot dry wt (g/plant)	Seed yield (g/plant)
(17 × 48)-22	60.8	1028.8	1680	58.6	31.5
(17 × 48)-17	52.7	911.8	1650	59.2	32.5
(48 × 65)-22	54.2	910.2	1678	67.1	26.3
(17 × 21)-09	53.3	791.7	1465	54.6	28.7
(17 × 65)-02	55.5	779.3	1384	52.6	29.8
(17 × 21)-04	50.7	756.4	1484	58.0	24.1
(17 × 65)-06	49.3	751.0	1519	58.3	30.6
(17 × 48)-18	57.9	739.1	1270	46.4	24.4
(48 × 65)-23	46.4	736.9	1576	64.0	22.3
(17 × 65)-16	51.2	721.4	1416	54.8	27.8
(17 × 21)-06	47.3	718.2	1485	50.4	23.1
(17 × 65)-25	43.4	707.2	1511	63.0	38.6
(17 × 48)-19	42.5	675.2	1565	59.0	32.5
(17 × 65)-20	44.0	672.4	1518	58.5	25.9
(17 × 48)-16	49.9	667.0	1359	46.9	23.8
(17 × 21)-06	47.3	718.2	1485	50.4	23.1
(17 × 65)-25	43.4	702.2	1511	63.0	38.6
(17 × 48)-19	42.5	675.2	1565	59.0	32.5
(17 × 65)-20	44.0	672.4	1518	58.5	25.9
(17 × 48)-16	49.9	667.0	1359	46.9	23.8
24-17	45.9	573.4	1267	48.1	31.8
24-21	37.2	414.3	1142	46.5	23.3
24-48	37.3	426.2	1146	43.4	18.0
24-65	36.2	504.4	1348	59.9	29.8
Puebla 152	50.4	589.2	1128	46.9	42.3
Sanilac	19.0	142.5	729	31.8	16.0
$LSD_{0.05}$*	12.8	336.5	487	16.2	7.2

* LSD mean comparisons at ($p < 0.05$).
Source: St. Clair and Bliss, 1991.

with increased total plant N and seed N accumulation (Fig. 2). Since no fertilizer N was applied and non-nodulating soybeans were N deficient, it was concluded that increased seed N of the selections compared to the parents, resulted from more N_2 fixation.

This method of indirect selection for increased N_2 fixation can be practiced successfully where there are low levels of soil N and no fertilizer is added. Simultaneous improvement for seed yield is also possible depending on the amount of genotypic variability in the segregating populations.

If breeding for increased N_2 fixation is to receive high priority along with other improvement objectives, there must be a clear recognition that: 1) high N_2 fixation is desirable, 2) adequate genetic variability is available, 3) selection for increased N_2 fixation is feasible using suitable methods, and 4) practical methods are available for identifying high N_2 fixing lines. If it is not viewed as such, N_2 fixation probably will not be improved. When other traits are given higher priority and breeding lines are evaluated on N-fertile soils, the farmer growing beans on infertile soils with minimal N input is not likely to receive new cultivars able to derive much of the required nitrogen from N_2 fixation.

The soil N level on which segregating lines are evaluated is crucial to successful selection for increase N_2 fixation. Where inherent N fertility is high or substantial fertilizer N has been applied it is impossible to discriminate among lines for differences in N_2 fixation without the use of ^{15}N isotope techniques. The technical demands and costs of these techniques may preclude use on a large scale to evaluate numerous breeding lines. Furthermore, evaluation under high-N condi-

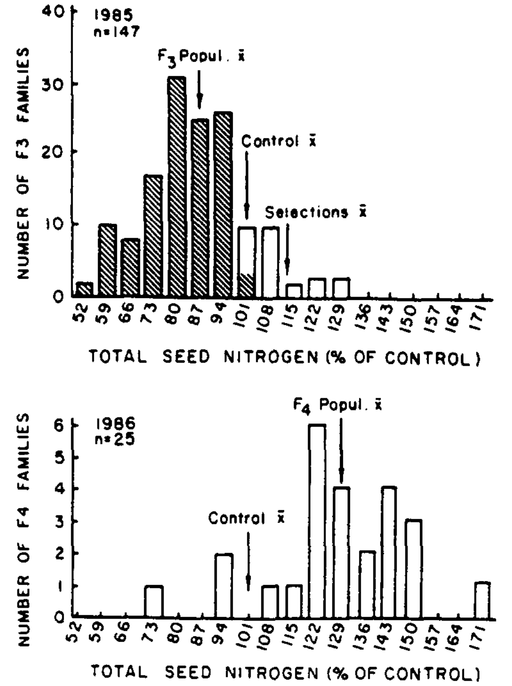

Fig. 2. Frequency distribution based on percentage of control of a population of F_3 half-sib families in 1985 and F_4 families resulting from the selected F_3's in 1986 for total seed nitrogen. Source: Miranda and Bliss, 1991.

tions will allow identification only of high N_2-fixing lines insensitive to combined N (if they exist); and those capable of fixing under low-N conditions will not be retained. Inclusion of an adapted, non-nodulating legume along with the breeding lines being tested provides a valuable visible means of evaluating the relative level of N coming from sources other than fixation.

Despite knowledge that selection among breeding lines for high N_2 fixation has been practiced and that the farmer is likely to grow beans on low-N soils, standard yield trials grown at experiment stations to evaluate lines prior to release, are often conducted with the addition of fertilizer N at high levels not likely to be used by the farmer. There is a belief among many researchers that a 'good trial' is a high yielding trial with a low coefficient of variation (CV). When high yields are achieved by adding N fertilizer it is difficult to evaluate promising lines selected for increased N_2 fixation, and these conditions are not likely to be encountered on the farms.

Future research priorities

Since ample genotypic variability for N_2 fixation in common bean has been identified in the primary gene pool, more effort in bean improvement programs should be placed on selection for increased N_2 fixation under representative field conditions and involving improved inoculant when possible. Accurate assay methods are available to allow discrimination between high- and low-N_2 fixing lines. The family mean rather than individual phenotype should be the unit of selection to allow replication and accommodate plant destructive methods used in the assay for increased N_2 fixation. Production of segregating families from planned crosses can follow either the modified pedigree selection method where lines are derived by single seed decent or the inbred backcross line method where near homozygous lines resembling the recurrent parent are produced. Evaluation of segregating progenies and of advanced lines should be on low-N soils representative of those that prevail in the target production area.

Both the rhizobia and plant genotypes often are sensitive to high levels of combined N, with N_2 fixation often being suppressed by added fertilizer N. There is evidence for both natural variants and induced mutants (Park and Buttery, 1989; St. Clair et al., 1988) of common bean able to tolerate combined N. This characteristic along with the capacity to fix large amounts of N_2 should be bred into high-quality, well-adapted lines with acceptable seed traits.

Despite extensive observation of many plant materials, no natural non-nodulating mutants of *Phaseolus vulgaris* have been identified. Several induced mutants incapable of forming effective nodules have been described, but non-nodulating lines of the major-classes, e.g., black, red kidney, carioca, etc., have not yet been developed. Although, of limited practical value, well-adapted non-nodulating lines would be useful in field selection and evaluation experiments to verify the level of soil N and to use as the non-fixing reference crop when estimates of N_2 fixation using the ^{15}N isotopic and total N difference methods are desired.

Few plant genes controlling expression of the different aspects of N_2 fixation in beans have been identified. Efforts should be made to elucidate plant genetic control more clearly and once identified, to place the genes on a genetic map along with other traits. If linkage with molecular markers can be established, marker-facilitated selection might be used to complement direct and indirect selection for increased N_2 fixation.

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