PN-AAS-613 3967

Summary Report of A Workshop

Biological Nitrogen Fixation for Tropical Agriculture

February 22-23, 1982

Washington, DC

Sponsored by the USDA-CSRS-AID

Program "Symbiotic Nitrogen Fixation:

Study of Factors Limiting N-Fixation for

LDC Crop Production"

Introduction

The Workshop was designed to bring together the principal investigators funded under the USDA-CSRS-AID program titled "Symbiotic Nitrogen Fixation: Studies of Factors Limiting N-Fixation for LDC Crop Production". In place since 1976, this project has involved some 35 research scientists at 22 U.S. universities and experiment stations. Though two earlier meetings in connection with this program had been held, this was the first attempt to bring all active researchers together at one time.

The Workshop was held in Washington, DC on February 22-23, 1982. It was organized to address three areas of major concern. Briefly, these were:

1. To identify and document the most effective and competitive strains of *Rhizobium* spp. for the major flood, forage and tree legumes;	**Work Group A**
2. To identify and document those varieties and/or selections of major agricultural legumes best adapted to tropical soils; and	**Work Group B**
3. To document the unique and valuable experiences of the Principal Investigators that have occurred as a result of cooperative agreements with scientists, institutions and agencies within emerging countries.	**Work Group C**

Participants at the Workshop included the Program officials, past and present Principal Investigators, and several guests from both NSF and AID.

A list of participants is provided in Appendix A.

Report of Work Group A

Work Group Chairman - David Bezdicek - Washington State University

The following criteria were agreed upon as a basis for selecting the strains of *Rhizobium* spp. to meet Objective 1.

a. Strain shown to be effective and/or highly competitive in greenhouse and preferably field trials.

b. "Serologically distinct".

c. Stability in culture and adaptability to certain environmental conditions.

d. Availability of supporting data in the literature.

The following represent those cultivars and associated *Rhizobium* strains that were discussed and selected as most effective.

Cultivar	Strains selected	Source
Beans (*Phaseolus vulgaris*)	Kim-5* (highly competitive)	Washington State Univ.
	C-05 (CIAT)	NifTAL
	TAL-182	NifTAL
	127K17	Nitragin
Cowpeas (*Vigna* spp.)	32H-1*	Nitragin
	TAL-169 (acid tolerant)	Beltsville
	THA-201 (good survival)	Texas
Peanuts (*Arachis hypogaea*)	32H-1*	Nitragin
	TAL-169	NifTAL
	T_1 (TAL 1371)	NifTAL
	TAL-1000	NifTAL
	176A34	Nitragin
Pidgeon pea (*Cajanus cajan*)	32H-1*	Nitragin
	IHP155	ICRASAT (Schroeder, Puerto Rico)
	IHP147	California (Focht)
Mung bean (*Vigna radiata*)	TAL-169(176A22)*	NifTAL
	THA-301	NifTAL
	M_6	NifTAL
Chickpeas (*Cicer arietinum*)	27A3*	Nitragin
	27A8	Nitragin
Peas (*Pisum sativium*)	N92A3*	Nitragin
	C1204	Washington State Univ.
	128A12	Nitragin
	128C53	Nitragin
Lentils (*Lens culinaris*)	N92A3*	Nitragin
	I11 (Hawaii 5-0)	NifTAL
	128A12	Nitragin
Fababeans (*Vicia faba*)	175 F12*	Nitragin
	175F10	Nitragin
Soybeans (*Glycine max* L.)	110*	Beltsville
	122	Beltsville
	138	Beltsville
	142	Beltsville
	143	Beltsville
	6	Beltsville
Lima beans (*Phaseolus linensis*)	?	Beltsville
Winged beans (*Psophocarpus tetragonolobus*)	?	NifTAL
Lupine (*Lupinus albus*)	?	NifTAL and
Lupine (*Lupinus angustifolius*)	?	Beltsville
Other seed legumes	?	NifTAL, Beltsville
Alfalfa (*Medicago sativa*)	?	Beltsville
Other Forage Legumes	?	?
Tree Legumes	?	NifTAL
Centrosema		
Stylosanthus		
Desmodium		

*Considered the standard of reference. All strains are considered to be effective, although the one listed first should always be included in comparative trials.

The lack of information for those legumes listed last occurred because of time constraints for further discussion and because few of the workshop participants were working on these species.

This list of strains is certainly not the final word and they are listed to be evaluated and challenged. Hopefully, these selections will find utility as standard strains for comparison to other new isolates in both laboratory, greenhouse and field studies. This list should also prove valuable for scientists beginning research work on BNF.

Finally, antisera is available for many of the strains listed but excess will have to be negotiated on a case-by-case basis from the person or agency identified with each strain.

In general, the objectives of Working Group A were met during this meeting. It is hoped that we can continue to communicate on the standard strains and exchange new data on additional strains. This may provide a justification for another meeting in the future. Some additional topics may include strategies in breeding for enhanced N_2 fixation and techniques for measuring N_2 fixation in the field. The latter topic is important in arriving at a N budget, especially in developing countries where modern techniques are not available. We should consider non-nodulating legumes, ineffectively nodulated legumes and even non-legumes when we are attempting to estimate N_2 fixation by difference in total plant nitrogen. Here again, the plant breeders may be able to provide input.

Report of Work Group B

Work Group Chairman - Johnny Wynne - North Carolina State University

Workgroup B spent considerable time discussing the following topics:

1) Factors affecting BNF by the macrosymbiont

The discussion of factors that may limit symbiotic fixation revealed little that was startling. However, there appears to be some evidence that the absence of mycorrhizae may limit nodulation under desert conditions.

The discussion raised two ancillary questions. One related to the response of well-nodulated legumes to fertilizer nitrogen. Is this an indication of insufficient fixation? The consensus was that virtually all annual legumes seem to respond to fertilizer N, even when nodulated; on the other hand, most perennials do not. For some, such as *V. faba* and mung, little information is available.

The other question revolved around the possibility of looking at the coevolution of host species and the rhizobial symbiont, as compared to those host species that may have evolved under conditions of high native soil-nitrogen. The conclusion seemed to be that such an effort would yield little worthwhile information, on one hand because of the paucity of good archeological informaton and, on the other, because of known variability in response within species, or genera, for which we

have "known" centers of origin. For example, all the wild species of limas, P. vulgaris and, perhaps, cowpeas appear to be perennials.

2. Methods for measuring BNF in legume symbioses

The next question that arose was, "What is it we are trying to measure, and how should we do it?" It was agreed that, for most pulse species, there is little correlation between grain yield and nitrogen-fixation efficiency. Therefore, some attempt must be made to evaluate symbiotic fixation per se. In ascending order of sophistication (and cost?), and assuming the crop is nodulated, estimates may be obtained by:

Recording nodule weight and color
Measuring Kjeldahl nitrogen
Analyzing by Acetylene reduction
Using Xylem-sap analysis (ureides)
Using N_{15} measurements

Each of these methods has its limitations; concern was expressed that these are too rarely considered. It was generally agreed that grain yield is the ultimate criterion but that, given reasonable yields, other variables must be measured. There was some discussion about the use of yield as such as the ultimate measure; some argument can be made for yield stability as being the more desirable objective.

3. Breeding strategies for enhancing BNF

Regarding breeding strategies for enhancing BNF it was generally agreed that measurements within individual genotypes in early generations are futile. For most crop species, reliable data and reasonable C.V.'s can only be obtained by evaluating pure or near pure lines in replicated trials at multiple locations. The feeling was that, from a breeding standpoint, nitrogen-fixing capacity (the efficiency of the host-strain interaction) is a quantitative trait similar to yield. Therefore, the selection and breeding strategies are comparable; there are no valid shortcuts. The breeders felt that their appropriate role is to create elite germplasm (lines) that have all the desirable characteristics of yield, disease resistance, etc., plus the ability to support a high level of N fixation, and then to use them or to release them for use as parents for local breeding program.

4. Identification of varieties, cultivars, and/or selections of major agricultural legumes as standards for breeding programs

Regarding the identification of specific genotypes that might be used as standards against which progress could be measured, the group was very reluctant to be too specific. Their feeling was that there is no single standard available for any crop species. The best approach, particularly for food legumes for which dietary preferences and taboos are usually strong, is to use the best local cultivars (agrotypes?) as the standard. If the area in question can be characterized climatolo-

gically and edaphologically, any competent U.S. breeder should be able to suggest a list of cvs. or P.I.'s that might be compared with the local standards. An alternative is to make a list for those species for which we have sufficient information from which the local investigator might choose. The main purpose such a list would serve would be to reduce the decisions a researcher would have to make. This is certainly a less desirable alternative than tailor-making a specific list for an identified site. Some tentative lists are below.

MUNGBEAN - Suggested genotypes from Asian Vegetable Research Development Center, Taiwan.
High nitrogen-fixing potential 2184
Low nitrogen-fixing potential 1484

PEANUTS*- Peanut cultivars suggested for:

High Nitrogen Fixation and Nodulation

Cultivar	Botanical Variety	Test Site
Florigiant	hypogaea	Texas, North Carolina, U.S.A.
Va 72R	hypogaea	North Carolina, U.S.A.
NC 5	hypogaea	North Carolina, U.S.A.
NC 6	hypogaea	North Carolina, U.S.A.
NC 4	hypogaea	North Carolina, U.S.A.
Florunner	hypogaea	Texas, North Carolina, U.S.A.
Robut 33-1	hypogaea	North Carolina, India
X-14-4-B-19-B (ICG 1561)		India
NCAcc 2821 (ICG 2405)	hypogaea	India, North Carolina

Acid tolerance

UF 78305	hypogaea	
UF 78307	hypogaea	

For selection of Rhizobium strains with broad adaptation to the host (lack of host specificity), strains should be tested with and perfo m well with diverse germplasm such as:

Florigiant - Virginia
NC 6
Argentine
Tamment
Tennessee Red
New Mexico Valencia - Valencia

*Contact Dr. Johnny Wynne, North Carolina State University

COWPEAS*

Screened for salt tolerance*. Inoculated with CB-756, granular.
Soil N (ppm) 5.05 - 22.80. Arizona.
Soluble salts (ppm) 5,327 - 29,407

	Ranking with respect to:		
	Yield	Nodule #	% Noduled Plants
P.I. 211642	1	3	4
353011	2	1	1
353380	3	2	2
180494	4	6	3
353332	5	7	7
293518	6	8	8
Ca. #5	7	5	6
Speckled Purple Hull	3*	4	5
*not mature			

Suggested Texas genotypes**. High nitrogen-fixing potential.

108	187	3795
113	1630	3837
129	2408	3848
174	3764	3851

Suggested P.I.'s

P.I.	180358	354861
	293458	382088
	354481	382115
	354858	

Commercial Checks.

Moderately high fixer - Brown Crowder
Moderately low fixer - Bush purplehull

*Contact Dr. Victoria Macarian, University of Arizona
**Contact Dr. J. Creighton Miller, Texas A & M University

Recommended lines of bean (phaseolus vulgaris L.) for biological nitrogen fixation experiments.*

Class	Line	BNF Potential[z]	Source[y]
White	1. Sanilac	L	1
	2. Seafarer		1
	3. Swan Valley		1
	4. Neptune		1
	5. C-20		1
	6. ExRico 23	I	1,3
	7. Bunsi		1
	8. Aurora		1,8
	9. BAT 340		5
	10. BAT 1061	I	5
	11. BAT 1280		5
	12. UW 24-21	H	3
	13. UW 24-55	H	3
	14. UW 24-5	H	3
	15. UW 24-65	H	3
Black	1. Black Turtle Soup	I	3
	2. T-39		1
	3. Midnite	L	1
	4. Domino		1
	5. Black Magic		1
	6. Puebla 152 Black	H	3,5
	7. Jamapa	I	3,5
	8. Porrillo Sintético	H	3,5
	9. ICA Pijao	H	3,5
	10. UW 20-46	H	3
	11. UW 20-55	H	3
	12. UW 21-58	H	3
	13. UW 21-43	H	3
	14. UW 21-19	H	3
	15. BAT 76	I	3,5
	16. BAT 1320	L	5
	17. BAT 304	L	5
Pinto	1. UI-111	L	7
	2. UI-114		7
	3. Olathe		7
	4. Pindak		7
	5. NW 590		4
	6. NW 410		4
Great Northern	1. UI-59	L	6
	2. Valley		6
	3. GN 1140		6

Class			BNF Potential	Source
Red Mexican	1.	Rufus	L	4
	2.	Big Bend		7
	3.	UI-36		4
	4.	NW 59		4
	5.	NW 63		4
Pink	1.	Viva	I	4
	2.	Sutter Pink		4
	3.	Rosa		4
	4.	A159		5
	5.	6R-1053		4
Red	1.	Montcalm	L	1
	2.	Charlevoix	L	1
	3.	Sacramento LRK		2
	4.	California LRK		8
	5.	California DRK		8
	6.	Redkloud		9
	7.	BAT 1249		5
	8.	BAT 1274		5
Snap	1.	BBL 240	L	3
	2.	Harvester		11
	3.	Cascade		11
	4.	Sprite		11
	5.	Triumph		11
	6.	Lake Superior		11
	7.	Astro		10
	8.	Eagle		10
	9.	Spurt		10
	10.	BBL 274		10
	11.	Goldrush		10
	12.	Kentucky Wonder Pole		10

[z]BNF potential as measured by acetylene reduction assay in Wisconsin. H = high, I = intermediate, L = low

[y]See Appendix B

*The list of recommended lines of P. vulgaris have been divided into 8 categories based on the commercial classes of most importance in the U.S.A. and represents either widely-grown and well-adapted cultivars or experimental lines of exceptional promise. Some of the lines have been characterized for BNF and most have shown excellent yield potential in the U.S.A. Yield and BNF will vary with experimental conditions. The recommended lines vary widely for many traits; questions concerning the characteristics and suitability of a specific line for a given region or set of experimental conditions may be answered by the plant breeders listed under Sources or other BNF workers. See Appendix B.

5. Future trends and goals for improved BNF through Plant Breeding

The discussion of future trends and goals for improved BNF through plant breeding was wide-ranging. It was agreed that BNF efficiency was pretty low on everyone's list of priorities. However, after other attributes such as disease resistance are accounted for, nitrogen-fixing capacity may come into play. No breeder in the room actually was selecting with BNF in mind; for some crops such selection may be fortuitous, particuarly if no N fertilizer is used on the breeding plots.

Questions that should be addressed are, "What effect does N fixation have on yield?" In peanuts, for example, it appears that 25% of the variation in yield was related to BNF. What effect does this have on associated crops in interplanting or on subsequent crops in the relay? Another point that requires more study is, "What plant traits (that might be more easily quantified) are associated with high N fixation capacity?" Again, with peanuts, high leaf weight, leaf area, or leaf area duration seem to be strongly correlated with high BNF.

We need to know more about the N-response curves of tree legumes. We need more support for Rhizobium, as well as plant, collections. We should not over-emphasize the utility of non-nodulating lines. We should be more careful to publish the plant germplasm, as well as the bacterial strains, used in our studies.

It was also suggested that we should be looking at patterns of plant growth and development with respect to their susceptibility to manipulation (management, as well as breeding) in support of higher nitrogen fixation. It was generally agreed that all components of BNF are genetically controlled and, therefore, heritable. Whether or not heritabilities are high enough to be reasonably exploitable remains to be seen. Under any circumstances, this is long-term work. Three years would represent just a beginning of such a program. Funding agencies must keep this fact in mind.

Finally, it was agreed that, speaking of most crop species, generalizations may cause more time to be wasted than would be invested in research to answer the specific question raised.

6. Miscellaneous discussions - Groups A & B

A discussion followed for both working Groups A and B which centered around strategies for the microbiologist and plant breeder to enhance N_2 fixation in legumes. Some questions and comments are as follows:

1. In genotype selection for enhanced N_2 fixation, should a single strain or multiple strains be used? One comment was that it is difficult to do both the genotype and strain evaluation adequately at the same time. One suggestion was to use a mixture of strains for the initial genotype evaluation

and then look for specific host-strain combinations in a later evaluation. However, we eventually run into the old problem of the added strains not being able to compete with indigenous ones.

2. Some discussion centered around using a high nitrogen control in greenhouse studies. One suggestion was to compare total plant growth and/or total plant nitrogen of the inoculated genotype to that of the same genotype under adequate nitrogen. This would seem to be a better approach than to compare the inoculated plant to the uninoculated control.

3. Some concern was expressed on the level of available soil nitrogen present in many plots used in the routine evaluation of new selections in legume breeding programs. In plots where legumes are planted continuously and not rotated with non-legume crops, soil nitrogen may build up. We should not expect to see a non-efficient host-strain combination to be expressed or identified if soil nitrogen levels are too high. It is therefore possible to carry along genotypes in a breeding program that are rather inefficient in N_2 fixation simply because of high levels of soil N. This is especially important in the LDC's where soil nitrogen may be low and even in the U.S. where costs of fertilizer N of $500/ton are estimated in the next three to four years.

4. There was some discussion on the selection of a few genotypes of each species to be used as standards by soil microbiologists in strain evaluation. The plant breeders generally agreed that is is not possible to pick only several 'standards" of genotypes. This is understandable in field studies where genotype adaptability is related to so many environmental and disease related factors. However, in greenhouse studies, it should be possible to select one or several genotypes the soil microbiologist can use.

Report of Workgroup C

Workgroup Group C was made up of all the workshop participants. This workshop had as its main objective the sharing of experiences with cooperative research in LDC countries which might be of benefit to other participants. Presentations were made by Dr. Bill Judy, USAID Africa Bureau; ~~Ms.~~ Mary Clutter, National Science Foundation; and Dr. Charles Smith, USDA-CSRS.

Topics discussed by the particpants included problems in providing appropriate training experiences for foreign graduate students; conducting research programs in LDC countries, communication of research results between scientists; and problems of transferring research grant dollars to LDC cooperating scientists.

Appendix A

List of Workshop Participants

Participant	Institution
D. F. Bezdicek	Washington State University
B. Ben Bohlool	University of Hawaii
William Bryan	West Virginia University
G. H. Elkan	N.C. State University
Dennis Focht	University of California, Riverside
H. D. Gross	N.C. State University
Dave Hubbell	University of Florida
William Lindemann	N. Mexico State University
Ron Lockermann	Montana State University
Thomas Loynachan	Iowa State University
James R. McFerson	University of Wisconsin
Victoria Macarian	University of Arizona
J. Creighton Miller	Texas A & M
Ian Pepper	University of Arizona
Harold L. Peterson	Mississippi State
Don Phillips	University of California, Davis
Walt Scudder	University of Florida
Larry Shuman	University of Georgia,(Exp. Station,GA)
Jim Sims	Montana State University
Carl Tucker	University of Califonria, Davis
Richard Weaver	Texas A & M
Barbara Webster	University of California, Davis
Davis O. Wilson	University of Georgia, (Exp. Station,GA
Arthur Wollum	N.C. state University
Sarah Wright	West Virginia University
Johnny C. Wynne	N.C. State University
Jake Halliday	NiFTAL, University of Hawaii
Deane Weber	USDA/ARS, Beltsville
Harold Keyser	USDA/ARS, Beltsville
Joe Burton	Nitragin Co. Milwaukee
Richard Graham	Univ. West Indies/Cornell University
Mohamid Hadad	Sudan/Iowa State University
Robert Miller	N.C. State University
Lloyd Frederick	US AID, Washington, DC
David Walker	Texas A & M

Appendix B

SOURCES: Seed and germplasm descriptions may be obtained from the following workers:

1. Dr. M. W. Adams
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6. Dr. D. P. Coyne
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7. Dr. J. J. Kolar
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11. Dr. Keith Zary
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