IMPROVING FOOD AND AGRICULTURAL PRODUCTION

THAILAND

BREEDING FOR SOYBEAN RESISTANCE TO ANTHRACNOSE DISEASE

UNITED NATIONS DEVELOPMENT PROGRAMME

INTERNATIONAL ATOMIC ENERGY AGENCY

VIENNA 1992
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Report prepared for
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by

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INTERNATIONAL ATOMIC ENERGY AGENCY

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MISSION REPORT

MISSION TITLE: FOLLOW-UP MISSION TO EVALUATE SEVERAL PROJECTS UTILIZING MUTATION BREEDING TECHNIQUES TO DEVELOP RESISTANCE IN SOYBEANS TO ANTHRACNOSE DISEASE

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PROJECT NO: THA/85/004-11.03
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PROJECT TITLE: IMPROVING FOOD AND AGRICULTURAL PRODUCTION WITH NUCLEAR AND RELATED TECHNOLOGY.

ASSIGNMENT DATES: OCTOBER 9, 1990- NOVEMBER 3, 1990

COUNTERPART: PATOOM SNITWONGSE
DEPARTMENT OF AGRICULTURE
BANGKEN, BANGKOK, THAILAND
FOLLOW-UP MISSION TO EVALUATE SEVERAL SUBPROJECTS UTILIZING
MUTATION BREEDING TECHNIQUES TO DEVELOP RESISTANCE IN SOYBEANS
TO ANTHRACNOSE DISEASE

Terms of Reference:
At the Department of Agriculture, Bangken, Bangkok and at
experimental sites in and around Chiang Mai and Khon Kaen
Thailand, I was engaged to carry out a follow-up mission to
evaluate and assist researchers seeking to develop soybeans with
resistance to the anthracnose disease.

Background Information:
At the time of my arrival, the project was very close to
termination, nearing the end of its 5 year term. In conversation
with Mrs. Patoom Snitwongse, the national chief technical advisor
for the project, it was indicated to me that the same group had
applied for renewal, though it was not known as yet whether or
not the project would be continued. The comments I will make in
this report are made with the probability that the original
project will be refunded in some modified form. Further, they
will reflect not only information and observations made during
the term of this mission, but will also be reflective of
perceptions gained during my previous mission made during October
of 1989. As for the present project, it was fully staffed, and
all facilities and equipment were operative and available.
Almost all researchers had irradiated lines under evaluation in field plots, some, 7-8 generations beyond their irradiation exposure. Since during the previous mission, I had spent a great deal of time observing and assisting with the evaluation of field plot experiments, I made the decision to make a stronger effort to interact with researchers in their laboratories, helping them with laboratory techniques that are supportive to the mutation breeding for the plant disease resistance objective.

Work Program: Chronological activities:

October 8-10: Travel from Auburn Alabama to Bangkok, Thailand, met at Airport by Dr. Siripong.

October 11: Meeting with Mrs. Patoom and counterparts to discuss the mission and the program prepared by counterparts in the various regions. Counterparts assigned were Drs. Chinda and Siripong for Bangkok and the Central region, Dr. Chatree (Chiang Mai University) and Mrs. Montha for the Northern Region, and Mr. Woolthisak for the Northeastern region. The time in Khon Kaen was reduced because Dr. Sanit was not available during time available at that location.

October 12: An all day visit was made to DOA's Chainat research center, where the primary activity is directed to the development and improvement of mung bean. Primary problem diseases were anthracnose, Macrophomina (charcoal rot), and Cercospora leafspot. An unusual aspect of the Macrophomina pathogen was the high frequency of fruiting in the upper plant parts that may indicate that the pathogen infects there directly, rather than moving there through the vascular system. Further
discussions were held with the station director and an entomologist who indicated problems in the region with *Heliothis* larvae and stem boring insects.

October 13: Transfer by air to Chiang Mai, met by Drs. Chatree and Vicha. Due to travel delays the Saturday program was cancelled.

October 15: Transported by Mrs. Montha to nearby Maejo and DOA's Chiang Mai Field Crops Research Center. Most of the day was spent in discussion with Dr. Supachai, the plant breeder working on soybean improvement. After touring his research plots and with further discussion the following points were concluded: Early selection criteria (M1 through M2) were directed to agronomic characteristics such as normal plants, heavy pod development, and branching. Additional criteria such as early maturity and clean leaves (no disease) were added later. Although these additional criteria might identify rust resistance, or resistance to other leaf damaging pathogens, it probably would miss stem and pod pathogen resistance. Further, by selecting those plants that mature early and have less disease on their leaves, Dr. Supachai may actually be selecting for plants that because of their short growing season give the pathogen less time to develop and that are therefore not resistant at all. The second confoundment is that if plants are maturing earlier, they may actually have more severe stem and root stress factors (greater disease). The suggestion was made that plants that had less disease usually lived longer (matured later) and that this might be a useful early selection criterion. Further comments were made that included the
suggestion that when planting mutation lines in the field for later disease severity evaluations, they should be planted with lines of similar maturity grouping, and that each maturity group planting should contain a known susceptible variety as a reference. Lines identified as disease resistant can later be back-crossed to attain the desired early maturity. Generally, after looking at Dr. Supachai's plots, I was not at all certain that any level of anthracnose resistance had been isolated. A final suggestion, given the problems in early selection criteria, was to plant saved seed from the M2 and M3 generations using the modified selection criteria suggested above.

October 16: Travel with Mrs Montha and Dr. Preecha Surin (DOA, Bangkok) to DOA's Pangda and Phrao research stations in the upland areas of North Thailand. Again, plantings included several different stages of maturity without a standard susceptible line. Suggestions were made to Mrs. Montha relating to grouping lines of similar maturity and planting a known susceptible of similar maturity to the lines under evaluation. Even given these problems there did seem to be enough differences at this site to indicate that resistance to both rust and anthracnose might be present in these plots. Samples were taken from these plots for later laboratory study. Several observations were made during the Maejo and upland station visits. These included the fact that symptoms were quite different from those that I had commonly seen in the United States. First, the anthracnose disease tended to defoliate the trifoliates well before the petiole was shed. Secondly, disease severity on the pods and petioles was much lower than in the
USA. Lastly, the severity of anthracnose-like symptoms on the petiolule (the leaf stem connecting the three leaflets) was more severe than I had previously observed.

October 17: Samples from Pangda were microscopically examined. One of the first observations made was the fact that 2/3-3/4 of the lesions on the pods were caused by *Colletotrichum gloeosporioides*, while *C. truncatum* the more recognized pathogen was much less common. There was evidence that *C. destructivum* was probably also present on the same group of pods. Although all of these pathogens are well known from the literature, and their presence in Thailand is also recognized, the high levels of *C. gloeosporioides* were unexpected, and may account for differences in symptom patterns between Thailand and the USA. Studies were initiated to determine if the frequency of the three species differs among different plant parts, and to isolate each pathogen to evaluate its individual pathogenicity.

October 18: A copy of a Statistical Analysis System (SAS 6.04) was donated to the research center by downloading the microcomputer (through the good services of Dr. Prawit Puddhanon) that I had brought along on the mission. Further a book on Soybean Diseases was donated to the center through Dr. Supachai. A seminar on soybean and peanut (groundnut) diseases with emphasis on disease epidemiology, assessment, losses, and control was presented to the researchers at the center and interested people from the Maejo Institute of Agricultural Technology. During the latter part of the day, I met with Dr. Tongchai Tonguthaisri discussing his research dealing with development of disease resistant *Phaseolus* through mutation breeding. Similar
to my observations during my previous mission, he seems to have identified useable resistance to bean rust, leafspot, and possibly *Cercospora*. His program seems to be progressing well. One comment he made did stimulate a change in methodology. He was attempting to grow bean rust on a synthetic medium, and had been unsuccessful. I advised him that no one had ever been able to culture bean rust, and the best way to secure inoculum would be to collect spores with vacuum bottles, freeze it, and later use this material for inoculum.

October 19: I was taken by Dr. Vicha Sarsud to Chiang Mai University's highland station near Chung Kien. Observations of soybeans in this area showed symptoms similar to those observed at Pangda. Some collections were made for later use. Dr. Chatree was not available, as he was attending a meeting in Mae Hong San.

October 20-21: A weekend tour of the Chiang Rai and Mae Kong River agricultural areas was arranged with Drs. Chatree and Sarsud accompanying. Many areas of rice, vegetable, tobacco, pineapple, and soybean culture were observed, with discussions centering around cropping systems during the rainy and dry seasons.

October 22: Dr. Chatree described his most recent plantings which were M2 and M3 irradiated lines. His previous plantings, which I had observed during 1989, had been severely damaged during heavy rains. He had therefore replanted his retained seed for reevaluation during 1990. In meeting with him and his student assistants, they agreed that selection for plants that lived longer as a primary selection criterion would be carried out on this year's lines. Suggestions for grouping plants by
maturity time, and planting a known susceptible line for comparison purposes will also be carried out. Dr. Chatree and I also discussed the multiple species of Colletotricum. He advised me that C. gleosporoides had many host species, many of which were locally grown crops (e.g. mango, coconut, etc.). A discussion was held on their role as possible primary inoculum sources. Since my visit in 1989, Auburn University and Chiang Mai University have developed an academic interchange agreement. I spent some time during the day interviewing a potential graduate student and advising here of financial support available from Auburn University.

October 23: Tours of Drs. Chatree and Vicha's laboratories indicated that some basic equipment was lacking or in short supply in the plant pathology department. Although they had good microscopes, they strongly need a laminar flow to maintain culture sterility when working with pathogens. The project would also benefit if the computer they presently use was equipped with a hard disc for data and program storage. In addition, they need a clean space or an incubator to grow out fungal and bacterial cultures. A preproposal to STDB (Thai Science and Technology Development Board) was developed relating to soybean disease research in the North.

October 24: Discussions continued on research techniques in evaluating anthracnose resistance in soybeans (similar to Maejo discussions). Additional time was spent preparing a grant proposal relating to biological control of plant diseases, one for STDB and a similar one for USAID. The plant pathology
department in Chiang Mai University was making every effort to initiate a research and teaching program in biological control. I departed for Bangkok in the afternoon.

October 25: An early morning flight was arranged to Khon Kaen with Mr. Wootthisak in charge of my program. We travelled to Loei, discussing soybean production practices in the region as well as diseases of peanuts and soybeans. I attended a soybean production meeting at the DOA center in Loei where I presented a short discussion on management of soybean diseases. While at Loei I discussed cotton breeding with Ms. Chuttima Panichsakphatana. Of particular interest were techniques she was using for cotton resistance screening to leaf roll virus and for soil borne disease resistance. A book on soybean diseases, and a second book on peanut diseases were donated to the DOA center at Khon Kaen. Due to Dr. Sanit being in Chiang Mai, I was unable to meet with him to discuss his program. However, on my previous visit it was obvious that he had a dynamic program on cowpeas and yard-long bean breeding that was exposing disease resistance to fungal, bacterial and viral diseases, as well as improving their agronomic traits. I returned to Bangkok on the same day.

October 26-27: These weekend days were used for report preparation, seminar preparation and touring.

October 28: A trip to Lop Buri province was arranged by Dr. Preecha Surin. There we collected soybean samples to determine severity and frequency of Colletotrichum species infections as compared to the Chiang Mai region. Collections were made at the
field crops station and 30-40 km from that location. On our return, samples were prepared by the paraquat dipping technique for later laboratory observation.

October 29: This day was spent in preparing the report.

October 30: Discussions were held with Drs. Siranut, Sman Keoboonrueng, and Arunee Wongpiyasatid, relating to field screening techniques and ways to utilize tissue culture grown plants for quick evaluations of resistance or susceptibility. The field screening techniques were similar to the discussions held in Maejo and Chiang Mai. The main points that were brought out in addition to those previously discussed was the establishment of differential cultivar sets so that races of pathogens can be determined. These researchers were not aware if the inoculum they were using for challenge was of a single race, or multiple races. This problem was evident in both Phaseolus breeding and the soybean breeding program. Ways to grow pathogens of a single race were discussed using differential cultivars as hosts. Plantings of differentials at various sites and/or collecting spores from various sites so that the race picture in Thailand could be clarified were suggested. These researchers were aware of the fact that a German researcher (Koch?) had identified 2 races of soybean rust in Thailand more than 10 years ago. He suggested that both races were present in the Northern region, while only one race was present in the central region. This would explain why the soybean cultivar (Doi Con?) released by Dr. Peerasak was resistant to rust in the south but not in the North. It also suggests that mutation research directed to developing resistance to diseases with known races
should be carefully designed so that the race to which the mutant is resistant can be identified. Further, it would be too much to expect to find double mutants resistant to 2 races, as would have had to occur to see resistance to soybean rust in the north. Dr. Siripong, in a later interview, advised me that differential cultivars were available for the soybean rust disease, and that he would make researchers aware of their availability. Dr. Sman described a method for disease screening using phytotoxins isolated from pathogens that sounded very interesting with the potential for quick accurate results. Facilities and equipment available to Drs. Arunee, Sman, and Siranut appeared to be very good, with good microscopes, tissue culture facilities and laminar flow hoods. They could use computer equipment however, and Dr. Sman could use an HPLC for his toxin work. Dr. Preecha Surin had much less suitable facilities. His microscope was poor, he did not have a laminar flow hood, and incubation facilities were poor. Should the IAEA project be renewed, some equipment money could be well spent to upgrade his laboratory. Generally speaking, DOA should build a seed storage facility for many of the promising lines they are producing. This could be a resource to the whole country assuring that promising novel germplasm would not be lost to the world.

November 1: A seminar was presented to DOA and Kesetsart University researchers on "biological control: potential for commercialization." A copy of The Statistical Analysis System was given to DOA through Dr. Amphai Satrusajang. Samples prepared on October 28 were examined, and it was decided that it was too
early to definitively determine the relative percentages of the various pathogens. DOA will evaluate these samples and mail the results to me.

November 2: Return by air to the USA (Bangkok to Atlanta, Georgia).

November 3: Atlanta, Georgia to Auburn, Alabama.
RECOMMENDATIONS:

COUNTERPART INSTITUTIONS:

At DOA's Chiang Mai crop research center, several changes in procedures are recommended if this group is to be successful in finding anthracnose resistance in soybean. First, they should select for plants that live longer in the early generations after irradiation. Secondly, they should compare promising soybean lines only to other soybean lines that have similar time requirements to reach harvest maturity, and with a known susceptible cultivar (nonirradiated) as a standard base for comparison when planting in the field. Thirdly, they will need to know which pathogen of the three Colletotrichum species is the greatest problem so that supportive laboratory challenges of germplasm can be developed. Several experiments were suggested that will help to reduce the confoundments that present experimental procedures are operating under. It was suggested to the plant breeder that he replant retained seed from M2 and M3 generations and select some lines showing extended survival (as an additional selection criterion) that might eventually be related to resistance to anthracnose or other stem or root diseases. The plant pathology program at this location could benefit by the purchase of a seed germination chamber that could double as a moist environment infection chamber. When evaluating irradiated lines for soybean rust resistance, experimenters should challenge the lines with individual races of rust. Tests
conducted in the north appear to be affected by at least two rust races, severely limiting the utility of field plantings until the race picture is clarified.

The Program at DOA Bangken, Bangkok is staffed with excellent personnel, but would benefit in the renewal period from the addition of one additional area to the project. This is the area of nematode diseases. Discussion with nematologists at this location indicated that in the upland and highland areas--wherever there are light, sandy soils--that nematodes are becoming a greater problem. Since mutation of germplasm will likely result in nematode resistance being exposed and since there is a nematode problem, this area should be evaluated. There is a high likelihood that with continued cropping of soybeans and beans that nematodes will be an increasing problem. A second need is to purchase or construct a seed storage facility with controlled humidity and temperature. Thailand's climate is too humid even under refrigeration to assure long term survival of valuable germplasm. Laboratories in Bangkok need some improvement. Mr. Preecha Surin's Lab needs new flooring, better microscopes, a laminar flow hood and at least one more incubator. On the other hand, the laboratory space and equipment at the Chainat Research Center are generally underutilized even though they are in good condition and well equipped. This stems largely from a shortage of trained manpower at that location. In the central region there seems to be a large problem caused by *Macrophomina* on soybean, mungbean and blackgram. The next
project should evaluate germplasm for resistance to this fungus. Experts on this pathogen should be brought in, and traineeships at their research locations should be developed.

Chiang Mai University has agreed to evaluate their present plantings of M2 and M3 lines for extended survival. Further, they will be observing which of the three *Colletotrichum* species are being affected by any observed resistance. Observations will also be made for plants resistant to bacterial pustule and rust. These changes should provide information to other members of the project on the relative value of proposed new methods for disease resistance assessment. Should the project be renewed, laboratories at the University should be improved by addition of a laminar flow hood so that aseptic fungal and bacterial cultures can be maintained. They also need an incubator for growing of microorganisms, and a hard disc for the laboratory computer that is already in place. Dr. Vicha Sardsud is deserving of a training period should the grant be renewed.
RECOMMENDATIONS

Agency:

Overall, the project during its first five years should be judged as a success. It has added to the general scientific infrastructure in Thailand by training numerous scientists at locations scattered across the world, frequently training these people in areas in which they were not previously well grounded. This will improve the teaching programs of Universities, and research capabilities will improve at all involved institutions. Generally, the involved scientists had very good academic credentials and should quickly incorporate their new found expertise. The scientists involved have generally performed their proposed experimental plans well. Many have identified beneficial genes offering agronomic, disease resistance, or insect resistance improvements that should serve to answer Thai agricultural problems. Where problems have been encountered, they have brought in appropriate experts to help find solutions. I make these points, because the project is ending and is up for renewal. A continuance of the project should allow many of the beneficial genes identified in plants to be incorporated into commercial cultivars. If funds are not forthcoming, progress will most certainly be slower.

Generally speaking, there is a shortage of good research books and journals at many locations. It would be beneficial if scientists sent outside the country for training could be given a large book budget, so that they can secure books and journals for their locations. If the project is renewed it should include a goal of identifying nematode resistance, particularly in crops
grown in light textured upland and lowland soils. This objective would be important in legume crops and cotton. Another soil borne pathogen that is causing damage on many crops is Macrophomina. Greater attention should be paid to this pathogen on several crops grown in Thailand. Secondly, a seed storage facility should be built at DOA so that unique germplasm exposed by these efforts would not be lost, but could be saved and made available to scientists all over the world. Such a facility could be very useful to agronomists throughout the country.
RECOMMENDATIONS

GOVERNMENT:

University scientists generally were found to have inferior laboratory facilities and were having a difficult time separating their research time from their teaching responsibilities without sacrificing some quality on the project. Again, they were well trained, but have different needs in order to function as effectively as DOA scientists. A portion of funds from the Thai government or IAEA funds might be directed to funding graduate students who can perform some of the research under their professors supervision. An alternative would be to provide technicians to these scientists. Several of the recommendations made under the institution heading, might more rightly be funded by government sources, because these institutions are funded from the government. The renewal might also consider whether a university from the southern region should also be included in the project.
SITES AND PEOPLE VISITED DURING MISSION
8/OCT/90--3/NOV/90

THAI DEPARTMENT OF AGRICULTURE, BANGKEN, BANGKOK THAILAND
Patoom Snitwongse, National Chief Technical Advisor
Siripong Kumphae, Plant Pathologist, Oil Crops
Preecha Surin, Plant Pathologist, Oil Crops
Chuteemun Panichsukpatana, Plant Pathologist, Cotton
Dr. Chinda, Cotton Breeder
Sman Keboonnnung, Plant Pathologist
Arunee Wongpiyastid, Plant Breeder
Surang Suthirawut, Plant Pathologist
Dr. Siranut, Plant Pathologist
Amphai Satrusajang, Soil Scientist

Chainat Field Crops Research Center--DOA
Charaspon Thavarasok, Director

Phra Phutthabat Field Crops Expt. Station--DOA
Amnart Chinchest, Director

Khon Kaen Field Crops Research Center--DOA
Wootthisak Budthanu, Plant Pathologist

Chaing Mai Field Crops Research Center, Maejo--DOA
Supachai Kaewmeechai, Plant Breeder
Montha Nuntapunt, Plant Pathologist
Sawant Kadkao, Entomologist
Prawit Puddhanon, Plant Breeder
Dr. Vichit, Director

Chaing Mai University, Faculty of Agriculture
Vicha Sardsud, Assistant Professor and Assistant Dean
Chattree Sitigul, Lecturer in Plant Pathology
Nakorn Nalampang, Dean of Agriculture

Kasetsart University, Faculty of Agriculture
Suebsak Sontirat, Plant Pathologist