PROJECT PROPOSAL

Genome Characterization of Whitefly-Transmitted Geminivirus of Cotton and Development of Virus-Resistant Plants through Genetic Engineering and Conventional Breeding

CFC/ICAC 07

Location of Project and Countries where Developed Technologies can be Applied[1]

[1] The colors, boundaries, denominations, and classifications in this map do not imply, on the part of the Common Fund for Commodities or its Members, any judgment on the legal or other status of any territory, or any endorsement or acceptance of any boundary. The projections used for maps may distort shape, distance, and direction.

GENOME CHARACTERIZATION OF WHITEFLY-TRANSMITTED GEMINI-VIRUSES OF COTTON AND DEVELOPMENT OF VIRUS-RESISTANT PLANTS THROUGH GENETIC ENGINEERING AND CONVENTIONAL BREEDING

Financing Summary

Sponsoring ICB	:	International Cotton Advisory Committee (ICAC)
Recipient	:	ICAC
Project Executing Agency	:	National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
Supervisory Body	:	ICAC
Location of the Project	:	Pakistan, with supportive research in the United Kingdom and the United States of America
Duration of the Project	:	Five years
Objective and Scope of the Project	:	Whitefly-transmitted (WFT) geminiviruses are recognized as the most widespread and most severe viral pathogens of cotton, and they are increasingly developing resistance against presently used chemical pest control methods. WFT-geminiviruses are known to cause, *inter alia*, cotton leaf crumble disease, cotton leaf curl disease and other leaf distortions responsible for severe cotton yield reduction. Based on existing knowledge and experience in the field of biotechnology and genetic engineering, research will be undertaken to define and characterize the biological and genetic particularities of the WFT geminiviruses; collect, characterize and preserve germplasm resistant to cotton viruses; develop transformation and regeneration systems for selected cotton varieties; produce geminivirus-resistant genotype of cotton; make available geminivirus-resistant genotype of cotton to interested cotton growing countries for hybridization to develop commercial varieties

resistant to virus attacks; produce a technical manual and disseminate the technology. The project will be focused on economically important WFT geminiviruses, in particular on the cotton leaf curl viral (CLCuV) disease, which has already caused massive damage in various cotton producing countries (e.g. Egypt, Nigeria, Pakistan and Sudan).

Total Project Cost	:	US$ 3,926,518		
Amount of Fund Financing	:	Grant: SDR ..... (equivalent to approximately US$ 1,549,770)		
Counterpart Contribution (by Sources)	:	Pakistan	US$	1,324,629
		United Kingdom	US$	791,226
		USA	US$	245,608
		ICAC	US$	15,285

		Total	**US$**	**2,376,748**

Previous Assistance to the Sponsoring ICB:

(1)

Title of Project	:	Study of Cotton Production Prospects for the Nineties
Amount of Assistance	:	SDR 389,879 (Grant)
Board Approval Date	:	13 October 1992
Effectiveness date	:	25 November 1992
Closing Date	:	31 March 1995

(2)

Title of Project	:	Integrated Pest Management for Cotton
Amount of Assistance	:	SDR 2,192,274 (Grant)
Board Approval Date	:	29 March 1994
Effectiveness Date	:	21 September 1994
Closing Date	:	30 September 1998

(3)

Title of Project	:	Integrated Pest Management of the Cotton Boll Weevil
Amount of Assistance	:	SDR 1,360,329 (Grant)
Board Approval Date	:	7 September 1994
Effectiveness Date	:	10 February 1995
Closing Date	:	30 September 2000

ABBREVIATIONS AND ACRONYMS

CLCrV	**-**	**Cotton Leaf Crumple Virus**
CLCuV	**-**	**Cotton Leaf Curl Virus**
CRI	**-**	**Cotton Research Institute (Faisalabad, Pakistan)**
DNA	**-**	**Deoxyribonucleic Acid**
ICAC	**-**	**International Cotton Advisory Committee**
ICB	**-**	**International Commodity Body**
IPM	**-**	**Integrated Pest Management**
NIBGE	**-**	**National Institute for Biotechnology and Genetic Engineering (Faisalabad, Pakistan)**
PCR	**-**	**Polymerase Chain Reaction**
PEA	**-**	**Project Executing Agency**
RNA	**-**	**Ribonucleic Acid**
SB	**-**	**Supervisory Body**
SDR	**-**	**Special Drawing Rights**
SOE	**-**	**Statement of Expenditure**
TOR	**-**	**Terms of Reference**
WFT viruses	**-**	**Whitefly-transmitted viruses**

APPRAISAL AND RECOMMENDATION OF THE MANAGING DIRECTOR TO THE EXECUTIVE BOARD

Genome Characterization of Whitefly-Transmitted Geminiviruses of Cotton and Development of Virus-Resistant Plants through Genetic Engineering and Conventional Breeding

1. The Managing Director hereby submits the following Appraisal and Recommendation on a proposed financing assistance to the International Cotton Advisory Committee (ICAC) for SDR ..... (equivalent to approximately US$ 1,549,770)[2] in the form of a grant to assist in financing the project "**Genome characterization of whitefly-transmitted geminiviruses of cotton and development of virus-resistant plants through genetic engineering and conventional breeding**". The International Cotton Advisory Committee would be the Supervisory Body.

PART I. INTRODUCTION

A. Project Background

2. The project was first submitted to the Fund by the International Cotton Advisory Committee in December 1993 and presented to the Consultative Committee at its Tenth Meeting, in April 1994. The Committee recognized that the WFT viruses are a rapidly spreading menace for the vast majority of cotton growing areas and observed that the development of disease resistant varieties was relevant and supportable. The Committee noted the experience and expertise of the institutes involved. The Committee sought clarification on the technical basis for applying genetic engineering for cotton breeding, and the elaboration of processes for producing genetically engineered cotton resistant to whitefly-transmitted geminiviruses; as well as to specify possible complications with regard to the regeneration of specific cotton varieties.

3. A revised version of the proposal was presented to the Committee at its Twelfth Meeting in January 1995. The Committee concluded that the revised proposal largely met the concerns expressed by the Tenth Consultative Committee Meeting. The Committee noted in particular that, in spite of its highly technical and scientific nature, the objective of the project appears feasible, given the recent progress made in this field of research. An elaboration of mid-term evaluation arrangements were recommended. The Committee agreed with the objectives, scope and design of the project. The Committee concluded that the project adequately falls within the formulated strategy of the ICAC and that the project is in accordance with the objectives and the mandate of the Fund. The Committee, therefore, recommended that the project be submitted to the Executive Board for consideration and approval.

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[2] For SDR valuation of the project, please see document CFC/EB/13/INF.5 (copy attached).

B. Overview of the Commodity and the Related Background[3]

4. Production and Demand - Cotton is a major agro-industrial crop produced in developing countries as well as in developed ones. The developed countries account for 55% of the world cotton output. Total world output was recorded at 18.47 million tons in 1994 and estimated to reach 19.32 million tons in 1995. The United States and mainland China are expected to produce 44.5% of the 1995 output. Forecasts for increases in output during the last half of this decade from the USA, India, Pakistan, Australia, Argentina, China, Brazil, Egypt, Turkey and the Central Russian Republics, however, may not materialize due to boll-worm pest in China and leaf curl virus damage to the cotton crops in India and Pakistan. Output in Uzbekistan, one of the world's five biggest cotton producers, has fallen due to environmental degradation and economic difficulties[4]. Based on the average of the last three years' figures, cotton is one of the few major commodities whose global production more or less match world mill consumption. In 1995, production and demand for cotton are expected to be in balance with consumption estimated at 19.31 million tons against 19.32 million tons of production.

5. The cotton market is dominated by major consumers, with China accounting for about one-fourth of the world's mill consumption. Other major buyers of cotton are South Korea, Taiwan, Japan and the EU, with USA coming out as a net exporter despite its high mill consumption. Mill consumption in the developing countries, particularly in Africa, is limited due to the lack of modern textile mills.

6. Due to the decline in China's production in 1995, this country is expected to import 600,000 tons of cotton to meet its domestic demand. This is a considerable volume as it represents 10% of the world exports. Three years ago, China was a net exporter but this shift in China's trade accounted for up to one half of the 70% increase in world prices in the past three years[5].

7. Consumption and Stocks - ICAC's cotton consumption forecast for this year is at 18.9 million tons, 400,000 tons above this year's forecasted production and hence, global stocks are expected to fall from 6.9 million tons last year to 6.8 million tons this year. Although production is expected to rise to 19.3 million tons next year, consumption is also expected at about the same level as the anticipated output. Thus the current situation will maintain a strong market pressure on global cotton stocks as the world economic growth gains momentum. The expected 10% drop in Chinese cotton production to about 4 million tons could also keep imports high until the end of the crop season in August 1995.

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[3] See Appendix I for basic commodity data.

[4] According to ICAC, harvested area in Uzbekistan fell by one-fourth between 1987/88 and 1993/94 crop seasons and reached 1.53 million hectares. The Government of Uzbekistan has set a planting target of 1.5 million hectares in 1995, 2% less than actual planted area in 1994. Less cotton will be available from Central Asia under barter arrangements and could lead to increasing prices for Central Asian cotton in Europe.

[5] Cotton: Review of the World Situation, published by ICAC (selected issues: September-October 1994; November-December 1994 and January-February 1995) Washington, D.C. U.S.A.

8. Prices and Prospects - The major market for cotton trading is the New York Cotton Exchange. Prices in this market serve as reference for other centres of cotton markets. Regional cotton trading centres, such as the Bombay Cotton Exchange, use the New York Cotton Exchange for their price discovery mechanism. Cotton prices in 1995 are expected to reach their average highest level in 11 years due to expected production shortfall, compounded by a sharp increase in imports and shrinking global stocks. ICAC estimated that world cotton prices would average about 88 cents a pound between August 1994 and July 1995, rising to 90 cents per pound next season. As at 1 February 1995, however, the Cotlook "A" Index (the industry bench mark), already reached 98.8 cents a pound compared with 58 cents a pound three years ago. The last time the Cotlook 'A' Index broke through the $ 1.00 mark was in September 1980, but industry analysts and statisticians predict that this barrier will be broken before the end of the second quarter 1995.

9. As at 16 February 1995, the New York Cotton Exchange settlement price for March cotton was quoted at 95.95 US cents per pound at 50,000 pounds lot-size on heavy volume of trading. Quarterly projection based on the futures market indicates a relatively firm market for the next three quarters, with only a 3.5-cent price differential for July 1995 cotton, with a relatively narrow spread (less than one cent) between bid/ask quotations.

C. Project Related Institutions

10. The International Cotton Advisory Committee, which is the sponsoring agency, will be the Supervisory Body. It has the capacity and resources to assume the supervisory role for this project. The ICAC is an association of governments having an interest in the production, export, import and consumption of cotton. It is an organization designed to promote co-operation in the solution of cotton related problems, particularly those of international scope and significance. Its members account for more than 80% of the world's cotton exports and for more than half of the imports.

11. One of the key concerns of the members of the ICAC is the ongoing competition with synthetic fibres. Lower relative cotton prices vis-à-vis those of synthetic fibres should ensure that cotton remains competitive. In order to maintain or possibly increase the competitiveness of cotton, producer incomes, as well as the market share of cotton, yields (both in quantity and quality) will have to increase while costs of production are reduced. The members of the ICAC have, therefore, given highest priority to projects which address the negative impacts caused by cotton diseases (e.g. loss in yield, increased costs of disease control, reduced quality of cotton and increased use of costly and environmentally hazardous chemical pest controls). Given the fact that most developing countries *cum* cotton producers lack the means of carrying out their own advanced research in this field, the ICAC has acknowledged the importance of exchange of technical information between member countries and the need for greater co-operation in the solution of mutual problems. The proposed project is an example of this prioritization and the recommended international co-operation.

12. The National Institute for Biotechnology and Genetic Engineering (NIBGE) located in Faisalabad (Pakistan) will be the Project Executing Agency (PEA) for this project. The Institute is a federal research institute, engaged in fundamental and applied research in the areas of health, agriculture, environment and industry. It has adequately trained scientific and technical staff and

has experience in networking and international co-operation. The institute is headed by a director. The activities envisaged by this project are closely related to activities presently ongoing in the Institute. The project will thus benefit from the experiences already gained. The Institute is autonomous in its functions and is controlled by a governing body having local scientists and external experts as members. The director as the Chief Executive, will be responsible for the project and will act as the project manager responsible for the overall co-ordination and implementation of the project.

13. The two key collaborating scientific institutes in this project are the John Innes Centre (Norwich, United Kingdom) and the Department of Plant Sciences, University of Arizona (Tucson, Arizona, United States of America). Both institutes have extensive experience in studying and elucidating biological and (molecular) genetic characteristics of whitefly-transmitted geminiviruses. The proposed activities in the project (and in particular the pertinent activities for which the primary responsibility is assigned to both institutes) are an integral part of their ongoing research work. Good working relations already exist and frequent exchange of staff has taken place between the John Innes Centre, the Department of Plant Sciences and the NIBGE. The University of Arizona will be represented by the Head of the Department of Plant Sciences, while the John Innes Centre will be represented by the Head of the Department of Virus Research.

14. In addition to the three institutes mentioned, the project will closely involve the Cotton Research Institute (CRI) in Faisalabad, Pakistan, which belongs to the Punjab Government and which has been engaged in breeding new cotton varieties over many years. CRI has adequate staff trained in plant breeding, agronomy, entomology, plant pathology and fibre technology. It has infrastructure for large scale experimentation under field conditions. CRI maintains a wealth of cotton germplasm collections ready for screening. Between NIBGE and CRI close working relations exist, in particular with regard to germplasm provision and epidemiological studies. These working relations extend also to the Central Cotton Research Institute in Multan, Pakistan. Other organizations involved (be it less closely in the core activities of the project) are the Advanced Centre for Plant Virology in New Delhi, India and relevant organizations like the USDA and the FAO.

D. Previous Support to the Commodity/ICB

15. The Executive Board has approved earlier three projects which were sponsored by the International Cotton Advisory Committee. The first one, entitled: "Study of Cotton Production Prospects for the Nineties", was approved in 1992. It consists of a nine country/case study examining the factors contributing to the differential cotton industry performance and it will outline the present and future challenges as well as the opportunities facing the cotton industry in the countries that are being studied. Particular attention will be given to the apparent influence of government policies on the strategic patterns of development and adoption of technology, on farm-level productivity and on the efficiency and effectiveness of processing and marketing operations. The Study is particularly policy oriented. The draft country reports and related analysis were presented at an international workshop held in Egypt, in November 1994. The project is envisaged to be completed in March 1995. It has been implemented in a satisfactory way and the ICAC has adequately provided the necessary supervision.

16. The second project, entitled: 'Integrated Pest Management for Cotton', was approved in March 1994. The project focuses on the development of environmentally sound pest management practices aimed at reducing the impact of two major cotton pests, namely, the

sweet potato whitefly and the cotton aphid, which are responsible for the contamination of cotton with a sticky honeydew. This contamination has a negative impact on both yield and quality of the cotton. The project is located in Israel and Egypt, with field trials in Ethiopia and Zimbabwe. The project is operational since September 1994.

17. The presently proposed project and the IPM project are related in the sense that both deal with the same insect, i.e. the whitefly. The IPM project is meant to develop a less expensive and environmentally safe technology to control the whitefly, in order to avoid or reduce stickiness in cotton. For this purpose, it is envisaged that thresholds of 4 - 5 whiteflies per leaf will be recommendable and achievable. In the case of viruses transmitted by the whitefly, infection can still occur in a threshold as low as one whitefly per leaf. The present project is thus not meant specifically to control the whitefly, but its objective is to understand the geminiviruses and to develop resistance to these whitefly-transmitted viruses. Transgenetic plants developed in the proposed project will be resistant to the geminiviruses and provide a sustainable, long-term solution for the protection of the cotton crop. The proposed project is therefore complementary.

18. The third project 'IPM of the Cotton Boll Weevil in Argentina, Brazil and Paraguay', was approved in September 1994 and is expected to be operational as of March 1995. The project aims in particular at the development and subsequent introduction of integrated pest management methods for the control of the cotton boll weevil. Focus of the project activities is on the three countries indicated above, as they are under severe threat of this cotton pest. This project is expected to start by mid-April 1995.

19. The total amount of funding made available for these three cotton projects is SDR 3,942,482 which is approximately 24% of the total assistance of the Fund for all approved projects as at 31 December 1994.

PART II. PROJECT DESCRIPTION

A. Project Rationale and Objectives

20. Whitefly-transmitted (WFT) geminiviruses are a major threat to the productivity and quality of cotton grown in the subtropical and tropical regions of the world. The present wide spread of diseases caused by WFT geminiviruses is expected to increase in importance as a result of the development of more and more resistance to insecticides, which are so far used to control the *Bemisia tabaci* (whitefly) known to be the major transmitter of geminiviruses. WFT geminiviruses are now recognized as the most abundant and most severe viral pathogens of cotton, worldwide. The most important diseases known to be transmitted by the whitefly vector are the cotton leaf curl viral (CLCuV) disease and the cotton leaf crumple viral (CLCrV) disease. The CLCuV has previously caused heavy losses in cotton producing countries like Sudan, Egypt and Nigeria. Recent years have already shown an upsurge in the recorded outbreaks of the whitefly vector, leading to dramatic losses in cotton production in Pakistan, Chad, India and Mali as a result of the virus epidemics. Other affected countries are the USA, the Dominican Republic, Guatemala, Nicaragua and Togo.

21. In particular, the situation in Pakistan is serious and could be illustrative of the impact of WFT geminiviruses on cotton production. The whitefly transmitted CLCuV disease in Pakistan has spread at increasing speed. In 1987 it was considered a minor disease, but in 1992/1993 it covered an area of over 420,000 hectares of the 2.8 million hectares used for cotton production.

The infected area increased the next year to 1.2 million hectares. The impact on cotton production was a fall in production from 2.2 million tons in 1991/1992 to 1.6 million tons in the next year, and a further reduction to 1.3 million tons in 1993/1994. This implies a fall in production of 40% over two years; an amount which is recorded as having an estimated commercial value of about US$ 1,285 million.

22. Although there is some evidence that the destructive 'B' biotype of *B. tabaci* is present in the Middle East and possibly India, most evidence suggests that indigenous populations are more virulent because of population pressures resulting from insecticide failures. It is, however, now known that *B. tabaci* populations from any world locations are capable of transmitting geminiviruses, despite the differences in biological or behavioural characteristics. Thus, there is a great potential for the introduction of exotic WFT geminiviruses throughout the world through whiteflies transported on plants in trade. Hence, the development of virus resistance in cotton now requires a cooperative approach between international laboratories. A current problem is the lack of information about the biology and genetics of most of these viruses, thus a concerted effort is now needed to characterize these viral pathogens and to use such characterized isolates in resistance efforts.

23. In order to suppress the incidence of the virus diseases, use was made in the past of cultural practices to reduce the whitefly population. The present high level of prevalence of the whitefly vector and the related viral diseases has led to reliance on massive use of pesticides. Unfortunately, *B. tabaci* has demonstrated a high capacity to develop pesticide resistance, which resulted in ineffective control. The most effective and economic method of reducing crop losses due to viral diseases is now acknowledged to be the cultivation of virus-resistant varieties. This requires, however, an understanding of the fundamental characteristics of the geminiviruses pathogens. In order to achieve a broad-spectrum control strategy one needs to develop:(i) a thorough knowledge of the identity and biological characteristics of the viruses; (ii) a means for differentiating between viruses; (iii) the ability to manipulate the viruses experimentally; (iv) the ability to identify which of the viruses are of primary economic importance; and (v) a definite knowledge of the genetics and subsequent relationships between the geminiviruses.

24. Advances in genetic engineering techniques have made it possible to introduce resistance against a virus without compromising other agronomic characters of existing cotton varieties. These complex scientific techniques use part of the viral genome for the induction of resistance against a virus. These methods, using technologies like 'defective interfering' or 'antisense RNA', have been shown to result in resistance against geminiviruses. With the use of the recently developed 'biolistic gun' method such source of resistance may be introduced into the cotton plant. This resistance is then inherited in the cotton seed and the virus-resistant seed can be multiplied by normal methods. Another method of developing virus-resistant seed is through the transformation of easily regenerable varieties (like the American 'Coker' and the Australian 'Siokra' varieties) by means of the so-called *Agrobacterium* method, whereby the character for viral resistance can be transferred by the so called 'back-crossing' procedure. Development work in these areas has been carried out in several research institutes. The proposed project will build on these past efforts and establish collaboration in research efforts to expedite the process of breeding resistant cotton varieties.

25. Recent efforts have been devoted to screening and selecting cotton germplasm with tolerance to CLCuV in Pakistan and CLCrV in the USA. Two cycles of field trials of several selections showed promising tolerance to CLCrV. The extensive use of pyrethroid in Pakistan

during the 1980s is regarded as the cause for the now prevalent insecticidal resistance and for the related wide scale appearance of the disease. Germplasm screening activities against CLCuV are ongoing in two cotton research institutes in Pakistan (in Faisalabad and Multan), but so far no definite resistant material has been reported. However, some genotypes have shown varying degrees of tolerance and are being used in breeding programmes. This research needs to be strengthened and extended in order to determine whether these selections also contain broad spectrum resistance to geminiviruses from other regions of the world.

26. The particular objective of the project is to use modern biological and genetic engineering techniques to develop geminivirus-resistant cotton varieties that will enable the economic production of high quality cotton while reducing the need for continuous/increasing dependence on environmentally harmful (and increasingly less effective) chemical crop protection methods. Attainment of this objective will enable cotton producing countries to maintain and possibly expand their competitiveness in the market vis-à-vis competing synthetic fibres.

B. Description of Project Components

27. The project would comprise the following four components: (a) biological and genetic characterization of WFT geminiviruses of cotton; (b) development of *in-vitro* transformation systems of cotton; (c) development of geminivirus-resistant genotypes of cotton by conventional breeding and genetic engineering and carry-out of field trials to establish the validity of resistance in different locations; and (d) project management, monitoring and evaluation.

(a) Biological and Genetic Characterization of WFT Geminiviruses of Cotton

A prerequisite for the actual development of virus-resistant varieties, is the analysis and definition of the pertinent characteristics of economically important WFT geminiviruses that have been detected in infested countries like Chad, Egypt, Mali, Pakistan and Sudan. The envisaged activities will result in confirmation of WFT diseases under controlled laboratory conditions and in their host range studies. These latter studies will identify the best bioassay and experimental hosts for the viruses, which will be critical for the development of methods for virus manipulation required for all later stages of the work proposed to be undertaken in the project. Besides these activities, which are of a biological nature, the genetic characterizations of selected WFT geminiviruses will be defined by DNA sequencing (through the technique of cloning PCR-amplified DNA) and subsequently be compared with known geminiviruses. Full length infectious clones of CLCuV will be generated by cloning of viral sequences in *Agrobacterium* vectors and confirmation of their infectivity will be obtained by Agro-inoculation. DNA probe/PCR based diagnostic tests for geminiviruses of cotton will be developed through the design of new sets of primers based on DNA sequence and their use in detection of geminiviruses of cotton. Also cloned viral DNA will be used as DNA probe for determination of virus titer in infected plants. The infectivity of virus clones through inoculation by biolistic gun will be demonstrated. Plants inoculated with the biolistic gun methods will be scored for virus titer by using DNA probes. Successful implementation will eliminate the need for whitefly transmission for the infection of cotton plants.

(b) Development of *In-Vitro* Transformation Systems for Cotton

Obtaining transgenetic plants was until recently hampered by complications in the transformation and regeneration of particular cotton varieties. Introduction of the technology using biolistic gun transformation has resulted in the development of a variety-independent method for transformation and regeneration of cotton plants. Scientific staff of the PEA has already achieved initial results in the transformation and regeneration of major cotton varieties in Pakistan. Regenerated plants have been transferred to glasshouses and have shown normal growth.

Activities to be undertaken will focus on the establishment of an *Agrobacterium* mediated transformation system for regenerable cotton varieties (Coker 312 and Siokra 1-3). Modified varieties will be used for the evaluation of gene constructs for virus resistance. For the non-Coker/Siokra varieties, the establishment of *Agrobacterium* mediated and biolistic gun transformation/regeneration systems is envisaged. This will be achieved through callus induction and determination of optimum conditions for regeneration of selected elite cotton cultivars for *Agrobacterium* transformations as well as through the establishment of shoot tip culture and biolistic gun transformation.

Key activities related to the Asian ('Old World') varieties will take place in the PEA's scientific centre in Pakistan, while in the Tucson laboratory of the University of Arizona activities will be focused on American ('New World') and African varieties.

(c) Development of Geminivirus-Resistant Genotypes of Cotton by Conventional Breeding and Genetic Engineering, and Carrying out of Field Trials to Establish the Validity of Resistance in Different Locations

Transformation of plants with gene constructs based on antisense RNA technology or on defective interfering DNA technology have shown to result in resistance against geminiviruses. Activities to be implemented in the framework of this project will ultimately lead to the identification of virus (in particular CLCuV) resistant germplasm by using biolistic gun mediated infection with geminivirus DNA. The resistant germplasm will be used in the hybridization programme to develop virus-resistant, high yielding material. Genetic engineering will be used in the cloning of viral genes in plant transformation vectors, which should result in the establishment of the link with viral genes or viral sequences for developing virus resistance. Subsequently the gene constructs will be transformed in regenerable cotton varieties.

After the evaluation of the transgenic plants by Agro-inoculation or biolistic gun mediated transformation with viral DNA (through selection and testing of the resistant material), cross breeding of the genetically engineered material with local varieties will take place. Subsequently field trials of the resistant genotypes will be implemented, initially at the testing fields at the research centres, afterwards in various regions in Pakistan. Finally the resistant breeding material will be made available to other cotton producing countries for hybridization and multiplication.

The ultimate findings and achievements of the project will be adequately documented and will be made widely available through the existing channels of ICAC, FAO, IPGRI, etc., ensuring a wide distribution of the results of the project.

(d) Project Management, Monitoring and Evaluation

The implementing institutes need to coordinate their operations and consult regularly on the progress of implementation. Necessary financing has been provided for exchange visits, coordinated preparation of annual work programmes and budget, and for annual joint review and evaluation of implementation. Arrangements are also included for the Fund and ICAC to participate in the annual reviews and evaluation. A comprehensive review of the achievements of implementation is planned for the end of the second and fourth implementation years. Such comprehensive reviews will provide for a critical evaluation of all aspects of the project implementation and to reorient implementation if required. A final evaluation is planned for the fifth year. In preparation for the evaluation, a comprehensive technical manual will be prepared and will include assembled data on germplasm screening and breeding; an assessment of risk of transgenetic cotton release; and epidemiology data. The developed resistant varieties will be registered. A final dissemination workshop will be held to release the results of the project. During this workshop, breeder seeds will be available to interested countries.

C. Benefits

28. Tolerable threshold levels for the whitefly as a virus vector stand at one insect per plant; thus, even if new insecticidal compounds are soon developed to reduce whitefly pressure, there are no virus-resistant cultivars available that can be mobilized rapidly for cultivation in these regions. Thus, for the first time in over fifty years, cotton growers are faced with widespread virus epidemics in developing countries for which no promising defenses are available. Losses of millions of dollars in revenue will be realized along with world market shortages of cotton if this problem is not resolved in the immediate future. Unfortunately, the problem has appeared in the most important cotton growing region of the world. Cultivation of virus resistant varieties has been recognized as the most effective, ideal and economical method of reducing crop losses. In addition to the losses due to the virus disease problem, the new 'B' biotype of whitefly threatens to reduce the quality of fiber as a result of unprecedented honeydew production and contamination of bolls, lint and stems. This deterrent reduces prospects that gins will ultimately purchase the fiber and oil seed processors, the seed.

29. A successful development of geminivirus-resistant cotton varieties and their subsequent large scale introduction in the cotton producing countries will have a substantial economic impact on the national economies of the countries involved. As indicated earlier, for Pakistan alone the production losses as a result of the whitefly-transmitted cotton leaf curl virus (CLCuV) in the two production years 1992/93 and 1993/94 amounted to some 900,000 tons, which, when valued at 67 cents/lb for 'B' index cotton is equivalent to approximately US$ 1.3 billion. In addition to that, one should add the losses and incomes-foregone for workers in the cotton production and processing sectors in the country. Similar estimates can be made for other affected countries, be it that the magnitude of the losses would be less as a result of lower production levels.

30. Apart from the present production losses experienced to a greater or lesser extent in the cotton producing countries, due consideration should be given to the longer term danger caused by the increasing resistance of WFT geminiviruses against chemical based pest control methods. Increasing resistance is expected to lead to more intensive use of toxic insecticides, which meanwhile have shown to have at best a temporary crop protecting effect. The increased application of chemicals has also severe negative environmental implications as these chemical insecticides are recognized as major environmental polluters and often hazardous to producers.

Furthermore the high mobility of the vectors is a reason for concern, when taking into account that four geographically closely located countries (Pakistan, India, China and Uzbekistan) account for more than 50% of the world cotton production. Spread of the WFT viral diseases from Pakistan to the other three countries, on a scale as presently experienced in Pakistan will have severe implications for the cotton supply to the textile industry.

31. The use of geminivirus-resistant varieties will enable a considerable reduction of pesticide spraying presently required for crop protection. Less spraying (implying lower crop protection costs) will have a favourable impact on the production costs. Beside this positive effect from the financial point of view, the possible reduction of the number of sprayings will also have a positive impact on the ecological balance in the cotton production areas. Present spraying does not only affect the virus-transmitting whitefly vector, but affects also in general not-harmful animal life, including natural biological control agents for other pests, resulting in increased pest attacks on other crops. These effects would in particular impact on the small cotton farmers as they are likely to practice intercropping as a risk minimization strategy (e.g. in Pakistan, where more than 70% of the cotton farmers own less than 5 hectares of land). The tolerant/resistant cotton varieties, once developed and widely available, represent an environmentally safe and long term solution to the control of plant virus diseases. Their availability will be an important step in paving the way for alternative approaches to crop management in order to reduce the impact of pathogens and pests.

32. One basic requirement for the realization of the expected benefits of the tolerant or resistant cotton cultivars is that the varieties should be available at an acceptable cost to all cotton producers. It should be avoided that the improved varieties will be priced in such a manner that they will be only available for large scale producers, thereby excluding smaller scale producers or family farmers. It is therefore important that the scientific and technical findings of the project will be adequately protected against unwanted, commercial patenting. This issue will be a point of continuous attention for the project parties.

33. Lastly it should be mentioned that the project will also contribute to the further development and refining of scientific methods in the field of biotechnology and genetic engineering. The experiences to be gained through the project in that respect will transcend the specific field of cotton research alone.

D. Project Target Beneficiaries

34. The target beneficiaries will be the cotton producers all over the world, in particular those producers who are presently experiencing heavy production losses due to the WFT viral diseases. Immediate benefits will go to Pakistan, Sudan, Nigeria, Egypt, India, Togo, Chad and Mali which have shown increased viral diseases in cotton in recent years. There is potential that the leaf curl disease will spread to Central Asia from Pakistan. Other countries with similar viral diseases that will benefit include the United States of America, the Dominian Republic, Nicaragua and Guatemala. The cotton industries will benefit from improved quality and increased output of cotton. Already as a result of decline in production, cotton prices have more than doubled between November 1993 and June 1994.

35. In developing countries, cotton is produced by millions of smallholders. These smallholder producers will benefit from the project not only in terms of improved yields but also in terms of increased family income resulting from improved productivity.

36. It is foreseen that the scientific findings of the project will be put in the public domain in order to make the final outcome of the project, the resistant varieties, available to all cotton producers without being 'monopolized' by profit oriented organizations. In this manner it will be reasonably ensured that the results are accessible to all interested cotton producers.

37. The scientific research institutes that are co-operating in this project will also benefit. They will be able to continue and expand their initial research in the field of developing improved cotton varieties. The researchers involved will be provided with sufficient funds from the Common Fund (which will complement the funding made available through counterpart contributions and co-financing) to implement the project and produce ultimately the envisaged geminivirus-resistant, high yielding cotton varieties.

E. Project Costs and Financing

38. Project costs: The total costs for this five year project are estimated to be US$ 3,926,518. This amount includes a contingency provision of approximately 5% of the non-personnel inputs. The summary of the total project costs (by component) is presented in Table 1 below. A breakdown of the project cost by year (for each project component) is given in Appendix II.

Table 1

Project Cost Summary
(US$ 000)

Component		Cost	% of Total Project Cost
(i)	Biological and genetic characterization of WFT geminiviruses of cotton	1190	30
(ii)	Development of *in-vitro* transformation systems for cotton	1134	29
(iii)	Development of geminivirus-resistant genotypes of cotton by conventional breeding and genetic engineering, and carrying out of field trials to establish the validity of resistance in different locations	1017	26
(iv)	Project management, monitoring and evaluation	586	15
Total		**3927**	**100**

39. Financing: The project will be financed by contributions in cash and in kind from the participating institutions in Pakistan, the UK and the USA; and from the Fund and the ICAC.

The total contribution of Pakistan amounts to US$ 1,324,629. It originates from:

*	a cash grant by the President of Pakistan to NIBGE for research in the field of virus resistant cotton	US$	163,132
*	a contribution by the Progressive Cotton Growers Association	US$	32,626
*	a grant by the Ministry of Food and Agriculture	US$ 97,871	
*	a grant by NIBGE	US$	281,000
	Total Local Grants	**US$**	**574,629**

*	in kind contribution by NIBGE (equivalent to)	US$	360,000
*	in kind contribution by CRI (equivalent to)	US$	390,000
	Total contribution in kind (equivalent to)	**US$**	**750,000**

* The contribution of the UK consists of an in kind contribution equivalent to US$ 791,226.

* The USA is contributing US$ 245,608 (consisting of a grant of US$ 152,657 by the Cotton Incorporated and an in kind contribution by the University of Arizona of US$ 92,951 representing half of the overhead normally charged for the University's involvement in these type of activities)[6].

40. The total level of the 'local' counterpart contribution (provided by Pakistan, the UK and the USA) is approximately 60% of the total project cost. The ICAC contribution amounts to US$ 15,825 for supervision activities, while the Fund's contribution is envisaged to be US$ 1,549,770. The summary of the proposed financing plan is given in Table 2 below.

Table 2

Financing Plan
(US$ 000)

[6] The University will make an additional contribution in kind through the provision of staff time, facilities, resources, etc. It is envisaged that this contribution will reach an amount of approximately US$ 700,000 (which is about the same level as the contribution by the UK institute). However, as the University's contribution has not been detailed in financial equivalents, it has not been reflected in the detailed, itemized cost tables. Hence they are also not reflected in the presented summary cost and financing tables.

Component	CFC	Pakistan	UK	USA	ICAC	Total
(i) Biological and genetic characterization of WFT geminiviruses of cotton	430	392	292	76	---	1190
(ii) Development of *in-vitro* transformation systems for cotton	428	372	258	76	---	1134
(iii) Development of geminivirus-resistant genotypes of cotton by conventional breeding and genetic engineering, and carrying out of field trials to establish the validity of resistance in different locations	297	560	158	2	---	1017
(iv) Project management, monitoring and evaluation	395	---	83	93	15	586
Total	**1550**	**1324**	**791**	**247**	**15**	**3927**
% to be financed	**40**	**34**	**20**	**6**	**---**	**100**

F. Procurement, Disbursement, Accounts and Audit

41. Procurement would be in accordance with the Fund's 'Regulations and Rules for the Procurement of Goods and Services of the Second Account'. Procurement of expendable and non-expendable equipment items and materials would be bulked as much as possible to attract International Competitive Bidding. Contracts costing the equivalent of US$ 100,000 or more would be subject to International Competitive Bidding. Contracts costing less than the equivalent of US$ 100,000 but more than US$ 50,000 equivalent would be procured through local competitive bidding satisfactory to the Fund. For contracts costing US$ 50,000 equivalent or less, or for specialized equipments, prudent shopping procedures (whereby at least three quotations are required) would apply. Contracts to be awarded to consultants or institutes will require prior agreement of the Supervisory Body which will ensure that the contracts are relevant and based on clearly defined Terms of Reference (TOR). The contracts will require clearance of the Fund prior to being awarded. The consultancy services under the project will follow internationally acceptable guidelines. Consultants/service providers shall have experience and qualifications, and be recruited under conditions of service, satisfactory to the Fund.

42. Disbursements against the purchase of equipments and materials costing the equivalent of US$ 1,000 or more, and against contracts and services will be fully documented. Operating expenses, workshops and supplies will be disbursed against certified Statements Of Expenditure (SOE). Documentation for withdrawals under SOE would be maintained in a central location by

the PEA for review during supervision missions and for authentication by the auditors. Since the PEA and the collaborating institutions will not be in a position to pre-finance expenditures eligible for Fund financing, a Project Account will be opened by the PEA in a bank satisfactory to the Fund, and in convertible currency. The Fund will make an initial deposit of the equivalent of US$ 250,000 equivalent to an estimated six months' worth of expenditures eligible for the Fund's financing. The Project Account will be replenished in accordance with the Fund's procedures for operating a Project Account. Based on the agreed work programme and allocation of responsibilities, the PEA shall provide funds from the Project Account to the collaborating institutions for the implementation of their part of the programme. In case it is established that this method of allocating funds to the collaborating institutions is not the most efficient and effective one, then appropriate alternative arrangements will be made by the Fund, in close consultation with all project parties concerned. The ICAC, in its role of Supervisory Body (SB), will ensure prior to the signature of the Project and Grant Agreements that the envisaged inputs of the collaborating institutes are confirmed in the quantity foreseen under the project

43. Accounts and Audit: The PEA and the collaborating institutes will maintain independent and appropriate financial records and accounts, in accordance with sound accounting practices. All project accounts, including the Project Account, will be audited annually, except otherwise agreed by the Fund, by independent auditors satisfactory to the Fund. The audited accounts and the auditors report, including separate opinions on SOE and on the utilization of the funds in the Project Account, would be submitted within three months after the end of the related project's fiscal year.

G. Organization and Management

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44. The National Institute for Biotechnology and Genetic Engineering (NIBGE), located in Faisalabad, Pakistan, will be the Project Executing Agency (PEA) and it will have the overall responsibility for the management and implementation of the project. NIBGE will be accountable for all expenditures made under the project. For this purpose NIBGE will establish protocols with the collaborating institutions indicating clear procedures for receipts of funds, expenditures including procurement guidelines, record keeping, accounting, financial statements preparation and audits. Establishment of such protocols will be a condition for disbursement by the Fund. The project management, including co-ordination of the planning and budgeting of the various activities as well as reporting on the progress made will be the responsibility of NIBGE. NIBGE will also assume overall responsibility for the project administration, including, *inter alia*, keeping of accounts, effecting or control of procurement, disbursements and audits.

45. The project will be implemented on the basis of an agreed detailed annual work plan and budget. Such annual work plan and budget should be prepared by the PEA in consultation with the participating institutions. The PEA will submit the work programme and budget to the SB and the Fund two months before the start of the implementation of the activities. This programme shall cover in sufficient detail the activities to be carried out by each of the institutions involved and the task assignments for the key staff involved. The work programme shall also include a time schedule and a framework for the annual progress reports to be submitted by NIBGE. The draft work programme and budget will be reviewed and commented upon by the ICAC and made available to the Fund for any further comments it might have. Agreed-upon comments from the Fund and ICAC will be incorporated in the final work programme and budget.

46. The other collaborating institutes will implement activities assigned to them as indicated in the detailed yearly work programme/annual budget prepared by the PEA and agreed upon by

all institutes and organizations involved. The parties responsible for the implementation of specific activities will report thereon to the PEA. The PEA will in turn report to the Supervisory Body (ICAC) and to the Fund on the overall progress made by the project.

47. Regular meetings will be organized by the PEA for discussion and review of implementation progress and results of the project. Monitoring visits to project sites will be made by ICAC and the Fund once a year, but more often should such be considered appropriate in order to ensure adequate monitoring of implementation progress. Based on these monitoring visits, the work programme can be amended in consultation with the project partners.

48. The detailed implementation arrangements indicating the envisaged timing of the identified activities to be implemented in the frame work of this project, is attached as Appendix III and provides an overview on the responsibilities of the three key institutes involved in the project, with regard to the realization of the envisaged objectives and outputs.

H. Monitoring, Reports and Supervision

49. The PEA will submit annual progress reports on the achievements of the overall project to the ICAC and the Fund. The PEA's reports will contain an assessment of the achievements in relation to the targets set (as reflected in the project appraisal and the work programme). Variances will be accounted for and remedial actions will be proposed if required. Major constraints and problems which may hamper the achievements of the project objectives will be highlighted and suggestions to resolve them will be provided. The ICAC shall provide its comments on the report to the PEA and to the Fund. The Fund will carry out regular supervision of the project, such in co-ordination with the ICAC which will also carry out supervision in its capacity as Supervisory Body. In the event that the PEA is anticipating any substantial delay in the implementation of (part of) the work programme or in its reporting as scheduled, it will inform the SB and the Fund accordingly.

50. Towards the end of the second year an interim review mission will be fielded, which will review the achievements of the project so far. The project will be subject to a further evaluation in the fourth year of the project. The actual timing of the review and evaluations, their composition of participants and their TOR will be agreed upon by the PEA, SB and the Fund.

51. A final workshop/seminar will be organized by the PEA in consultation with the project parties, to highlight the scientific/technical achievements of the project and to present the results of the project to an international audience of professionals in this field, from cotton producing countries.

52. Towards the end of the fifth project year, the PEA will prepare a project completion report (PCR) to highlight the project achievements, constraints and experiences gained in the design and implementation of the project. The technical manual (para 28 d) will be submitted along with the draft PCR. The reports will be reviewed by the SB and the Fund, after which it will be finalized by the PEA, taking into account the comments received. The PCR in its final form, along with the final accounts and audit, will be submitted to the SB and the Fund not later than three months after the completion of the implementation activities. The SB will prepare a separate report containing its observations on the overall implementation and achievements of the project. This report of the SB will be submitted, along with the final PCR and technical

manual, to the Fund. The Fund's management will provide its comments and conclusions with regard to project implementation to the Executive Board not later than six months after the closing date of the project.

I. Risks

53. Inherent to a research and development project is the risk that the objectives of the project cannot be achieved due to disappointing technical and scientific results. Although this risk cannot be completely neutralized, it is minimized by the fact that considerable progress has been made in the technology to be applied, and the implementing institutions are fully involved in the application of genetic engineering and conventional breeding of cotton. The institutes involved will each contribute in those fields where they have specific expertise. These institutes are acknowledged to be at the forefront of biotechnology and genetic engineering and have built up adequate experience and expertise in these fields. The project will build on past efforts and will benefit from other, ongoing, research activities in the institutes involved.

54. Achievement of the objectives of the project will, to a large extent, be contingent on the co-ordinated implementation of the research and development activities of the participating institutes in Pakistan, the UK and the USA. This requires carefully prepared organizational arrangements, a detailed, agreed-upon research agenda, and a flexible, but carefully adhered to, implementation and reporting schedule. All these requirements are included in the project design. Collaboration between the institutes concerned has already been established and will be further strengthened by this project. The process of coordination will be fostered by a joint preparation of the annual work programme and budget and annual review of implementation.

55. Genetic engineering of cotton has been the subject of a patent application by a commercial US research firm. After initial awarding of the patent by the US Patent and Trademark Office, the patent (after having been challenged by, *inter alia*, the US Department of Agriculture) has been reexamined and subsequently suspended. The patent remains valid, however, until a final ruling is made. This final ruling is not expected within two/three years from now. At the request of the Fund, the ICAC has been in touch with both the Patent and Trade Office and with the patent holder. The subject matter of the proposed project does not constitute part of the patent in dispute. Further, ICAC has obtained assurances from the patent holder that, should a licence be required for any of the unforeseen areas of the project, free license will be issued to all government and academic researchers upon request. The patent does not apply in Pakistan where the PEA is located. The activities and results of the project will, therefore, not clash with the existing patent held by the private firm. It will be the task of the SB to closely follow developments in this field and to ensure that appropriate steps are taken in order to avoid possible infringements on existing patents and to ensure that the project results can be made available to the cotton producing member countries of the Fund and the ICAC, as presently envisaged.