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FINAL REPORT

AID Grant No. AID/DSAN-G-0089

Biological Nitrogen Fixation in Tropical Agriculture

Grant Period: January 1, 1979 to October 1, 1983

Funding LeveL: $361,627

Principal Investigators: Drs. G. H. Elkan
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A. G. Wollum

This report summarizes the work done under the cited 211-d Grant that had as its broad objective:

to expand and utilize NCSU's response capability in BNF as applied on small farms in LDCs.

This activity grew out of an interest that had developed under the aegis of a grant in support of Tropical Soils Research. Under our grant four universities, Cornell, Hawaii, NCSU and Puerto Rico, worked in concert to improve their collective expertise in confronting the broad spectrum of Biological Nitrogen Fixation (BNF) phenomena and their application in tropical agriculture. NCSU's specific objectives were to:

expand the knowledge base on rhizobial inoculum, and improve evaluation techniques;

train cooperators in LDCs;

expand its advisory capacity for potential utilization by AID and LDCs;

accumulate an accesible literature file; and

establish firm linkages and networks within the BNF community and among the LDCs.

Thus, NCSU's responsibility to the overall effort was in the area of symbiotic nitrogen fixation and tropical legume inoculation. Our progress in this regard is discussed by specific objectives in the following report.

OBJECTIVE 1. Expanded Knowledge Base. To develop methodologies designed to quantify the role of N-fixation in improved soil management and crop production in developing countries.

The initial thrust under this objective was to describe the base as it existed at [illegible] time. To this end three state-of-the-art essays were written an[illegible]shed as NCSU Technical Bulletins. They addressed the questions of (a) evaluating the need for, and response to, inoculation, (b) selecting efficient rhizobium strains for use in the tropics, and (c) evaluating the quality of rhizobial inoculants. The titles are listed in the Appendix.

Some 1500 copies of these bulletins were printed. About 1300 of these have been distributed to date, and requests are still coming in. Over and above regular mailing lists, they have been sent to over 180 cooperators in about 36 DCs and 44 LDCs.

Laboratory and field studies on Rhizobium strain selection, host/strain interactions, host and bacterial response to temperature and drought stress, and environmental effects on carbohydrate partitioning and symbiotic fixation were initiated under this grant. (Proposed field studies in Colombia, Perú and Venezuela have been delayed because of local problems with both funding and personnel). Most of these studies are still in progress, but results to date are summarized below.

A methodological study on the factors influencing the enumeration of rhizobia in soil (using Rh. japonicum as a test organism) indicated no difference between the most-probable-number (MPN) and most-likely-number (MLN) estimates. It is felt that MLN may be easier to use in an LDC context. An ancillary sampling study showed that the variation among

samples from a given site was greater than that among subsamples within a sample. Therefore, it seems unwise to pool samples if the objective is to characterize a field population. Season of the year, depth of sampling, moisture status of the soil and past cultural practices each influenced population values. However, populations do not appear to vary with pH, within the limits studied. For soybeans, the rhizobia populations rose from a minimum in the Spring, prior to planting, to a maximum after flowering. The maximum appeared to coincide with the time of root and nodule degradation. From December till Spring the population was virtually stable. The population after a soybean crop was about one log number higher than the previous Spring, following a corn crop. Populations fell as the soil water potential dropped below -0.5 bar (slight stress). Water stress and temperature were probably the preponderant factors in influencing the variation with depth. As moisture in the surface soil decreased, population levels in the 0-6 cm stratum declined while those at 6-12 cm were relatively stable. Averaged across the season the 6-12 cm sample was 55% higher than the 0-6 cm level (both at the 10^4 level).

Soil temperature is a factor often cited influencing the behavior of rhizobia in soil. However, there is little information on the temperature relationships one might encounter during preinfection periods in soil supporting soybeans. Data from a field soybean site serves to highlight potential temperature problems. During preinfection periods soil temperature at 3 cm reached 46°C and 40°C for significant periods of the day at depths of 7.5 cm. These temperatures are beyond the optimum growth range of most *R. japonicum*. We have found, however, that there is a natural range of temperature tolerance among 40 *R. japonicum*

individuals with several strains capable of some growth at 43°C. Some of the more tolerant strains are being evaluated for their efficiency under temperature stress conditions. Results from these studies are indicative of a significant interaction between root temperature, nodulation, dry matter accumulation, and nitrogen fixation and the strain used as an inoculant. A close relationship was also found between the relative effect of temperature on growth of the strains in pure culture and that on the soybean-*Rhizobium* association. Continuing studies show a positive correlation between optimum temperature as measured and strain survival in soil.

Water potential and soil texture have a profound effect on survival of *R. japonicum*. Differences in ability to tolerate low water potential were noted among 10 serogroups tested. In general, isolates 110, 122, 138 tolerated moisture stress better than isolates 24, 31, 94, and 123. Soil *R. japonicum* populations were also depressed under the extremes of soil texture. Populations were lowest in Lakeland sand and Davidson clay loam and highest in Walla Walla silt loam, Duplin sandy loam and Iredell sandy clay loam.

In a field study on the influence of irrigation and *R. japonicum* inoculation on yields of soybeans, it was found that irrigated soybean yields were significantly greater when inoculated than yields from non-inoculated but irrigated plots. Inoculation without irrigation did not increase soybean yields, and in some cases actually decreased yields on non-irrigated plots.

These trials, developed with soybean rhizobia, are being expanded to include strains (the cowpea miscellany) appropriate to peanut inoculation.

A number of projects designed to strengthen our capability in working with rhizobia of the "cowpea miscellany" were initiated. These bacteria are the symbionts in most of the tropical legumes, but much less is known about the biology of these rhizobia. Specifically, we have started a culture collection of "cowpea miscellany" rhizobia. These have been isolated, purified, are undergoing testing for effectiveness and competitiveness.

Some 234 isolates from 78 plants have been isolated and screened. Additional strains are being evaluated.

Promising strains from greenhouse tests were evaluated in the field. Some strains produced greater nodulation and nitrogenese activity than the native rhizobial populations. Moreover, a significant correlation between field and greenhouse nitrogenese activity was realized. Promising cultures have been sent to various LDC cooperators and others (CIAT, Kenya, Beltsville, ICRISAT, IITA, Boyce Thompson, Tanzania, etc.). Using more than 250 peanut genotypes as hosts, we have established host/strain interactions between the rhizobia and legume. This is being followed up in further field testing in India (ICRISAT) through a cooperative project. The most dramatic result of this exchange was that an Israeli peanut cultivar, Robut 33-1, inoculated with a South American isolate, NC-92, gave a 30% yield in increase when tested in India (ICRISAT).

Methodological studies aimed at finding short-cuts to evaluating legume germplasm for early root growth (a drought-avoidance mechanism) and drought stress have resulted in a technique that permits the imposition of drought (composed of rate of drying-out, intensity of stress, length of stress period, stage of plant development at which stress occurs, and rate

of stress relief) on young legume plants. Field correlations are now being tested. The root-growth studies established the fact that greenhouse root growth is largely a function of the planting medium; in media like greenhouse potting mixtures, everything does well. Only when the root's extension potential is challenged, by using clay-based media, are cultivar differences expressed. Correlations of greenhouse seedling data and field performance of the same cultivars were low.

The effects of radiation level on symbiotic fixation was also studied. Using reflectors, along with both screens and black boards to evaluate temperature and turbulence effects, in the soybean field, we found that the periods during which light enhancement is most critical are right at flowering and, later, at mid pod-fill. The reflectors tended to increase light about 30% throughout the canopy. An effect of enhanced light on growth and production has been documented. Our studies have included the effects on fixation. Increasing incident radiation during the four-week period at flowering increased pod-set 92%, yield 79%, and C_2H_2 over 500%. Light enhancement during the four-week period around mid pod-fill resulted in 21% more pod, a 13% yield increase, and a 9-fold increase in acetylene reduction. Actual yield levels were: control 2.9 t/ha, flowering enhancement 5.3 t/ha, and enhancement at mid pod-fill 3.3 t/ha. Perhaps more importantly, enhancement at flowering increased the duration of a relatively high level of fixation. The beans that had received increased radiation at flowering, and then had the reflectors removed, continued to fix nitrogen at a level almost six times that of the checks. Nodule weights were also increased by the increased radiation as were nodule numbers, though these latter were not quantified.

OBJECTIVE 2. Expanded education and training capabilities. To train individuals in the technology and methodology of evaluating nitrogen fixation for improved soil management and crop production in the developing counties.

The major output under this objective was the synthesis of a short-course on symbiotic nitrogen fixation and legume inoculation designed specifically for field workers. Though several courses on the same subject matter are offered by other institutions, most of them are designed for microbiologists or technicians with considerable background in rhizobium work. Our course had as its target audience those workers who have responsibility for legume-based programs (forage or grain) but who have little or no formal training in microbiology or legume/rhizobium ecology.

The course was structured, a training manual has been assembled, and the material has been field-tested in both Lima, Peru, in 1981, and Bangkok, Thailand, in 1983. These represented vastly different environments, in all possible meanings of the word, and provided excellent proving-grounds for the short-course. A list of participants is appended. In both instances the course was enthusiastically received and given high grades at the subsequent evaluation sessions.

A Bill of Materials and appropriate schedules, including the establishment of demonstration plots, is included with the course manual.

The state-of-the-art papers, mentioned above, have also proven to be excellent training materials and have been requested by more instructors, and in greater quantities, than we have been able to satisfy.

A course titled "Food Production in the Tropics" was prepared under the auspices of this grant. It is taught at NCSU at the graduate level. It emphasizes food, as compared to plantation, crops and is based on the premise that there is nothing mysterious about producing crops in the

tropics; the principles of soil science, plant physiology and microclimatology are the same as in temperate zones. This course has been well received by both foreign-born and U.S. graduate students.

Another training activity involved a trip by our Soil Microbiologist and his technician to Puerto Rico to help conduct a workshop on Rhizobium strain identification, using microagglutination techniques, and the evaluation of the high-temperature tolerance of pigeonpea rhizobia to high-temperature stress. Puerto Rican BNF and some INTSOY personnel were among those participating.

Our Rhizobium microbiologist spent several months at the Universidad del Oriente, Jusepin, Venezuela, teaching graduate courses and assisting in setting up a rhizobium research project for that section of the country.

This same faculty member at another time, gave a series of lectures on Rhizobium genetics and physiology for the Instituto Internacional de Estudios Avanzados in Caracas.

The agronomist on the project gave talks at both the CIAT BNF Technology Workshop, Cali, Colombia, and the Bangkok BNF course taught by NifTAL personnel. The first of these was on training extension staff in BNF technology and the second was on plant/legume interactions as they affect symbiosis.

Graduate students from both the U.S. and abroad have been supported wholly or in part under this grant. A list of those so subsidized is in the Appendix. Also, grant moneys were used to underwrite the participation of foreign students at several workshops or RELARs (Latin American Rhizobium Workers Conferences) in Maracay, Venezuela, and Lima, Peru. Participants from Argentina, Brasil, Peru and Uruguay were so supported on one or another occasion.

OBJECTIVE 3. Expanded Advisory Capacity. To provide a readily available pool of capable individuals willing to provide technical assistance for the exploitation of BNF.

Several of the activities mentioned earlier, particularly under Training, might equally well be cited in this section. Virtually any contact with a developing-country technician, either here or abroad, involves providing advice on methodology, sources of materials and information, project formulation and budgeting, and the like. Each of the P.I.'s on the project have attended several of the meetings of the Latin American rhizobiologists (the RELAR). Usually each of us has presented a paper on current developments, research methods, etc. Such contacts have always resulted in a post-conference exchange of correspondence regarding the expansion of symbiotic-fixation technology in the classroom, the research plot, and the farmers' fields.

The Soil Microbiologist made a trip to the Ministry Tropical Soils station in Yurimaguas, Peru, to help them with project formulation, laboratory construction, and technique evaluation for their proposed expansion in the area of BNF. Unfortunately, this has not gone much further because of internal problems.

The advisory activities of the Rhizobium Microbiologist with colleagues in Venezuela has already been mentioned. More rapid progress in that country has been constrained by local funding and personnel changes.

The Rhizobium specialist and the Agronomist attended a planning workshop held in Trinidad. This was held in conjunction with a proposed amplification of Trinidad's long-standing relationship with Cornell University. Contacts made at that time continued to be used by both parties.

Early in the life of the project, we were involved in urging the incorporation of BNF technology in several of the CRSPs that were then in the planning stages. Specifically the Bean/Cowpea, Peanut and Tropical Soils Management Planning Groups called upon us for input on several occasions.

NCSU personnel were heavily involved in planning sessions held by NifTAL in 1976 and 1979. These were useful in structuring not only NifTAL's future activities but also in defining the roles that each of the four universities funded under this 211-d grant would cooperate.

We were invited to participate in a workshop convened by the East-West Center, Honolulu, in 1982, to establish guidelines for their activities under an NSF grant. The broad outlines of both future working papers and research projects on the impact of BNF on global agriculture were sketched at that workshop.

OBJECTIVE 4. Expanded Information Capacity. To develop a library relative to BNF and tropical legume species.

Specific activities under this objective included subsidizing subscriptions to the now-defunct Rhizobium Newsletter, formerly published in Brisbane. Our support provided subscriptions to 97 rhizobium workers in 48 different countries. Unfortunately, staffing problems resulted in the cessation of publication in 1982.

A newsletter written in a more popular style than the RN, mentioned above, was also subsidized under this grant. Published by NifTAL, Maui, Hawaii, the BNF Bulletin is now wholly supported by that organization. However, its inception was underwritten from June, 1979, to June, 1982.

The Rhizobium Microbiologist has been actively involved in trying to establish a linkage whereby appropriate abstracts could easily be made available to counterparts in Latin America. Satellite and computer linkages, as well as local printout with subsequent mailings, have been considered. Each of the options evaluated to date strain both budgets and communications systems beyond reasonable limits. He continues to explore alternative means of providing this service that is absolutely vital to keeping our Latin counterparts reasonably current with developments in BNF.

OBJECTIVE 5. Linkages and Networks. To develop and strengthen linkages in support of the above objectives.

Each of the activities mentioned previously has served to either establish a new contact or firm up a pre-existing relationship. The degree to which each of these has flourished has been more dependent on the availability of funds or the stability of professional positions than on the motivations of the people involved.

Specific and active relationships have resulted from the provision of selected strains from the "cowpea miscellany" selections to CIAT, Kenya, Tanzania, ICRISAT, IITA, the Philippines, Malaysia and Thailand. Further refinement has been achieved by coordinated testing on different peanut strains under local conditions. Those relationships are firm.

Contacts made with the MIRCEN in Brasil, with NifTAL, and with cooperators at the RELAR have resulted in on-going relationships and information exchange.

A steady stream of foreign visitors, some of whom are listed in the contacts made under the aegis of this grant have led to firm commitments, several funded by AID, with Venezuela, Colombia, Malaysia, the Philippines, Thailand, and Peru. Further, linkages with such IARCs as ICRISAT, IITA, and CIAT as well as entities such as Boyce Thompson and AVRDC are in place and on-going. Several of these have led to the submission of proposals to ROSTID/AID or other granting agencies.

Relevance to the Developing Country situation. The output from these grant activities is, for the most part, directly applicable to the crop-production situation in most developing nations today. Grant funding has provided the impetus to establishing a strengthened response capability in the areas of training of personnel at levels of expertise from the extension agent in the field to the potential PhD candidate in the lab, in developing techniques that do not require expensive hardware or high-level technology for their application, in isolating and culturing strains of rhizobia that show promise under several different tropical field situations, and in providing reference material for use by colleagues in areas where library resources are limited or non-existent.

NCSU has improved its response capability. To the degree that AID sees fit to call upon this resource we, and our colleagues in the other three universities involved, are markedly better prepared to help in the expanded exploitation of symbiotic nitrogen fixation in tropical farming situations.

APPENDIX TABLES

I. Logical Framework - Project Design Summary

II. Utilization of Grant Outputs

III. Papers published

IV. Students trained

V. Participants, Symbiotic Fixation Short-courses.

TABLE I.

PROJECT DESIGN SUMMARY

LOGICAL FRAMEWORK

Life of Project -
CY79 through CY81
Total US Funding - $361,627
Date Prepared - 5/78

Project Title: Biological Nitrogen Fixation in the Tropics

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program or Sector Goal: Improve capability to increase food production on small farms in the tropics.	Measures of Goal Achievement: Greater use of BNF under tropical conditions.	Agronomic results in cooperating countries.	LDCs give high priority and support to appropriate commodities.
Grant Purposes: To expand and utilize NCSU's response capability in BNF as applied on small farms in LDCs.	End of Project Status: Enhanced reputation as US center of excellence for BNF and tropical legume management. Amplified supportive involvement in LDC agronomic problems.	Acceptance by LDC cooperators. Peer evaluation. On site application of results.	Continued support of AID and LDCs for grant objectives. Continued commitment of NCSU to international involvement.
Outputs: 1. Expanded knowledge base on rhizobial inoculum. Improved evaluation techniques. 2. Trained cooperators in LDCs. 3. Expanded and utilized advisory capacity. 4. Accessible literature file. 5. Firm linkages and networks.	Magnitude of Outputs: 1. Applicable techniques for use in LDCs. 2. Courses and materials developed. Cooperators better educated. 3. Local staff aware of and prepared to advise on LDC problems. Availability to consult on-site in LDCs. 4. Literature maintained for all collaborators. 5. Enhanced complementarity with International Centers, LDCs, and US institutions.	1. Methodologies developed. 2. Materials prepared. Course taught. 3. Annual reports. 4. Literature on hand. 5. Trainees in place. Annual reports.	1. Cooperation of Centers and LDCs in evaluation of techniques. 2. Support by LDCs of potential trainees. Training facilities available. 3. Proper timing of requests; appropriateness of requests. 4. None. 5. Continued support of grant purpose.
Inputs: NCSU: 1. Staff with expertise in and sympathy for BNF problems in the tropics. 2. Administrative support, physical facilities, laboratories, library, office and computer availability. Consortium Institutions: 3. Continued cooperation in furtherance of stated objectives. AID: 4. Financial and administrative support.	Implementation Target: 1. Forty work-months of faculty, 156 work-months of subprofessional staff. 2. Adequate for proposed work. 3. Nine work-months or more. 4. $361,627 over a three-year period.	1. Annual reports. 2. Site visits. 3. Annual reports. 4. Institutional financial records.	1. Stability of staffing pattern. 2. Support will be provided. 3. Open collaboration within consortium. 4. AID funding and backstopping provided.

TABLE II.

UTILIZATION OF GRANT OUTPUTS

OUTPUT	PRODUCT	IMPACT	FOLLOW-UP (PAN-TROPICAL) TAB/AGR	LDC AND USAID'S	NCSU
1. Expanded knowledge base.	Methodologies appropriate to BNF research in the tropics.	Immediate application to problem-solving activities.	Publication as Technical Series Bulletins.	Direct application to local situation.	Grant funding of appropriate cooperative research.
	Scientific publications.	Detailed information available to LDC research establishments.		Specialized knowledge for direct use.	
2. Expanded education and training capabilities.	Trained personnel available on-site. Course development here and in LDC.	Better problem-oriented research.	Provide opportunity to train technicians and educate scientists in BNF and in LDCs.		Expanded and modified course offerings at NCSU.
3. Advisory capacity.	Cadre prepared to support LDC problem-solving efforts.	Solution of specific LDC problems related to BNF.	Grants, contracts, and cooperative agreements supported by AID, LDC, or other agencies.		On-going involvement with LDC research, training and extension problems.
4. Information capacity.	Up-to-date source of information related to BNF in tropics.	Availability of information, time and money saving.	Maintenance of current information bank for use by AID, LDCs, cooperators, staff and students.		
5. Linkages and networks.	Firm up current and open new contacts with interest in BNF.	Assure exchange of information, avoid duplication, enhance utilization of research results.	Strengthen AID response.	Linkage in research, training and out-reach.	Enhanced capability and involvement with BNF problems.

APPENDIX TABLE III

Papers published (at least partially grant-supported)

Ball, S. T., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. 1983. Effect of inoculum and nitrogen on yield, growth and nitrogen fixation of peanut cultivars. Field Crops Research. 6:85-91.

Chong, K., J. C. Wynne, G. H. Elkan, and T. J. Schneeweis. 1983. Effects of soil acidity, Al content and Rhizobium inoculation in growth and nitrogen fixation of peanuts and other grain legumes. Jour. Trop. Agric. (in review).

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Isleib, T. C., J. C. Wynne, G. H. Elkan and T. J. Schneeweis. 1980. Quantitative genetic aspects of nitrogen fixation in peanuts (Arachis hypogeae L.). Peanut Sci. 7:101-105.

Ligon, J. M., M. H. Scholla and G. H. Elkan. 1983. A spectrophotometric DNA-DNA hybridization technique for studying the genetic taxonomy of the cowpea Rhizobia. Proc. American Rhizobium Conf. 8:481-500.

Ligon, J. M., T. G. Isleib and G. H. Elkan. 1983. Characterization of the cowpea rhizobia incorporating DNA-DNA homology and symbiotic performance. Submitted to Appl. and Environ. Microbiol.

Mahler, R. L., and A. G. Wollum, II. 1980. Influence of water potential on the survival of rhizobia in a Goldsboro loamy sand. Soil Sci. Soc. Am. J. 44:988-992.

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Stowers, Mark D. and Gerald H. Elkan. 1980. Criteria for selecting infective and efficient strains of Rhizobium for use in tropical agriculture. N.C.A.R.S. Tech. Bull. 264. (SOTA)

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Scholla, Michael H. DNA homology among the Rhizobia.

Schroder, Eduardo Carlos. Host Specificity of Glycine max Genotypes with Antibiotic-Resistant Mutants and Phage-Typed Strains of Rhizobium japonicum.

Speidel, Karen Louise. Screening a native Rhizobium japonicum population for tolerance to acid soil stresses.

Stowers, Mark. Carbohydrate metabolism among the cowpea rhizobia.

APPENDIX TABLE IV

Students involved

Abu-Bakar, A. R., Malaysia

Ball, S. T.

Bowers, A. P.

Byalabeka B., J., Uganda

Chong, Kewi C-S., Malaysia

Farnham, M. W.

Garcia, Gabriel, Mexico

Henry, S. C., Jr.

Hallis, A. B.

Hughes, T. A.

Isleib, T. J.

Laast, J., Ghana

Ligon, J. W.

Lin, K-H., Malaysia

Mahler, R. L.

Meisner, C. A.

Mulongoy, K., Zaire

Munevar, F., Colombia

Scholla, M.

Schroder, E. C.

Sikurajapathy, M. R., Sri Lanka

de Souza, P. I., Brasil

Speidel, K. L.

Stowers, Mark D.

APPENDIX TABLE V

Participants, Short-course on Symbiotic Fixation and Legume Inoculation

Lima, Peru, May 30 - June 13, 1981

Technicians

Vicente Rimarachin Campos
José Zefia Ventura
Josué Rengifo Ruíz
José Muro Carrasco
César Marmanillo Villanueva
Roberto de la Cruz
Comber Rodríguez Cruz
Dimas Herencia
Juan Huamán
Raúl Aldana
Alfonso Cáceres F.
Cipriano Mantari Camargo
Rogelio Padilla
Justino Cutipa Condori
Leonel Cohaila Siles

June 20-July 4, 1981

Professionals

Ing. Agr. Raúl Mori Monja
Ing. Agr. Alcides Zárate
Ing. Agr. Antonio Sandoval
Ing. Agr. Antonino Lermo
Ing. Jorge Zevallos Mariazza
Ing. Flavio Torres Cárdenas
Ing. Max Millones Peso
Ing. Miguel Valdivia
Ing. Hugo Moreno Jeri
Ing. Jesús de la Cruz Rojas
Ing. Alberto Sandoval
Ing. Vidal Ortiz A.
Ing. Juan Zanabria
Ing. Diómedes Holgado
Med. Vet. Alejandro Alva Navarrete
Ing. César Cahuarjulca Gaona
Biol. Marcela de la Barrera Laca

APPENDIX TABLE V (Cont'd)

Bangkok, Thailand. May 1-13, 1983

Mr. Thawat Watkaew	Mr. Anan Pintarak
Mr. Anan Suriyagarn	Mr. Chiraphant Laongsri
Miss Pattamarat Redkachane	Miss Vinita Atipirin
Mr. Supote Chaivimol	Mr. Wirote Cholwiriyakul
Mrs. Porntip Polapukde	Mr. Waravuth Iamgumpang
Mr. Bancha Soparat	Mr. Sanayh Kraokau
Mr. Prasat Stensaovapak	Mr. Preecha Prammanee
Mr. Sompong Meeklai	Mr. Somsak Srisombun
Mrs. Narisara Kamragal	Mr. Vudhisak Pornprompratan
Mr. Pornchai Suthatorn	Mrs. Nujanart Kungpisadan