PN-ABF-544
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FINAL TECHNICAL REPORT

SUBMITTED TO

AGENCY FOR INTERNATIONAL DEVELOPMENT

GRANT TITLE: Unconventional Gene Transfer and Somatic Cell Manipulation of Food Legumes

GRANT NUMBER: DPE-5542-G-SS-6014-00 (8-29-86 to 8-28-89)

PRINCIPAL INVESTIGATORS: David W. S. Mok and Machteld C. Mok
Department of Horticulture
Oregon State University
Corvallis, OR 97331

OVERSEAS COLLABORATORS: Luis Vieira
Fundacao Instituto Agronomico Do Parana
(IAPAR), 86100
Londrina, Parana, Brazil 23-2525

I. BACKGROUND INFORMATION:

Beans (Phaseolus species) and other food legumes are important sources of food and dietary protein worldwide. Legume improvement via conventional plant breeding has been a major objective to increase world food production. The most recent efforts include the Bean/Cowpea CRSP involving Botswana, Brazil, Cameroon, Dominican Republic, Ecuador, Guatemala, Honduras, Kenya, Malawi, Mexico, Nigeria, Senegal and Tanzania. Such efforts center on solving immediate food production problems but do not address more advanced technologies of plant cell manipulations. These techniques, when appropriately applied, can facilitate and complement conventional plant breeding. For example, desirable traits such as resistance to diseases, tolerance to environmental stress and efficient biological nitrogen fixation contained in other Phaseolus species but not present in common beans (P. vulgaris) can be incorporated via interspecific gene transfers. More specific targeting of particular genes using mutant selection and gene modification may also be applied. As income and scientific capabilities of developing countries improve, these modern tools of plant biotechnology will become relevant to the overall efforts of plant improvement. The research work of the grant merges conventional plant breeding with current techniques of cellular manipulation in an effort to solve some fundamental problems and to derive practical benefits in the form of improved bean varieties. The approaches combine basic and applied techniques used by US and foreign scientists to achieve effective technology transfer, training and utilization of the immense genetic diversity available in South America.

II. LONG TERM OBJECTIVES:

The long term goals of the grant were to achieve gene transfer between species and genera of beans via embryo and cell culture techniques and to attain plant regeneration from tissue cultures of food legumes. Specifically, interspecific hybrids involving parents of

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diverse geographic origins but adapted to South America and Africa were to be generated to facilitate gene transfer. These interspecific plants could then be used to incorporate desirable traits of undomesticated forms into the cultivated beans. Another goal of the research was to examine the biological effects of novel and embryo-specific plant hormone, on somatic embryogenesis of cell cultures of food legumes. Information derived from this portion of the research would be useful in designing methods to obtain redifferentiation from cell cultures of these large seeded legumes which is not yet possible at present. Results of the research could provide a more immediate approach to facilitate genetic exchange between species of beans and reduce some limiting factors precluding the application of current biotechnologies, such as cell selection, protoplast fusion and vector mediated gene transfer, to food legumes.

III. SPECIFIC OBJECTIVES-TECHNICAL

1. To generate interspecific hybrids of Phaseolus using embryo culture techniques:

2. To assess the causes of phenotypic reversion to parental types of P. vulgaris-P. coccineus crosses and to evaluate useful traits in hybrid populations.

3. To determine the possible causes of embryo abortion by examining cytokinin metabolism in selfed (P. coccineus and P. acutifolius) and hybrid (P. vulgaris-P. coccineus and P. vulgaris-P. acutifolius) embryos.

4. To investigate the effects of novel zeatin metabolites on embryo development and somatic embryogenesis.

IV. SPECIFIC OBJECTIVES-SCIENTIFIC COOPERATION AND TRANSFER OF TECHNOLOGY

1. To transfer methods of generating interspecific hybrids of Phaseolus to collaborating institutions with the goal of utilizing such techniques for the improvement of indigenous varieties.

2. To provide advanced training in tissue culture techniques to selected scientists from LDCs.

3. To advise on the formation of research groups dealing with genetic research of food legumes and to secure critically needed scientific instruments.

V. PROGRESS

V-A. TECHNICAL

1. To generate interspecific hybrids of Phaseolus using embryo culture techniques:

Interspecific hybrids between P. vulgaris and P. coccineus, P. acutifolius and P. lunatus have been generated with the aid of embryo culture. The parents used included North American varieties as well as

those adapted to South America (work performed at IAPAR, Brazil). Advanced progeny populations of P. vulgaris-P. coccineus and P. vulgaris-P. acutifolius were found to contain a higher degree of resistance to diseases (root rot and white mold) and more efficient nitrogen fixation (wide Rhizobium host range). A summary of the results was published in a paper entitled "Interspecific hybridization of the food legume Phaseolus: current status and potential.".

Hybrid embryos of P. vulgaris x P. lunatus usually develop only to the heart shape stage and a long embryo culture period is required to obtain hybrid plantlets. However, during the course of the research, it was discovered that some Brazilian varieties of P. vulgaris could give rise to hybrid embryos capable of growing to the early cotyledonary stage. The utilization of such germplasm shortens the time of hybrid generation considerably.

2. To assess the causes of phenotypic reversion to parental types of P. vulgaris-P. coccineus crosses and to evaluate useful traits in hybrid populations.

Interspecific hybrids of P. vulgaris-P. coccineus can be used most readily for the improvement of P. vulgaris due to the relatively high fertility, especially that of P. coccineus x P. vulgaris. However, progeny populations tend to revert back to the parental types. We were interested in determining the cause of such reversion as well as devising methods to identify individual progeny containing a high proportion of the hybrid constituion. As F_2 embryos can be either normal or abnormal, the possible relationship between embryo development and a number of biochemical markers (isozymes and DNA fragments) was examined.

Embryo development was found to correlate with specific isozyme patterns and the patterns in abnormal embryos resembled those of P. coccineus. This may provide a plausible explanation to the skewed distribution of progeny phenotypes, which may be due to the lower germination rate of seeds containing a higher proportion of P. coccineus genome. The research is continuing, using RFLP analyses to determine the genomic constitution of the two types of embryos and to identify genes controlling hybrid embryo development. Parts of this research have been presented in a publication entitled "Isozyme banding patterns and embryo development in interspecific crosses of Phaseolus".

3. To determine the possible causes of embryo abortion by examining cytokinin metabolism in selfed (P. coccineus and P. acutifolius) and hybrid (P. vulgaris-P. coccineus and P. vulgaris-P. acutifolius) embryos.

As the extent of hybrid embryo development in Phaseolus (from mature seed to four-celled embryos) is dependent on the parental species combination, we have examined the embryo-sepecific metabolism of one of the cytokinins, zeatin. Using the most diverse Phaseolus species (P. vulgaris versus P. lunatus) we were able to detect a qualitative difference in the formation of zeatin metabolites in immature embryos. For example, zeatin was converted to O-xylosylzeatin in P. vulgaris embryos whereas such conversion does not occur in P. lunatus. The intermediate species (in terms of hybrid fertility) between the two

(i.e. P. coccineus and P. acutifolius) had metabolism patterns resembling that of P. vulgaris. We are in the process of examining the metabolism of zeatin in hybrid embryos and to estimate the possible relationship between the developmental potentials of embryos and the occurrence of various metabolites. Parts of the research results were published in a paper entitled "Metabolism of ^{14}C-zeatin in Phaseolus embryos".

4. To investigate the effects of novel zeatin metabolites on embryo development and somatic embryogenesis.

A number of novel zeatin metabolites were identified in immature embryos of Phaseolus. Thus far, the major metabolite in P. vulgaris, O-xylosylzeatin, appears to be embryo specific. Due to this specificity, this chemical may be of interest in relation to redifferentiation processes of beans and other large-seeded legumes. We have synthesized sufficient quantities of this compound and found that it is biologically active in callus bioassays. However, no appreciable effect of this compound in inducing regeneration from callus was observed. In the process of pursuing this line of research, interspecific hybrid embryos of mungbeans (Vigna radiata and V. glabrescens) were found to be embryogenic. Preliminary results indicate that the adventitious embryony is associated with the interspecific nature of the embryos (selfed embryos of either species did not undergo adventitious embryogenesis). This observation in mungbean has redirected our efforts on beans and we are now focusing on interspecific hybrid embryos instead of selfed embryos. This part of the project is continuing. This research has resulted in two papers entitled "The biological activity and metabolism of a novel cytokinin metabolite, O-xylosylzeatin, in callus tissue of Phaseolus vulgaris and P. lunatus", and "Interspecific hybridization between Vigna radiata (L.) Wilczek and V. glabrescens".

V-B. SCIENTIFIC COOPERATION AND TRANSFER OF TECHNOLOGY

1. To transfer methods of generating interspecific hybrids of Phaseolus to collaborating institutions with the goal of utilizing such techniques for the improvement of indigenous varieties.

The research group under the direction of Dr. Luis Vieira of IAPAR was able to adopt techniques of embryo culture to generate interspecific hybrids of Phaseolus using indigenous varieties and land races. The primary objective of the breeding program was transfer of disease resistance. Germplasm generated has been incorporated into the conventional breeding program. Our methods of embryo culture are also being applied to other crops such as coffee.

2. To provide advanced training in tissue culture techniques to selected scientists from LDCs.

A three-week workshop in advanced tissue culture techniques was organized in 1987. Participants were research leaders from collaborating overseas institutions (Luis Vieira and F. Manetti of Brazil, S. Bose of Zimbabwe, S. Bunnay and P. Suriyajantratong of Thailand, N. Mathius of Indonesia, A. Rabakoarihanta of Madagasca, and Y. Song of Taiwan).

The topics included Aseptic techniques, Medium, Environment, Micro-propagation, Callus culturing, Somatic embryogenesis, Organogenesis, Embryo culture, Ovary and ovule culture, Anther culture, and Protoplast culture and fusion.

Drs. Bose and Rabakoarihanta were collaborators on the project in germplasm identification and have subsequently established programs on interspecific hybridization in beans. Dr. Suriyajantratong has applied similar techniques to the cowpea breeding program in Thailand.

3. To advise on the formation of research groups dealing with genetic research of food legumes and to secure critical scientific instruments.

Dr. Luis Vieira has established a food legume genetics program at IAPAR including two technicians and five assistants partly supported by the grant. In addition, scientific instruments (densitometer, scanner, spectrophotometer, gel electrophoresis sets and computers) and various research chemicals were purchased with funds of the grant. Research planning and review sessions were conducted during a visit of the US principal investigator to IAPAR and two visits of Dr. Vieira to the US (participation in the Tissue Culture Workshop and grantee's meeting at Washington D. C.). Cooperation will be continued through graduate student training and visits.

VI. PUBLICATIONS RESULTED FROM RESEARCH SUPPORTED BY THE GRANT

Mok, D. W. S., M. C. Mok and J. R. Baggett. 1987. Interspecific hybridization of the food legume Phaseolus: current status and potential. In: The Breeding of Horticultural Crops. Ed. J. B. Petersen. pp. 18-30.

Mok, M. C., D. W. S. Mok, K. E. Marsden and G. Shaw. 1987. The biological activity and metabolism of a novel cytokinin metabolite, O-xylosylzeatin, in callus tissue of Phaseolus vulgaris and P. lunatus. J. Plant Physiol. 130:423-431.

Mok, D. W. S. and M. C. Mok. 1987. Metabolism of ^{14}C-zeatin in Phaseolus embryos. Plant Physiol. 84:596-599.

Guo, M., D. W. S. Mok and M. C. Mok. 1989. Isozyme banding patterns and embryo development in interspecific crosses of Phaseolus. J. Hered. 80:29-32.

Submitted by:

David W. S. Mok Professor	9-7-89 Date	Machteld C. Mok Professor	9-7-89 Date

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X

November 5, 1987

Dr. Harvey Hortick
ST/AGR
Room 420, SA-18
Agency for International Development
Washington, D. C. 20523

RE: DPE-5542-G-SS-6014-00

Dear Dr. Hortick:

Enclosed are:

1. progress report No. 4 covering the period from July 1 to September 30, 1987;

2. a copy of the program of Legume Tissue Culture Workshop for our overseas collaborators;

3. reprints of two papers where AID support is acknowledged and

4. form SF 269

Best regards.

Sincerely,

David W. S. Mok
Professor of Genetics

Oregon State University is an Affirmative Action/Equal Opportunity Employer

PROGRESS REPORT

SUBMITTED TO

AGENCY FOR INTERNATIONAL DEVELOPMENT

GRANT TITLE: Unconventional Gene Transfer and Somatic Cell Manipulation of Food Legumes

GRANT NUMBER: DPE-5542-G-SS-6014-00

PRINCIPAL INVESTIGATORS: David W. S. Mok and Machteld C. Mok
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Corvallis, OR 97331

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(IAPAR), 86100
Londrina, Parana, Brazil 23-2525

PROGRESS REPORT NO. 4

Period: from July 1, 1987 through September 30, 1987

TECHNICAL:

Specific objective 1: To generate interspecific hybrids of Phaseolus using embryo culture techniques:

Interspecific hybrids of P. vulgaris and P. acutifolius are being generated in large numbers using the pod culture methods (see Progress Report No. 3). Hybrid embryos can be stimulated to germinate precociously in the pod. Rooting of the germinating plantlets is achieved by either direct treatment with mild concentration of auxins or by putting the plantlets on medium containing the growth regulator. To assist the identification of plants containing mixed genomes of both parents in later progeny generations, we are in the process of selecting isozymes as biochemical markers.

Specific objective 2: To assess the causes of phenotypic reversion to parental types of P. vulgaris-P. coccineus crosses and to evaluate useful traits in hybrid populations.

Four isozymes (6-phosphogluconate dehydrogenase, malic dehydrogenase, esterase and acid phosphotase) exhibiting polymorphism between P. vulgaris and P. coccineus have been used to analyse the genomic relationship in F_1 and F_2 populations of P. vulgaris-P. coccineus hybrids. Abnormal (shrunken and under-developed embryos) and normal embryos can be clearly distinguished by the characteristic banding pattern. Over one hundred F_2 progeny embryos have been examined and the initial observation was substantiated. These findings will be used for the early detection and selection of embryos containing a higher proportion of hybrid genome to enhance introgression between the

two species. The hypothesis will be further tested in the reciprocal cross, P. coccineus x P. vulgaris.

Specific objective 3: To determine the possible causes of embryo abortion by examining cytokinin metabolism in selfed (P. coccineus and P. acutifolius) and hybrid (P. vulgaris-P. coccineus and P. vulgaris-P. acutifolius) embryos.

The physiological basis of hybrid embryo abortion in P. vulgaris - P. acutifolus crosses was examined via pod culture. As selfed pods of both species can be successfully performed and mature seeds obtained from these pod culture, the methods and medium composition are appropriate to support in vitro development of Phaseolus embryos. However, identical procedures did not change the development of hybrid embryos; i.e. instead of mature seed, hybrid embryos germinate precociously. These results indicate that the arrest of hybrid embryo growth is not due to insufficient nutrient supply in vivo or to the presence of inhibitors. Most likely, the omission of seed maturation phase of the hybrid embryo is due to intrinsic genetic factors. Future experiments will involve the application of novel zeatin metabolites via pod culture to examine if further embryo development can be achieved.

Specific objective 4: To investigate the effects of novel zeatin metabolites on embryo development and somatic embryogenesis.

The addition of the new cytokinin metabolite, O-xylosylzeatin to callus cultures to examine its effects on somatic embryogenesis was performed. No appreciable difference was observed. The next sets of experiments will include different explants, using immature embryos and leaves for adventitious embryo formation in addition to callus tissues.

COORDINATION AND COMMUNICATION:

1. A Tissue Culture Workshop was conducted for overseas collaborators (September 15 to 23, 1987) . Drs. Luis Vieira and Filho Manetti from LAPAR and legume project leaders, Drs. Aimee Rabakoarihanta (Madagasca), S. Bose (Zimbabwe), P. Suriyajantratong (Thailand), S. Bunnay (Thailand), S. Yu (Taiwan), Marita Mathius (Indonesia) and T. Sekioka (Hawaii) participated. The detail program of the Workshop is also enclosed. Laboratory techniques in tissue culture, protoplast fusion, enzyme extraction, electrophoresis, germplasm conservation, virus eradication techniques were taught by the principal investigators and other OSU scientists (refer to program). In addition, the commercial application of tissue culture in the production and research of vegetables, field crops and woody species were examined by touring seed companies and nurseries. Progress reports were given by collaborators and future research plans were developed.

Signatures: 11-5-87 11-5 '87

David W. S. Mok Professor	Date	Machteld C. Mok Professor	Date

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DEC 31 1987

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July 31, 1987
Dr. Harvey Hortick
ST/AGR
Room 420, SA-18
Agency for International Development
Washington, D. C. 20523

RE: DPE-5542-G-SS-6014-00

Dear Dr. Hortick:

Enclosed is a copy of the **Progress Report No. 3** covering the period of April 1 to June 30, 1987. The SF 269 is also attached.

I hope the Progress Report No. 2 and the letter dated April 17 were received. As I did not hear from your office as late as June, a duplicated packet was sent on June 8. At your earliest convenience, please review my request contained in the April 17 letter and let us know of your thoughts. Thank you and best regards.

Sincerely

David W. S. Mok
Professor of Genetics

Oregon State University is an Affirmative Action/Equal Opportunity Employer

PROGRESS REPORT

SUBMITTED TO

AGENCY FOR INTERNATIONAL DEVELOPMENT

GRANT TITLE: Unconventional Gene Transfer and Somatic Cell Manipulation of Food Legumes

GRANT NUMBER: DPE-5542-G-SS-6014-00

PRINCIPAL INVESTIGATORS: David W. S. Mok and Machteld C. Mok
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OVERSEAS COLLABORATORS: Luis Vieira
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Londrina, Parana, Brazil 23-2525

PROGRESS REPORT NO. 3

Period: from April 1, 1987 through June 30, 1987

TECHNICAL:

Specific objective 1: To generate interspecific hybrids of *Phaseolus* using embryo culture techniques:

The development of interspecific hybrid embryos of *P. vulgaris* and *P. acutifolius* was greatly enhanced using a pod culture technique (see Progress Report No. 2). Hybrid embryos can now be directly incubated after excision to stimulate precocious germination. The significance of these new procedures is the elimination of the more tedious embryo culture step. Moreover, large number of interspecific hybrids can now be obtained in much shorter time (four to five weeks after pollination). These findings are particularly relevant in developing countries where equipment and chemicals for embryo culture may be limited. We are now expanding the tests to include more genotypic combinations.

Specific objective 2: To assess the causes of phenotypic reversion to parental types of *P. vulgaris*-*P. coccineus* crosses and to evaluate useful traits in hybrid populations.

Five isozymes (6-phosphogluconate dehydrogenase, malic dehydrogenase, lactate dehydrogenase and acid phosphotase) are currently being used as biochemical parameters to ascertain genomic relationships in F_1 and F_2 populations of *P. vulgaris*-*P. coccineus* hybrids. Abnormal embryo development (shrunken and under-developed embryos) was found to correlate with particular isozyme patterns. Analyses of the progeny populations of one particular cross will be completed in the next three to four months.

Specific objective 3: To determine the possible causes of embryo abortion by examining cytokinin metabolism in selfed (P. coccineus and P. acutifolius) and hybrid (P. vulgaris-P. coccineus and P. vulgaris-P. acutifolius) embryos.

The metabolism of ^{14}C-labeled zeatin in embryos of P. coccineus cv. Scarlet Runner and P. acutifolius PI. 321637 has been examined. Two new metabolites were isolated from P. coccineus embryos. They were identified as O-xylosyldihydrozeatin and its riboside. Embryos of P. acutifolius form zeatin metabolites similar to those isolated from P. vulgaris (publication in press). These findings suggest the possible presence of a cytokinin reduction enzyme in P. coccineus embryos. Preliminary experiments are underway to isolate the enzyme from this species and to determine if similar enzyme activity is absent from P. vulgaris embryos. If this tentative hypothesis is proven correct, the genetic difference in cytokinin metabolic enzymes between different Phaseolus species may be one of the causes of abnormal growth of interspecific hybrid embryos.

Specific objective 4: To investigate the effects of novel zeatin -- metabolites on embryo development and somatic embryogenesis.

The novel zeatin metabolite O-xylosylzeatin, isolated from embryos of P. vulgaris, and its radioactively labeled isotope have been chemically synthesized (publication in press). We have completed the tests of its biological activity using callus bioassays. The activity of this novel compound was 30 to 100 times more active than zeatin in P. vulgaris callus culture and was equally active as zeatin in P. lunatus tissues (publication in press). We will be testing its effects on embryo development and embryogenesis from callus cultures.

COORDINATION AND COMMUNICATION:

1. Embryo culture techniques and the updated methods of pod culture and embryo incubation have been passed on to collaborators in Brazil to facilitate the generation of interspecific hybrids using locally adapted genotypes as parents.

2. Preliminary preparations for the tissue culture workshop (September 15 to 23, 1987) have been completed. Dr. Luis Vieira and Mr. Manetti from IAPAR will be participating. In addition, legume project leaders, Drs. Aimee Rabakoarihanta (Madagasca), S. Bose (Zimbabwe), P. Suriyajantratong (Thailand), S. Bunnay (Thailand), S. Yu (Taiwan), T. Sekioka (Hawaii) and W. Asano (Hawaii) will also attend.

Signatures: 7-15-87 7-15-87

David W. S. Mok Professor	Date	Machteld C. Mok Professor	Date

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DEC 31 1987

6.185

February 3, 1987
Dr. Harvey Hortick
ST/AGR
Room 420, SA-18
Agency for International Development
Washington, D. C. 20523

Reports 1, 2, 3 & 4

RE: DPE-5542-G-SS-6014-00

Dear Dr. Hortick:

Enclosed is a copy of the Progress Report (No. 1) for the grant entitled "Unconventional Gene Transfer and Somatic Cell Manipulation of Food Legumes". The report covers the period from August 29, 1986 through December 31, 1986. A copy of form SF 269 is also attached to the report as required.

The project is progressing well and I am planning on visiting Dr. Luis Vieira at IAPAR in March. As prior approval to countries involved in the project including Brazil has been granted, I am seeking in country travel approval by telex with the AID mission in Brazilia via the US Embassy. However, I was told that the position vacated by Dr. Howard Lusk is not yet filled. I wonder if this situation will dalay the response. Any advice you may have will be greatly appreciated. Best regards.

Sincerely

David W. S. Mok
Professor of Genetics

JAN 1 1 1988

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PROGRESS REPORT

SUBMITTED TO

AGENCY FOR INTERNATIONAL DEVELOPMENT

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GRANT NUMBER: DPE-5542-G-SS-6014-00

PRINCIPAL INVESTIGATORS: David W. S. Mok and Machteld C. Mok
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PROGRESS REPORT NO. 1

Period: from August 29, 1986 through December 31, 1986
(Award Notification received on September 17, 1986)

TECHNICAL:

Specific objective 1: To generate interspecific hybrids of *Phaseolus* using embryo culture techniques:

Seeds of selected genotypes of *P. vulgaris* adapted to Brazil and resistant to white mold and root rot have been obtained. Interspecific crosses between these selections and genotypes of *P. coccineus* and *P. acutifolius* will be attempted. Since it has been demonstrated by previous research that the development of interspecific hybrids is determined by the *P. vulgaris* parent, the diverse origins of the South American genotypes will facilitate the recovery of normal hybrids.

Specific objective 2: To assess the causes of phenotypic reversion to parental types of *P. vulgaris*-*P. coccineus* crosses and to evaluate useful traits in hybrid populations.

Protocols for the electrophoretic analyses of proteins and isozymes of immature selfed embryos have been developed. Protein patterns (on native and SDS gels) and selected isoenzymes will be used as markers to distinguish interspecific parents. The genomic relationship of the hybrid embryos to the parents will be estimated using these biochemical markers, and will be correlated with the extent of the embryo development.

Specific objective 3: To determine the possible causes of embryo abortion by examining cytokinin metabolism in selfed (*P. coccineus* and *P. acutifolius*) and hybrid (*P. vulgaris*-*P. coccineus* and *P. vulgaris*-*P. acutifolius*) embryos.

The metabolism of ^{14}C-labeled zeatin in embryos of *P. coccineus* cv. Scarlet Runner and *P. acutifolius* PI. 321637 is under investigation. The metabolites are being identified and will be compared with those previously obtained from embryos of *P. vulgaris* and *P. acutifolius*.

Specific objective 4: To investigate the effects of novel zeatin metabolites on embryo development and somatic embryogenesis.

The novel zeatin metabolites (O-pentose of zeatin and ribozylzeatin, discovered in embryos of *P. vulgaris*) have been identified as O-xylose of zeatin and its nucleoside (by isolating the enzyme (UDPxylose-zeatin xylosyltransferase) catalyzing the reaction *in vitro* and determining the pentose donor (UDPxylose)). We are in the process of determining methods to chemically synthesize sufficient amounts of these compounds in order to test the biological activities and their effects on embryo development and somatic embryogenesis.

COORDINATION AND COMMUNICATION:

1. Appropriate accounting procedures were set up with Dr. Luis Vieira of IAPAR. Equipment required to perform appropriate experiments at IAPAR were selected jointly by US and Brazilian investigators. Orders have been placed for such items (small centriguge, pipetter, electrophoresis set, densitometer and accessories).

2. Discussions with collaborators in Brazil, Madagascar and Zimbabwe resulted in the decision to hold a hands-on work shop on tissue cultures for overseas cooperators and their colleagues. It will most likely be in September 1987. Some details need to be finalized.

3. David Mok presented an invited paper entitled " Interspecific Hybridization of the Food Legume, *Phaseolus*: Current Status and potentials" at the International Symposium on Horticultural Breeding held in Taiwan. Companion visits to AVRDC, National Taiwan University and National Chung Hsing University regarding legume research programs have been beneficial. Potential collaborators have been identified and exchange of genetic materials will be arranged.

Signatures: 2-4-87 Machteld Mok 2-4-'87

David W. S. Mok Professor	Date	Machteld C. Mok Associate Professor	Date