RESEARCH ON PLANT VIRAL DISEASES IN PAKISTAN

Bibliography and Abstracts






Saif Khalid

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Dedicated to
my dear mother who wished
and prayed to see me as a Plant Scientist

and to

Dr. S.M. Mughal who laid the sound
foundation of systematic
plant virology research
in Pakistan

CONTENTS

FOREWORD

It gives me a great privilege and pleasure to introduce this monograph entitled "Bibliography and Abstracts" to the virologists working in Pakistan. Plant Protection is as important as Plant Production because it helps to increase the crop productivity and also saves the agricultural products from destruction. Although the foundation of plant pathology was laid in pre-partition days as a side subject but it assumed its full-fledged status after 1947 through the efforts of the late Dr. Abdus Star, who was rightly named as father of Plant Pathology in Pakistan. He produced many scientists of world fame.

Till recently very little systematic research work was carried out on plant viral diseases except some preliminary researches conducted on mosaics of sugarcane and potatoes because of lack of well-equipped laboratory facilities. It was in 1975 when the undersigned visited the university of Adelaide in Australia on an FAO mission and met Dr. S. M. Mughal who was doing his Ph.D. there. I met his Professor who appreciated his capabilities and strongly recommended Dr. Mughal's full utilization on his return to Pakistan. Dr. Mughal and myself together discussed the strategy. He was advised that in the absence of the proper facilities, he should try to screen various crop varieties against the present viruses and develop technology for producing virus-free seed potatoes. Dr. Mughal on his return to Tando Jam started the proposed work. In the mean time Pakistan Agricultural Research Council (PARC) opened a new Division of Virology and on the author's inducement Dr. Mughal joined as Head of the Division in PARC. Through his dedicated efforts basic facilities were made available and work on viral diseases took off the ground.

Dr. Saif Khalid soon (1984) joined the division and became its incharge in 1991 when Dr. Mughal left PARC.

During the interviewing period, the Virology Division at NARC has done quite satisfactory work which includes; identification of major viruses of banana, cotton, chillies, potato, tobacco and sugarcane, production of polyclonal antiserum and development of ELISA-kits to important plant viruses for detection. The division also carried out the most outstanding work on identification of the causes of two important viral epidemics i.e. banana bunchy top virus (BBTV) and cotton leaf curl virus (CLCuV); development of a method for the screening of cotton germplasm against CLCuV and management practices for BBTV which led to the development of crop management practices to contain these viral diseases. In the last decade two catastrophies BBTV and CLCuV hit Pakistan very badly causing huge damages to the crops and to the national economy. This evidently disturbing situation made the government to realize the real importance of Plant Viral diseases and provided additional facilities to improve the researches.

Dr. Saif Khalid has now compiled this reference monograph for the guidance of research workers in the field of plant virology, which will go a long way in improving and stimulating the researchers to safeguard our valuable crops, leading to the much needed increased agricultural productivity, growth rate for achieving self sufficiency and also for building up sizeable export surpluses. This monograph will also provide some useful information to the administrators and progressive farmers regarding the great importance of viral diseases. As a scientist my advice will be that all the virologists should join hands through establishing an Association for further improving

the researches and avoiding duplication through developing a national program; assigning the work to different centers according to the available manpower and the facilities.

Dr. Saif Khalid deserves appreciation and congratulation in compiling the useful monograph. It is earnestly hoped that he will continue endeavoring for the uplift of plant virology in Pakistan and many more scientists will qualify in this interesting discipline to serve the country.

Islamabad, Sept., 1999

DR. ABDUL HAFIZ
Former Director, Food & Agriculture
Organization of the United Nations

PREFACE

Despite early records of plant viral diseases in this part of the world the discipline of plant virology remained neglected for a long period of time. The reasons for this were the lack of trained manpower, unavailability of research tools and realization of the fact that disease of viral nature can be as destructive as banana bunchy top virus (BBTV) and cotton leaf curl virus (CLCuV). Although late, but Pakistan Agricultural Research Council, Islamabad, felt this importance and started work on plant viruses in 1982. However, its importance was recognized after the outbreak of BBTV and CLCuV epidemics in 1989 and 1991, respectively. Enough funds were provided for research on CLCuV, resulting in generation of sizeable information. It is anticipated that the discipline of plant virology will be further strengthened and funds will be made available on regular basis.

The idea of compiling this monograph was conceived in 1992, when the author had to write a review article on " Status of Plant Virological Research in Pakistan". Despite the utmost efforts, the author could not get hold of the needed information regarding the research done on plant viral diseases in Pakistan. The author therefore, continued efforts to collect all the possible information, which now, has taken the shape of this monograph. The object of compiling this publication is to bring together all the valuable contributions of researchers and provide a comprehensive reference source for those who have some interest in plant virology. Efforts have been made to collect all published information from national and international literature on plant virological problems pertaining to Pakistan till September, 1999. In this attempt, the author might have missed some

articles inadvertently but regretfully. All the virologists are requested to provide the missing information for inclusion in the future editions.

The targeted readers are plant virologists, pathologists, teachers, students, extenuation workers, policy makers and donor agencies. All of them are requested to make use of this monograph in their respective fields, helping this neglected discipline to play its full role improving the national economy. The pervious researchers who did some work without the needed facilities deserve appreciation.

The author is grateful to Dr. Zafar Altaf, Ex-Chairman, Pakistan Agriculture Research Council, and Federal Secretary for Agriculture & Livestock, Islamabad, for moral and financial support; Dr. A. Hafiz and Dr. M.H. Soomro for suggestions and encouragement during compilation; Mr. Abdul Khaliq for skillful typing, composing and giving present shape to this monograph and Moazzum Bin Akhtar and Shahid Aslam Siddiqui for proof reading and to all those who supplied material for inclusion in this monograph.

Islamabad: Sept., 1999

DR. SAIF KHALID

BANANA

BANANA

SOME STUDIES ON BIOLOGY OF *PENTALONIA NIGRONERVOSA* CONQUARREL – THE VECTOR OF BANANA BUNCHY TOP VIRUS

Yasmin, T., E. Haq, S. Khalid & S.A. Malik

Biology of viviparous black banana aphid, *Pentalonia nigronervosa* Coq., vector of Banana bunchy top virus (BBTV) was studied on banana under growth chamber at a photoperiod of 16:8 hours (light:dark), 55±5% humidity and at 20±5°C. From the time of its birth as a nymph, *P. nigronervosa* took a period of 10 to 14 days with an average of 10.6 days to develop to the final nymphal moult. Fecundity was 1-4 nymphs per day. Over 10 days it ranged from 9 to 26 nymphs/aphid, the average being 13.2. The average body length, width and length of hind tibia of adult aphid were 0.507 mm, 0.282 mm and 358.18 µm, respectively. Life span of *P. nigronervosa* ranged from 19 to 26 days with an average of 20.3 days.

Pak. J. Biol. Sci. (in press) 1999.

SOME STUDIES ON THE BIOLOGY OF *PENTALONIA NIGRONERVOSA* CONQUARREL- THE VECTOR OF BANANA BUNCHY TOP VIRUS

Yasmin, T., E. Haq, S. Khalid & S. A. Malik

Black banana aphid, *Pentalonia nigronervosa* Conq., is the only vector of banana bunchy top disease (BBTD). It is the most destructive and economically important viral disease of banana (*Musa spp.*) in the world including Pakistan. Banana bunchy top virus (BBTV) is transmitted in a persistent manner by *P. nigronervosa*. Some biological studies on *P. nigronervosa* were conducted under controlled conditions during 1998. Aphid cultures, collected from an apparently BBTV free banana plantation from district Hyderabad, Sindh, were established on healthy banana plants (Dwarf Cavendish) in a rearing room at a photoperiod of 16:8 hours (light:dark) with a mean maximum daily temperature of 24 to 30°C.

Development time and fecundity (mean number of nymphs per aphid over 10 days) on banana plants ranged from 10-14 days and 9-26 nymphs/

aphid, respectively. The average body length, width and length of Hind Tibia of adult aphid were 0.507 mm, 0.282 mm and 358.18 µm, respectively. Life span of *P. nigronervosa* ranged from 19-26 days.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p. 52.

FIRST REPORT OF CUCUMBER MOSAIC VIRUS IN BANANA IN PAKISTAN

Khalid, S., T. Yasmin & M.H. Soomro

Sporadic banana plant showing mosaic, chlorotic spot intermingled with dark green areas resembling to CMV infection, were observed in few places in the districts of Thatta, Hyderabad and Nawab Shah in the province of Sindh, Pakistan. These plants were uprooted and planted at National Agricultural Research Centre (NARC), Islamabad, in clay pots. These plants were tested for the presence of banana bunchy top virus (BBTV), cucumber mosaic virus (CMV), tomato mosaic virus (TMV), chili veinal mottle mosaic virus (CVMV), potato virus X (PVX) and potato virus Y (PVY) through double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). All tested plants (8) were only ELISA positive for CMV. Leaf dip and partially purified preparation revealed virus particles measuring 25-28 nm in diameter. Thus, on the basis of field symptoms, serology (ELISA) and particle morphology presence of CMV in banana was established. Other biological studies of the virus are in progress.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p. 53.

RECENT DEVELOPMENTS IN BANANA CULTIVATION IN PAKISTAN WITH EMPHASIS ON BBTV MANAGEMENT

The article describes background information about banana cultivation, appearance (1989) and identification of banana bunchy top virus - BBTV (1991) and its vector (1994). In addition to this, the present situation in respect of disease distribution, prevalence of banana aphid (*Pentalonia nigronervosa* Coq.), availability of

Soomro, M.H.

virus-free planting material, other recent developments and future prospects are discussed.

7th Regional Advisory Committee Meeting INIBAP, Asia & Pacific Network, Oct. 21-23, 1997, Hinoi, Vietnam, Appendix U, pp. 136-143.

DISTRIBUTION OF BANANA APHID IN SINDH, PAKISTAN

Soomro, M.H.

Banana aphid (*Pentalonia nigronervosa* Coq.) is present wherever in the world banana is grown, but its presence in Pakistan was confirmed only in 1993. The aphid is the only known vector of banana bunchy top virus (BBTV) which has seriously damaged the banana crop in Sindh over the last few years. Thus it is considered as a very important insect. For a better understanding of epidemiology of BBTV, a survey of banana plantations in Sindh was undertaken in 1994 to determine the distribution of the insect. At random 3-10 fields in each banana growing district were inspected for prevalence of the aphid. The aphid was widely distributed irrespective of BBTV and was present in all districts visited including Khairpur, Naushehro Feroze, Nawabshah and Hyderabad.

Proc. 15th Pak. Cong. Zool., Apr. 15-17, 1995, NARC, Islamabad, 15: 257-259.

FIRST REPORT OF BANANA APHID IN PAKISTAN

Soomro, M.H. & S. Khalid

During a survey of banana crop in Sindh, colonies of aphids were observed at a number of locations throughout the province. The insects were identified as the banana aphid, *Pentalonia nigronervosa* Coq. This is the first report of the aphid in Pakistan.

Pak. J. Zool., 26(3): 276, 1994.

FIRST REPORT OF BANANA BUNCHY TOP VIRUS IN PAKISTAN

Khalid, S.,

An unknown disease was observed in 1988 for the first time on banana, an important fruit crop introduced in 1913 to Sindh, Pakistan. The disease was observed first in coastal areas of Sindh province, then in northern areas, where it

M.H. Soomro & R.H. Stover

caused heavy losses. A survey of three to six fields at various locations in each of the six major banana growing districts in July 1991 revealed that one-half of the banana plantations had been destroyed. The disease was prevalent in Thatta, Karachi, Hyderabad, Badin and Mirpur Khas districts. Symptoms resembled those of banana bunchy top virus, including bunching and brittleness of leaves and dot-dash dark green streaks on petioles and lamina parallel to veins. The method of Wu and Su was used to purify the virus, and electronmicroscopy detected virus particles 20-22 nm in diameter. An ELISA kit for banana bunchy top virus (Bananase-96, General Biology Corporation, Taiwan) was used to confirm the etiology. Thus, the disease was identified on the basis of symptoms, particle morphology, and serology (DAS-ELISA).

Pl. Disease, 77(1): 101, 1993.

BANANA BUNCHY TOP VIRUS CONFIRMED IN PAKISTAN

Soomro, M.H. & S. Khalid

The article briefly describes the occurrence, distribution and identification of disease and its vector, as banana bunchy top virus and *Pentalonia nigronervosa*, respectively. Efforts of Pakistan Agricultural Research Council to rehabilitate banana industry by formation of a Banana Bunchy Top Management Bureau/ Board and availability of virus free banana plants are discussed.

INFOMUSA, 2(1): 17, 1993.

BANANA BUNCHY TOP DISEASE IN PAKISTAN

Khalid, S. & M.H. Soomro

The cause of a banana disease in the province of Sindh, Pakistan, was attributed to banana bunchy top virus. Identification was based on symptomatology, size/morphology of the virus particles (20-22 nm) detected in diseased tissue, and serology (DAS-ELISA).

Pl. Path., 42(6): 923-926,1993.

STATUS OF BANANA BUNCHY TQP DISEASE IN PAKISTAN

Khalid, S. & M.H. Soomro

Banana, an important fruit crop of Pakistan, is grown over an area of 23,000 hectares with annual production of 210,000 tones. More than 87% of the area under banana cultivation is located in the province of Sindh. At present, about 90% of area under banana is planted with "Dwarf Cavendish". In December, 1988 a serious disease was noticed in the coastal district of Thatta. The disease was first thought to be caused by nematodes and fungi, however, use of nematicides and fungicides did not improve the crop. The problem remained undiagnosed until 1991 when, in July and October, affected banana fields were visited, where disease incidence ranged from 0-100%. Samples at different locations were collected from plants showing classical symptoms of banana bunchy top virus (BBTV) including bunching and brittleness of leaves plus dot-dash dark green streaks on petioles and lamina parallel to veins. Virus particles measuring 20-22 nm in diameter were detected by electronmicroscopy when such material was used for virions purification, however, the virus concentration was very low. The disease was therefore, diagnosed as BBTV on the basis of symptoms produced and particle morphology.

5th Int. Pl. Virus Epid. Symp." Viruses, Vectors and Environment", Jul. 27-31, 1992, Valenzano (BARI), Italy, pp. 243-244.

OUTBREAK OF BANANA BUNCHY TOP VIRUS IN SINDH, PAKISTAN

Soomro, M.H., S. Khalid & M. Aslam

In July and October 1991, major banana growing areas of Sindh, Pakistan were surveyed for prevalence and incidence of banana bunchy top virus, a serious disease that up to now has not been verified to occur in the country. The disease was found to be widespread in the districts of Thatta, Badin and Hyderabad, and limited infestations were observed in the Karachi and Mirpur Khas districts. No symptoms were seen in

the Nawabshah, Naushehro Feroze and Khairpur districts. Incidence of the disease varied from 0.5 to 100%. In July the disease was prevalent in 16 fields, but by October the situation was improved. The possible causes of the outbreak are discussed, along with actions taken by the farmers to control the disease.

FAO Pl. Prot. Bull. 40(3): 95-99, 1992.

IDENTIFICATION OF BANANA BUNCHY TOP VIRUS IN PAKISTAN

Khalid, S. & M.H. Soomro

In late 1988 a mysterious disease of unknown etiology was observed in banana plantations in the province of Sindh, Pakistan, which became an epidemic by 1991. Infected plants showed the typical symptoms of bunchy top disease including bunching and brittleness of leaves and dot-dash dark green streaks on pseudostems, petioles, midribs, and laminae. Tissues with such symptoms were used for virus purification. Infected tissues were pulverized in liquid nitrogen and extracted in 0.1 M potassium phosphate buffer (pH 7.4) containing 2-mercaptoethanol and sodium diethyl dithiocarbamate. Chloroform/butanol were used as emulsifier. Emulsion was broken by low-speed centrifugation and supernatant subjected to two cycles of low- and high-speed differential, followed by 10-40% sucrose density gradient centrifugation. The virus zone was recovered by scanning tubes in an ISCO density gradient fractionator at 254 nm and concentrated by high-speed centrifugation. Such preparations were mounted on a carbon-coated copper grid and were stained with 2% uranylacetate. Virus particles (20-22 nm) were detected by TEM. DAS-ELISA, with monoclonal antibodies to Taiwanese BBTV isolate, was performed and the virus confirmed. Thus, on the basis of typical symptoms, particle morphology, and

serology, the cause of the devastating disease of banana in Pakistan was identified as banana bunchy top virus.

Proc: Int. Symp. on Genetic Improvement of Bananas for Resistance to Diseases and Pests, Sep. 7-9, 1992, Montpellier, France, p. 377.

DISTRIBUTION AND INCIDENCE OF BANANA BUNCHY TOP DISEASE IN PAKISTAN

Soomro, M.H., S. Khalid, I. Ahmad & M. Aslam

To determine the distribution and incidence of banana bunchy top disease (BBTD) in Sindh, Pakistan, a survey of banana-growing areas was carried out in July 1991 and another in July 1992. In total, 48 fields in 1991 and 80 in 1992 were visited. Diagnosis in the field was based on the presence of typical BBTD symptoms and incidence was estimated as percentage plants showing BBTD symptoms. In 1991, the disease was present in five southern districts only but, by July 1992, it had spread further north to other districts, wiping out the crop completely from two districts: Hyderabad and Badin. In other districts the incidence varied from 0 to 100% depending on the area, farmers' awareness about the problem and cultural practices. By late 1992 only two northern banana-growing districts were apparently free. However, if the situation continues, the crop may be destroyed completely.

Proc. Int. Symp. on Genetic Improvement of Bananas for Resistance to Diseases and Pests, Sep. 7-9, 1992, Montpellier, France, p. 381.

BUNCHY TOP: A VIRUS DISEASE OF BANANA AND ITS CONTROL

Khalid, S & M.H. Soomro

Banana is one of the most important fruit crops of Pakistan grown on an area of 23,500 hectares with annual production of around 210,000 tones. Over 87% of the total area under banana is in Sindh, mainly on the left bank of the Indus River, followed by Punjab (10.3%), North-West Frontier Province (NWFP) (2.2%) and Balochistan (0.4%). The major banana-growing districts of Sindh are Thatta, Badin, Hyderabad, Mirpur

Khas, Sanghar, Nawabshah, Naushehro Feroze and Khairpur. Cause of the disease, symptoms in banana and management methods are discussed.

Progressive Farming, 12(2): 29-31, 1992.

PREVALENCE AND INCIDENCE OF BANANA BUNCHY TOP VIRUS IN SINDH

Soomro, M.H., S. Khalid, I. Ahmad & M. Aslam

A survey of banana fields for banana bunchy top virus was undertaken in five districts, viz., Thatta, Karachi East, Hyderabad, Mirpurkhas and Nawabshah during 1991. A total of 21 fields at 18 locations were visited. The disease was prevalent in 17 fields at 14 different locations. Disease incidence varied from 0.5% at Saedabad to 100% at Tandojam and Khisano Mori in Hyderabad district, while the fields north of Saedabad and around Sakrand in Nawabshah district were found apparently free from the virus.

Nat. Symp. on Status of Pl. Pathol. in Pakistan, Dec. 3-5, 1991, Univ. Karachi, (Abst.) p. 92.

DIAGNOSIS OF AN UNKNOWN BANANA DISEASE IN SINDH

Khalid, S., M.H. Soomro & R.H. Stover

Banana, one of the most important fruit crops of Pakistan was struck by an unknown deadly disease in 1988 for the first time since introduction of the crop in 1913. The disease was first observed on coastal areas of Sindh and spread northwards causing heavy crop losses. A number of investigations were made by various workers mainly in Thatta area and the problem was thought to be caused by different organisms including nematodes and fungi. However, nematicidal and fungicidal treatments did not improve crop health and thus was suspected to be something else. The authors surveyed five southern districts of Sindh during July 1991 with the cooperation of Sindh government. The disease was identified as "banana bunchy top virus (BBTV)" on the basis of symptoms characteristic of the virus. Diseased plants showed clear-cut bunching, dot-dash streaks on

petioles and lamina parallel to veins, and brittle leaves. Symptoms were more pronounced in Tandojam, Hyderabad district than the other places visited. Further studies on purification of virus were conducted at the National Agricultural Research Centre, Islamabad and virus particles were seen. Thus the presence of BBTV in banana is confirmed.

Nat. Symp. on Status of Pl. Pathol. in Pakistan, Dec. 3-5, 1991, Univ. Karachi, (Abst.) p. 92.

BARLEY

BARLEY

DETECTION OF FIVE BARLEY YELLOW DWARF LUTEOVIRUS SEROTYPES IN PAKISTAN

Bashir, M., L. Bertschinger, N.S. Kisana, M.Y. Mujahid & N.I. Hashmi

Forty-five leaf samples from plants with symptoms suggestive of barley yellow dwarf (BYD) of wheat (*Triticum* sp.), barley (*Hordeum vulgare* L. sub sp. *vulgare*), oat (*Avena sativa* L.) and triticale (× *Triticosecale* Wittm. ex *A. Camus*) in experimental and farmer's fields in North Western Frontier Province (NWFP) and Punjab province of Pakistan were collected during the main wheat-growing seasons in winter 1993 and 1994. These samples were tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with broad- spectrum antibodies against five strains of barley yellow dwarf luteoviruses (BYDVs: PAV, MAV, RPV, RMV, and SGV). Thirty-three samples were infected with BYDV. PAV-like isolates were the most prevalent (64.4%), followed by MAV-like isolates (40%). For the first time, SGV-like isolates were detected in Pakistan (4.4%). The samples which did not react with any of the antibodies used suggest either the presence of other BYDV isolates or factors such as nutritional disorders that are not detected with these antibodies.

Rachis 16(1,2): 47-49, 1997.

DEETECTION OF FIVE BARLEY YELLOW DWARF VIRUS SEROTYPES IN CEREALS OF PAKISTAN BY ENZYME-LINKED IMMUNOSORBANT ASSAY

Bashir, M., L. Bertschinger,

Barley yellow dwarf virus (BYDV) is becoming more important in North West Frontier Province (NWFP) and northern parts of the Punjab. For detection of BYDV-like virus particles, 45 leaf samples of wheat, barley, oat and *triticale* from plants with BYDV-like symptoms were collected from experimental plots and farmers' fields from NWFP and Punjab. These samples were tested in the Virology Laboratory at CIMMYT, Mexico, by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with anti-

N.S. Kisana, M.Y. Mujahid & N. I. Hashmi

immunoglobulin against five strains of barley yellow dwarf viruses (BYDVs) namely; PAV, PMV, MAV, RPV and SGV. The results showed the presence of all five BYDV serotypes in Pakistan. PAV-like isolates were more prevalent and widely distributed than other isolates. Detection of SGV-like isolates from wheat samples is being reported for the first time in Pakistan. The symptomatic samples which did not react with any of the antibodies used suggest either the presence of BYDV isolates that could cause BYDV-like symptoms or factors such as nutritional disorders that were not detected with these antibodies.

5th Nat. Conf. for Pl. Scientists, NARC, Islamabad, Mar. 28-30, 1995, (Abst.) pp. 74-75.

SCREENING OF BARLEY AND OATS GENOTYPES AGAINST BARLEY YELLOW DWARF LUTEOVIRUS UNDER NATURAL INFECTION CONDITIONS IN PAKISTAN

Bashir, M., M. Aftab, S. Khan, A. Hussain, D. Muhammad & M.B. Bhatti

Barley yellow dwarf virus (BYDV) is a potential disease of major cereals in Pakistan. Recently the disease has been recognized as of significance importance due to its sporadic occurrence in major cereal growing areas of Punjab and North Western Frontier Provinces. On the basis of serological tests two BYDV isolates; PAV and MAV have been recognized in Pakistan. PAV-like isolates are more common and wide spread than MAV-like isolates. BYDV infects all major species of cereals in Pakistan. BYDV may cause high damage if severity is high.

Barley Yellow Dwarf Newsletter, 5: 13-14, 1994.

RECENT RESEARCH STATUS OF BARLEY YELLOW DWARF LUTEOVIRUS IN PAKISTAN

Bashir, M., N.S. Kisana,

Barley yellow dwarf luteovirus (BYDV) was first observed in measurable intensities about 4 to 5 years back in northern parts of the country. In later years the disease was never seen in critical intensities. The disease, however, was seen in high intensity during 1993 in wheat crop and its occurrence has been confirmed serologically.

M. Aftab,
M.Y. Mujahid &
N.I. Hashmi

As we know, amongst viral diseases of cereal crops, BYDV is known to be the most damaging world wide. In severe form it can cause losses up to 75% of the crop. Almost all Pakistani wheat cultivars particularly grown in northern parts are susceptible to BYDV with little variability which could be exploited for breeding purposes. This disease is a potential threat to our wheat crop and therefore, a systematic/concerted research effort is warranted by Wheat Improvement Program, NARC supplemented by CIMMYT support.

Barley Yellow Dwarf Newsletter, 5:11, 1994.

DETECTION OF FIVE BARLEY YELLOW DWARF LUTEOVIRUS SEROTYPES IN PAKISTAN

Bashir, M.,
L. Bertschinger,
N.S. Kisana,
M.Y. Mujahid &
N.I. Hashmi

Forty-five leaf samples from plants with barley yellow dwarf (BYD)-like symptoms of wheat, barley, oat and *triticale* in experimental and farmer's fields in North Western Frontier Province (NWFP) and Punjab province of Pakistan were collected during the main wheat growing seasons in winter 1993 and 1994. These samples were tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with broad spectrum polyclonal antibodies against five barley yellow dwarf luteovirurses (BYDVs) such as PAV, MAV, RPV, RMV, and SGV. Seventy three percent of the samples were found to be infected by BYDV. The results showed the presence of five BYDV serotypes. PAV-like isolates were the most prevalent (64.4%), followed by MAV-like isolates (40.0%). For the first time, SGV-like isolates are reported (4.4%) from Pakistan. The symptomatic samples which did not react with any of the broad spectrum polyclonal antibodies used suggest either the presence of viruses that cause BYD-like symptoms but do not react with these antibodies or other abiotic reasons such as nutritional disorders.

Barley Yellow Dwarf Newsletter, 5: 25, 1994.

PURIFICATION AND ANTISERUM PRODUCTION OF BARLEY YELLOW DWARF LUTEOVIRUS

Aftab, M., M. Bashir, S. Khalid & I. Ahmed

A purification protocol was developed for barley yellow dwarf luteovirus (BYDV) previously identified as PAV and MAV isolates, to get an adequate amount of virus to produce antiserum for serological tests. Leaf samples from virus – infected plants of wheat, barley and oats were collected during survey of winter season of 1992 from fields at different locations in Punjab and NWFP. The bulk frozen leaf samples were powdered in liquid nitrogen and homogenized (1 g tissue/2ml extraction buffer) in a blender in 0.1 M potassium phosphate buffer (pH 7.0). The homogenate was emulsified with chloroform and butanol (20% v/v each) prior to clarification. The virus was precipitated in 9% (w/v) polyethyleneglycol (PEG, MW: 6000), and 2% NaCl (w/v), and was re-suspended in the same buffer and concentrated by two cycles of differential centrifugation. The partially purified virus was run on 10-40% sucrose density gradients, and the virus band was collected using Isco density gradient fractionator. The preparation had a A 260/280 nm ratio in range of 1.7 to 1.9. A yield of 210 µg of purified virus was obtained from 100 g of leaf tissue. The partially purified virus preparation was observed under electronmicroscope and a large number of isometric virus particles of about 25-30 nm in diameter were observed.

In order to produce antiserum against BYDV, a New Zealand white rabbit was injected intramuscularly with 50 µg of virus emulsified with Freund's complete adjuvant. A total of four injections each of 50 µg of virus were injected at 10 days interval followed by a booster injection of 60 µg virus emulsified with Freund's incomplete adjuvant at 45 days after the initial immunization. A total of three cardiac bleeding were taken and serum was separated and stored at –20°C with one drop of 0.2% NaN_3 as a

preservative for serological tests. The produced antiserum reacted positively when tested in SDS-immunodiffusion tests. However, determination of antiserum titer and standardization for direct and indirect enzyme-linked immunosorbent for crude antiserum and purified immuno-gamma globulins is under process.

Proc. Biotech. Sustainable Dev., Dec. 15-20, 1993, NIBGE, Faisalabad, pp. 96-97.

DETECTION OF BARLEY YELLOW DWARF VIRUS IN PAKISTAN

Khalid, S., M. Aftab, I. Ahmad & M. Aslam

Barley yellow dwarf virus (BYDV) is an important virus disease of small-grain cereals in the world. The infection of BYDV is characterized by several symptoms produced on barley, oat, wheat and other cereals, which include leaf discoloration and reddening, leaf necrosis, stunting, and delay and lack of heading.

In Pakistan BYDV was first noticed on the basis of symptoms in 1964, in wheat crop near Pak-Afghan border, but it was in 1987 when the presence of virus was confirmed by scientists at Rothamsted Experiment Station, England in leaves of diseased plants of wheat, barley, *triticale* (X *Triticosecale* Witmack), and oats collected from different localities. Following 1987, surveys in various years indicate that incidence of BYDV is increasing in cereals in Pakistan. The situation necessitated the need for a systematic study on the virus. The first step in this direction was to standardize the ELISA technique for detection and identification of the virus which is reported here.

Symptoms of BYDV infected plants were observed and recorded at three different planting sites at National Agricultural Research Centre (NARC), Islamabad. Samples for ELISA were collected from patches on the basis of symptoms i.e., stunting various degrees of yellowing/

chlorosis, mosaic type in barley and dwarfing with leaf reddening in oats. Samples were prepared and double antibody sandwich ELISA (DAS-ELISA) was performed for detection of the virus.

A total of 60 samples (20 barley and 40 oats) were collected from different patches and tested through ELISA, of which 25 (9 barley and 16 oats) gave clear-cut positive ELISA values to BYDVPAV isolate. All the samples having red leaf symptoms or chlorosis with dwarfing gave positive ELISA values except in few barley plants with mosaic and chlorosis in which virus was not detected. In our study most of the samples in which PAV was detected gave very high ELISA values, which suggest that the concentration of virus (PAV) was high in these plants. On the other hand, weak reaction to MAV isolate indicates that the reaction was heterologous. Griesbach, et al., (1990) also noticed this and they considered MAV-positive only if it was also negative for PAV. In case of low PAV and MAV-positive, the low MAV-positive value was taken as heterologous and sample was considered only as PAV-positive. So far, RMV, RPV and SGV isolates have not been tested for, but the results against PAV and MAV showed that PAV-like isolates are present in Pakistan. And no mixed infection was detected. On the other hand, samples giving negative ELISA values, especially those showing BYDV-like symptoms, suggest that they may be infected with other BYD isolates.

Pak. J. Bot., 24(2): 225-226, 1992.

BARLEY YELLOW DWARF DISEASE IN PAKISTAN

Barley yellow dwarf (BYD) is an important viral disease of small grain cereals in the world. In Pakistan, sporadic occurrence of a disease with BYD-like symptoms is known since 1964.

However, it was not formally reported in literature until 1987. From 1985 onward the disease became more pronounced with incidence ranging from 0.5 to 1.0%. Heavy incidence of aphids was also noticed on wheat, among which a known vector of BYDV *Macrosiphum (Sitobion) avenae* Fabr., was commonly identified. The presence of BYDV was first confirmed in 1987 at Rothamsted Experiment Station, England, in leaf samples of wheat, barley, *triticale* and oats, collected from different localities of Pakistan. The analysis at Rothamsted indicated the presence of both PAV and RPV like strains of virus.

Recent surveys indicate that the disease is on the increase in cereals in Pakistan. It is more pronounced in the Northern Punjab and North West Frontier Province (NWFP). Almost all the commercial wheat varieties presently under cultivation in Pakistan are infected with BYDV. The disease has also been observed in various international wheat nurseries. Systematic studies have been started at Plant Virology Laboratory, NARC, Islamabad in collaboration with Crop Disease Research Institute (CDRI), PARC, Islamabad. The first step in this direction was standardization of the detection technique for BYDV locally through DAS-ELISA for which IgG and IgG-conjugate was kindly supplied by Dr. P. Gugerli. The adopted technique working satisfactory using which PAV like isolates were readily detection in oat and barley samples.

During surveys in 1991-92 aphids *Rhopalosiphum padi* L. and *R. maidis*. Fitch have been also found associated with wheat, barley and oats in different locations in the province of Punjab and NWFP.

Further work on characterization of BYDV

isolates of local origins including purification, serological and physico-chemical properties is in progress in collaboration with other laboratories working with BYDV.

5th Int. Pl. Virus Epid. Symp. Viruses, Vectors and the Environment, Jul. 27-31, 1992, Valenzano (BARI), Italy, p. 201

BARLEY YELLOW DWARF IN PAKISTAN

Aslam, M. & I. Ahmad

In Pakistan, barley yellow dwarf (BYD) occurs in all the major cereals. Surveys during the last 2 years have shown that almost all the commercial wheat (*Triticum aestivum* L.) varieties presently under cultivation in Pakistan are infected by barley yellow dwarf virus (BYDV). The incidence ranges between 0.5 and 1.0%. The disease is more prevalent in the plains than in the hills. Aphids, including the known BYDV vector, *Sitobion avenae* (Fabricius), have also been found in fairly high densities in areas where BYD was observed. BYD has also been observed in various international wheat nurseries. Studies on BYD are now being undertaken.

Proc. Int. Workshop on World Perspectives on Barley Yellow Dwarf, Jul. 6-11, 1997, Udine, Italy, p. 85.

CHICKPEA

CHICKPEA

SURVEY FOR CHICKPEA AND LENTIL VIRUSES IN PAKISTAN

Bashir, M., R. Jones, K.M. Makkouk, S. Kumari & M.H. Munawar

To know the viral disease situation in chickpea and lentil in Pakistan an extensive survey was conducted during first week of March 1997. More than 6505 samples were collected and tested by tissue-blot immunoassay. Overall one in five plants of lentil were virus infected with 15% of fields having incidence over 50%. Whereas, level of infection in chickpea was low. Pea seed-borne mosaic virus (PSbMV) and cucumber mosaic virus (CMV) were common in lentil, but also occurred in chickpea. Faba bean necrotic yellow virus (FBNYV) and beet western yellow virus (BWYV) were detected for the first time in Pakistan. The other viruses were chickpea chlorotic dwarf virus (CCDV), chickpea luteovirus (CpLV) and alfalfa mosaic virus (AMV). The annual loss due to viral diseases in lentil was estimated as 1.5 million US$.

Proc. 3rd European Conf. on Grain Legumes, Nov., 14-19, 1998, Valladolid, Spain. Part III Posters Diseases and Pests, p. 491.

SURVEY OF CHICKPEA (*CICER ARIETINUM* L.) FOR CHICKPEA STUNT DISEASE AND ASSOCIATED VIRUSES IN INDIA AND PAKISTAN

Horn, N.M., S.V. Reddy, J.F.J.M. van den Heuvel & D.V.R. Reddy

Chickpea chlorotic dwarf geminivirus (CCDV) and some luteoviruses were associated with chickpea stunt disease in India and Pakistan. One thousand eight hundred and four plants with stunt disease symptoms were collected and tested with poly- and monoclonal antibodies. Bean leaf roll luteovirus (BLRV)-like luteoviruses and viruses reacting with an antiserum to a luteovirus isolate from chickpea, tentatively referred to as chickpea luteovirus (CpLV), were involved. Relative prevalence of the viruses varied among the different chickpea-growing areas. The BLRV-like viruses were of minor importance, while CCDV and CpLV-like

viruses were widely distributed. The reaction patterns of the luteoviruses from chickpea with monoclonal antibodies different from those of some known luteoviruses. In addition to CpLV, BLRV, and other luteoviruses, an unidentified, graft-transmissible agent may be involved in the etiology, which is more complex than reported initially.

Pl. Disease, 80(3): 286-290, 1996.

NATURAL OCCURRENCE OF CUCUMBER MOSAIC VIRUS IN CHICKPEA IN PAKISTAN

Bashir, M. & B.A. Malik

In recent surveys, some plants of chickpea breeding material sown at the National Agricultural Research Centre, Islamabad, Pakistan, showed symptoms of a virus disease such as stunting with mild mosaic and reduced terminal buds. Of the 56 samples, assayed eight (14%) reacted positively with the cucumber mosaic virus (CMV) antiserum in repeated tests. None of the samples reacted with the chickpea luteovirus, whereas the antiserum of chickpea chlorotic dwarf virus (CCDV) did not react by the ELISA procedure. The eight samples which reacted positively with the CMV antiserum were further tested with antisera produced against three strain of CMV, the tobacco strain (obtained from NARC, Pakistan), peanut strain (obtained from ICRISAT, India), and bean strain (obtained from Dr. R.O. Hampton, USA). These eight samples reacted strongly with the CMV antiserum of the bean strain and weakly the antiserum of the tobacco strain. No reaction was obtained with the antiserum of the peanut strain of CMV.

The presence of CMV was also confirmed by the symptoms produced on tobacco cvs. Samsun and Xanthi (systemic infection) and on *Chenopodium amaranticolor* (local lesions) after mechanical sap inoculation. Natural occurrence of CMV in chickpea has been reported from Iran, India,

Morocco and Spain. To our knowledge, this appears to be the first report of natural infection of CMV in chickpea in Pakistan.

Int. Chickpea Newsletter, 29:10, 1993.

A SURVEY OF CHICKPEA 'STUNT' DISEASE IN PAKISTAN

Horn, N.M, H.R. Khan, S. Khalid & B.A. Malik

Chickpea 'stunt' is the most important virus disease of chickpea. The symptoms of this disease are leaf reddening in the case of desi chickpeas and yellowing in kabuli chickpeas, internode shortening, plant stunting, and phloem discoloration in the collar region. Thirty three chickpea fields from four stations in the districts of Attock, Chakwal, Thal and Islamabad were visited. Total 217 samples were collected out of which 73 were positive for chickpea chlorotic dwarf virus (CCDV) and 32 for chickpea luteovirus (CpLV). The potential importance of CpLV is shown by its prevalence at the stations at Attock and Chakwal districts.

There is an obvious need to monitor the viruses causing chickpea 'stunt' in Pakistan. Information on the occurrence and distribution of viruses in this crop is essential for developing strategies for breeding and to provide diagnostic tools in case of any calamity.

Int. Chickpea Newsletter, 29:8-10, 1993.

SCREENING OF CHICKPEA GERMPLASM AGAINST *ASCOCHYTA* BLIGHT, *FUSARIUM* WILT AND CHICKPAEA STUNT VIRUS DISEASE

Shakoor, M.A.

Ascochyta blight, *Fusarium* wilt and Stunt virus disease being the major diseases of chickpea in Pakistan, cause appreciable losses and now and then upset the production statistics of the crop in the country. The cheapest and economical control of these diseases is the use of resistant cultivars which are not so commonly available in the country. Therefore, efforts were directed for the screening of available chickpea germplasm for the identification of sources of resistance against these diseases.

The evaluation of 64 chickpea germplasm lines to natural infection of stunt virus disease revealed that ten lines such as 15-1, 48-1, 936, 984, 1114, 1115, 1431, 14174 and 14177 remained completely free (immune) from the disease. None was highly resistant. Six lines such as 46, 270, 832, 908, 1434-1 and 14144 were found to be resistant as they exhibited less than 10 % stunt virus incidence. Twenty lines such as 1-3, 6-1, 113-1, 128, 283, 588, 660-1, 662-1, 816, 854, 896, 907, 910, 914, 927, 942, 985, 1435, C-44, and CM-72 behaved as moderately resistant while the remaining were susceptible to highly susceptible.

Some of the test lines revealed the expression of multiple resistance. Thus one test line i.e. 14177 was found to possess resistance against blight, wilt and stunt virus disease. Two test lines i.e., 14133 and 14177 were resistant to blight and wilt six lines such as 662-1, 816, 914, 936, 85 and 14177 were resistant to both wilt and stunt virus disease while nine test line such as 113-1. 283, 270, 984, 1435, 14144, 14174, 14177 and C-44 were resistant to both blight and stunt virus disease.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad, 1991.

EFFECT OF CHICKPEA STUNT VIRUS ON VEGETATIVE AND YIELD COMPONENTS OF CHICKPEA PLANTS

Ayub, M., M.B. Ilyas, M.N. Bajwa & M.A. Ayub

The effect of chickpea stunt virus on chickpea plants revealed that the virus may affect both the vegetative and yield components. Thus, various chickpea cultivars suffered from 13.88 to 53.3% decrease in plant height, 46.93 to 63.68% decrease in branch length and 30.03 to 94.53% decrease in dry plant weight. Usually the cultivars did not suffer any decrease in number of branches. Similarly, various chickpea cultivars suffered from 58.13 to 98.19% reduction in pod number, 55.11 to 98.21% reduction in number of

seeds per plant, 45.36 to 98.14% reduction in 100 seed weight and 62.40 to 99.81% reduction in plant yield.

Pak. J. Phytopath., 2(1-2):68-73, 1990.

STUDIES ON THE INCIDENCE OF CHICKPEA STUNT VIRUS AND ITS EFFECT ON CHICKPEA PLANTS

Ayub, M.

Twenty seven chickpea cultivars were planted in the field areas of the Department of Plant Pathology, University of Agriculture, Faisalabad, to visualize the incidence of chickpea stunt virus disease in these cultivars. The incidence of chickpea stunt virus infection in different cultivars varied with the cultivar sown. The extent of stunt virus infection varied from 0 to 21.42%. Cultivars 6104-E.57 and 6105-E. 20 remained 100% free from stunt virus infection. The most affected cultivars, in descending order, were 1431, ICC-918, 6102- E.61, 6153, ILC-2956, A-945 and AUG-426 which exhibited 21.42, 20.00, 20.00, 18.75, 18.86, 15.68 and 15.38% disease incidence respectively. Fourteen cultivars exhibited intermediate disease incidence ranging from 7.14 to 13.72% infection. The least affected cultivars were F.81-75, C-44, F.83-2C and 6103-E.22 and they exhibited 5.88, 5.88, 4.00 and 4.00% incidence of the stunt virus, respectively.

Studies on grown responses of ten chickpea cultivars to chickpea stunt virus infection revealed that the virus may affect both vegetative components (plant height, number of branches, length of branches and dry plant weight) and yield components (number of pods, number of seeds per plant, 100 seeds weight and seed yield) of the chickpea plant. The degree of effect, however, varied with the cultivars and probably depended on genetic make up of the cultivars affected. Thus various chickpea cultivars suffered from 13.88 to 53.58 % decrease in plant height. Most of the cultivars did not suffer any decrease

in number of branches. However, two cultivars i.e. ICC-7520 and CM-1917/80 suffered from significant decrease while one cultivar i.e. C-727 exhibited significant increase in number of branches. Various cultivars suffered from 46.93 to 63.68% decrease in length of branches and 30.03 to 94.53% decrease in dry plant weight.

The effect of PLRV on yield components also varied greatly and depended upon the cultivars infected. Various chickpea cultivars suffered from 58.13 to 98.19% reduction in pod number, 55.11 to 98.21% reduction in number of seeds per plant, 45.36 to 98.14% reduction in 100 seeds weight and 62.40 to 99.81% reduction in seed yield.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad, 1988.

CHICKPEA LEAF SMALLING DISEASE

Kausar, A.G.

A disease of gram characterized by smalling of the leaves appeared at Agricultural Farm, Cambellpur. In general, the incidence of the disease was low in the fields of farmers in the locality and appeared seriously in some plots at the farm. Preliminary studies indicated the probable virus nature of the disease as it could be transmitted through sap from affected plants in some cases. Some of the blight resistant varieties appeared to be more susceptible to the disease at the farm than others with the exception of C. 12/34, which was comparatively less susceptible.

Fifty Years of Investigations on Pl. Diseases, Agric. College Res. Inst., Lyallpur, 1960, p. 32

CHILLIES

CHILLIES

EVALUATION OF PEPPER LINES AGAINST TOMATO LEAF CURL VIRUS

Siddiqui, S.A., S. Khalid, M.B. Ilyas & M.A. Aulakh

In search of resistance/tolerance source against tomato leaf curl virus (TLCV), a whitefly (*Bemisia tabaci*) transmitted Geminivirus, seven pepper (*Capsicum annuum* L.) lines were inoculated with TLCV through *B. tabaci* in controlled conditions. Symptoms started to appear seven days after inoculation. Downward leaf curling and stunting of plants was the most common symptom observed. Symptom severity matched with ELISA values. All lines were highly susceptible (high ELISA values), except PBC-491, which showed milder symptoms and low virus titer.

Pak. J. Biol. Sc. (in press), 1999.

DETERMINATION OF PEPPER AS ALTERNATE HOST OF TOMATO LEAF CURL VIRUS (TLCV) AND GREENHOUSE SCREENING OF TOMATO GERMPLASM AGAINST TLCV

Siddiqui, S.A.

Tomato leaf curl virus (TLCV) is a severe disease of tomato *(Lycopersicon esculentum* Mill) throughout the world, including Pakistan. Pepper (*Capsicum annum* L.) is among the alternate hosts of TLCV. In pursuance of a resistant/tolerant source against TLCV, fourteen lines of tomato and seven lines of pepper, received from AVRDC were screened against TLCV under controlled conditions. Nurseries of both (tomato and pepper) were raised in greenhouse of Plant Virology Program at National Agricultural Research Centre, Islamabad. Plants of each line of tomato and pepper were inoculated through its vector-the cotton whitefly (*Bemisia tabaci*) at two to three leaf stage. Data were collected after three and then eight weeks of inoculation as recommended by AVRDC.

Symptoms in pepper and first trial of tomato started to appear eight days after inoculation. Leaf curling, vein thickening and stunting of

plants was the most common symptom observed. Three lines of tomato i.e., ATY-1, ATY-10 and ATY-11 were found to be resistant to TLCV. Although plants of these lines visually displayed mild symptoms but responded negatively to Elisa. In case of pepper lines, reaction in PBC-491 was mild. After three weeks, only twenty percent plants exhibited symptoms of moderate curling and reaction strength in ELISA was 2 to none. However, after eight weeks susceptibility increased upto ninty percent and hybridization results were also positive. All other lines of tomato and pepper showed 100% infection after three weeks and gave positive response to ELISA.

In case of second trial of tomato, symptoms started to appear nearly after three weeks of inoculation. Symptoms were generally severe curling and rolling of leaves. Although same culture of infected tomato was kept for second trial but in first trial rolling is mild and in second trial it become severe. Two lines i.e., ATY-22 and ATY-23 were found to be resistant to TLCV. These varieties remained symptom less after eight weeks of inoculation and also gave negative results to ELISA. All other lines of tomato showed 100% infection after eight weeks of inoculation and gave positive response to ELISA.

Thus in two trials, collectively five lines of tomato were found to be resistant to TLCV. Out of these five lines, only ATY-1 is breeding line while all other four lines are wild species. So we may use ATY-1 as such for cultivation but regarding other lines, their resistance may be incorporated, in breeding program, into commercial cultivars of tomato. It was interesting to note that ATY-23 was disliked by whiteflies as it was not visited by them.

However, all other resistant lines of tomato were frequently visited by whiteflies.

As only five lines of tomato and none of the pepper line was found to be resistant/tolerant to TLCV, so there is a need for further screening of tomato and pepper germplasm to find out resistant/tolerant lines of tomato and pepper against TLCV.

M.Sc.(Hons.) Thesis. Dept. Pl. Path., Univ. Agric., Faisalabad, 1999.

EVALUATION OF PEPPER LINES AGAINST TOMATO LEAF CURL VIRUS UNDER CONTROLLED CONDITIONS

Siddiqui,S.A., S. Khalid & M.B. Ilyas

Tomato leaf curl virus (TLCV) is a severe disease of tomato (*Lycopersicon esculentum* Mill.) throughout the world, including Pakistan. Pepper (*Capsicum annum* L.) is among the alternate hosts of TLCV. In pursuance of a resistant/ tolerant source against TLCV, seven lines of pepper, received from AVRDC, were screened against TLCV by inoculation through its vector- the cotton whitefly (*Bemisia tabaci*), in controlled conditions. Symptoms started to appear eight days after inoculation. Downward leaf curling and stunting of plants was the most common symptom observed. Occasionally twisting of leaves was also noticed. Reaction in PBC-491 was mild. Only 4 out of 20 inoculated plants of line PBC-491 showed mild symptom ten days after inoculation, while mild symptoms in remaining 16 plants developed after 8 weeks. All other lines showed 100% infection.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) pp. 54-55.

PREVALENCE OF CHILLIES VIRUSES IN PAKISTAN

Hameed, S.,

Chilli (*Capsicum annum* L.) is among the important cash crops of Pakistan. It is mainly grown in Sindh and Punjab provinces. Viral disease complex is considered to be the major constraint in its production. The complexity of

H. Shah, H. Ali & S. Khalid

problem was apparent from the varied symptoms of mosaic, mottling, leaf distortion, vein etching, yellowing and narrowing of leaves observed in the field. Direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) based survey conducted in 1994 indicated that chilli veinal mottle virus (CVMV) and cucumber mosaic virus (CMV) were prevalent in almost all locations. Other viruses detected were tobacco mosaic virus (TMV), potato virus Y (PVY), potato virus X (PVX), pepper virus etch (PVE) and pepper mild mottle virus (PMMV). Their incidence was 21.03, 19.6, 8.9, 7.3, 3.17, 1.38 and 1.0%, respectively, while mixed infection was found in 21.23% of the total samples tested.

5th Nat. Conf. of Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, p. 128.

SCREENING OF CHILLI VARIETIES FOR YIELD TOLERANCE AGAINST VIRUS DISEASE 'SMALLING OF LEAVES'

Hussain, A., M. Nazir, A. Chaudhry & Q. Latif

Eight local and exotic chilli varieties viz. Skyline, Guk Chosaeng, Long Green, Chosaeng Mud, Dhing Shah No. I, Sanam, Cluster and Rasco were compared for yield performance and tolerance against virus disease called 'smalling of leaves' during 1987-88 and 1988-89. Skyline and Guk Chosaeng produced higher yield during both the years. Skyline showed the lowest incidence of virus infection while Dhing Shah No. I, Long Green and Rasco were found highly susceptible.

J. Agric. Res., 32(2): 201-205, 1994.

SCREENING OF *CAPSICUM* SPP. FOR TOBACCO MOSAIC VIRUS RESISTANCE

Hameed, S., M.A. Khan, S. Khalid,

Eight cultivars/accessions of *Capsicum* were screened for TMV resistance. Experiments were carried out both under controlled and field conditions. Inoculations were made eight weeks after sowing. Based on symptoms expression and ELISA test, cultivars. Longhi, Ghotki, Qaiser, and Hungarian Hot Wax, and two selections of chillies namely, Bhawani and NARC-4 were

K.M. Khokhar & M. Banaras

found to be susceptible, whereas Anaheim TMR-23 and an accession of *C. chinense* (Chi-3) were scored as resistant both under controlled and field conditions. Symptoms were not indicative of the virus titre in case of susceptible cultivars. Efforts are underway to transfer the resistance into local cultivars.

Pak. J. Phytopath., 5(1-2): 78-81, 1993.

SCREENING OF CHILLI VARIETIES FOR YIELD AND THEIR TOLERANCE AGAINST VIRUS DISEASE "SMALLING OF LEAVES" UNDER FAISALABAD CONDITIONS

Hussain, A., M. Nazir, A. Chaudhary & Q.L. Cheema

The local and exotic Capsicum annuum varieties Sky Line, Guk Chosaeng, Long Green, Chosaeng Mud, Dhing Shah 1, Sanam, Cluster and Rasco were evaluated during 1987-88 and 1988-89 for yield and tolerance of an unspecified virus disease causing small leaf size. Sky Line showed the lowest incidence of viral infection while Dhing Shah 1, Long Green and Rasco were highly susceptible. Mean yields over both seasons were highest in Dhing Shah 1 (1837 kg/acre).

Capsicum Newsletter, Special issue, EUCARPIA 8th meeting on Genetics and Breeding on Capsicum and Eggplant, Sep. 7-10, 1992, Rome, Italy, pp. 86-91.

SYMPTOMATOLOGY, HOST RANGE AND TRANSMISSION OF CHILLI MOSAIC VIRUS

Aftab, M.

Typical symptoms on mosaic affected chillies (*Capsicum annum* L.) in field and in the growth chamber artificially inoculated plants were chlorotic and darkgreen areas on leaves, sometimes blistering the color. In case of severe attack puckering of leaf surface and wrinkling of lamina occurs. Distortion of the growth, shortening of the internodes is an important symptom. Plants produce less flowers and fruits. Sometimes stunting the whole plant.

PeMV was identified on symptom basis including five differential and five indicator plants. Differential hosts were *Capsicum annuum*,. *C.*

frutescens, N. glutinosa, Solanum nigrum of family *Solanaceae* and *Beta vulgaris* of family *Chenopodiacae*. Hosts used as indicator were *Nicotiana rustica, N. tabacum* var. White burley, *Physalis floridana. Datura stramonium* of family *Solanaceae* and *Cucumis sativus* of *Cucurbitaceae*.

All those test plants develop systemic symptoms except *Beta vulgaris* which developed only necrotic local lesions. Plants raised from nursery were inoculated at the age of six to eight weeks. Those not raised from nursery were inoculated at cotyledonous or three leaf stage. Incubation period of virus after inoculation till appearance of symptom was six to twelve days. Mostly the systemic infections were severe than localized.

A total of 57 species of different host including differential and indicators were tested against this virus, which were grouped in 46 genera and 14 families, out of which 30 species were proved susceptible. Some other local lesion hosts were *Trigonella foenum graecum* L., *Chenopodium amaranticolor* L., *Vigna unguiculata, Trifolium alexandrium* and *Midicago sativa* L.

This virus was unable to be transmitted through infected chilli seeds as well as from seeds of *Nicotiana glutinosa*. Only *Aphid gossypii* was used for vector transmission which transmitted PeMV efficiently.

M.Sc. (Hons) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad, 1984.

CITRUS

SURVEY OF CITRUS TRISTEZA VIRUS IN PUNJAB (PAKISTAN)

Anwar, M.S & M.S. Mirza

A survey of some citrus growing areas of Punjab was conducted to establish the presence of citrus tristeza virus (CTV). Fourteen localities in five Districts viz; Sahiwal, Sargodha, Faisalabad, Lahore and Sheikhupura were surveyed. ELISA tests indicated the presence of CTV in Faisalabad, Sheikhupura, Sargodha and Sahiwal districts where as no infection was recorded in Lahore district. Infection varied from 7.14 to 18.18% in various localities. Highest infection (18.18%) was recorded in Sahiwal district, while lowest (7.14%) was in Sheikhupura. Infection in Faisalabad was 13.13% and in Sargodha district, it was 13.20%.

Proc. 1st Int. Sem. on Citriculture in Pakistan, Dec. 2-5, 1992, Univ. Agric., Faisalabad, pp. 413-416.

A SURVEY FOR TRISTEZA AND GREENING IN PUNJAB (PAKISTAN)

Catara, A., A. Azzaro, M. Davino, V. Grimaldi, M. Hussain, A. Saleem & M.S. Mirza

A survey for citrus tristeza virus (CTV) and greening was carried out in Punjab province. More than fifty orchards and ten nurseries were sampled in different areas of the province. ELISA tests and electronmicroscopic observations showed that CTV was present in different districts in the varieties: Mosambi, Bloodred and Pineapple sweet orange. Mosambi variety was the most affected (7 positive out of 35). Many orchards showed symptoms of greening on different species (lemon, grapefruit, sweet orange and mandarin). Electronmicroscopic observations revealed the presence of the greening organism in phloem cells. *Diaphorina citri* was found widespread in the province.

11th IOCV Conf., California Riverside, USA, pp. 166-170, 1991.

DETECTION OF CITRUS TRISTEZA AND GREENING IN PAKISTAN THROUGH ELECTRON-MICROSCOPY

Grimaldi, V. & A. Catara

An electronmicroscopic investigation was carried out on citrus fruits and leaves collected during a survey in Pakistan. Thread-like particles of citrus tristeza virus (CTV) were ascertained in phloem tissues of the columella, whereas the bacterium associated with greening was detected in sieve tubes of the columella and in leaf midribs of trees showing typical yellow vein, fruit malformation and decline. CTV was also confirmed by ELISA tests.

J. Phytopath., 126: 17-21, 1989.

VIRUS, VIROID AND PROKARYOTIC DISEASES OF CITRUS IN PAKISTAN

Catara, A., A. Azzaro, S.M. Mughal & D.A. Khan

A survey for citrus virus and virus-like diseases has been carried out in NWFP and Punjab provinces. Exocortis has been observed on different trees grafted onto citranges and sweet limes, whereas *cachexia-xyloporosis* has been detected on Willow leaf and Feutrell's early mandarin. Severe infectious variegation and ring spot were present on Eureka lemon in many orchards and nurseries. Tristeza was detected, only in few trees, in both provinces. CTV was confirmed by ELISA and electron-microscopy. Many orchards showed symptoms of greening and infestation of *Diaphorina citri*. The causal agent of the disease was observed under the electronmicroscope in the phloem cells. Brief informations on other diseases, such as stubborn and ring pattern, are also given.

Proc. 6th Int. Citrus Cong., Mar. 6-11, 1988, Tel Aviv, Israel, pp. 957-962.

COTTON

COTTON

RELATIONSHIP OF COTTON LEAF CURL VIRUS SYMPTOMS WITH VIRUS CONCENTRATION AND EPITOPE PROFILE

Khalid, S., H. Shah & M.A. Masood

Vein thickening, enation formation and curling of leaves (upward or downward) are characteristic symptoms of cotton leaf curl virus, a whitefly (*Bemisia tabaci*) transmitted geminivirus, in cotton. To ascertain the effect of virus titer on type of curling and their relationship with epitope profile we determined virus concentration in 26 samples (13 each upward and downward curling) and established epitope profile of 34 samples (17 each) by TAS-ELISA using polyclonal antisera to Indian Cassava Mosaic Virus (ICMV) and Monoclonal Antibodies (MAbs) to African Cassava Mosaic Virus (ACMC) & ICMV and Okra Leaf Curl Virus (OLCV). No relationship between virus concentration and epitope profile was found with type of symptoms.

Pak. J. Biol. Sc. (in press), 1999.

GENETIC VARIABILITY OF NATURAL POPULATIONS OF COTTON LEAF CURL GEMINIVIRUS, A SINGLE-STRANDED DNA VIRUS

Sanz, A.I., A. Fraile, J.M. Gallego, J.M. Malpica & F.Garcia-Arenal

Reports on the genetic variability and evolution of natural populations of DNA viruses are scarce, in comparison with the abundant information on the variability of RNA viruses. Geminiviruses are plant viruses with circular ssDNA genomes that are replicated by the host plant DNA polymerases. Whitefly-transmitted geminiviruses (WTG) are the agents of important diseases of crop plants, and best exemplify emerging plant viruses. In this report we have analyzed the genetic diversity of cotton leaf curl geminivirus (CLCuV), a typical emerging WTG. No genetic differentiation was observed between isolates from different host plant species or geographic regions. Thus, the analyzed isolates represented a unique, undifferentiated population. Genetic variability, estimated as nucleotide diversities at

synonymous positions in ORFs for the AC1 (=replication) protein and coat protein (CP=AV1), was very high, exceeding the values reported for different genes in several plant and animal RNA viruses. This was unexpected in a virus that uses the DNA replication machinery of its eukaryotic host. Diversities at non-synonymous positions, on the other hand, indicated that variability may be constrained in the genome of CLCuV. Ratio of non-synonymous to synonymous substitutions varied for the different ORFs: they were higher for CP than for AC1, and lesser still for the AC4 and AV2 ORFs, that overlap AC1 and CP ORFs, respectively. Analysis of nucleotide diversities at synonymous and non-synonymous positions of the AC4 and AV2 ORFs, suggest that their evolution is constrained by AC1 and CP, respectively. Data suggest that AC4 and AV2 are new genes that may have originated by overprinting on the pre-existent AC1 and CP genes. Evidence for recombination was found for the AC1 and CP ORFs, and for the non-coding intergenic region (IR). Data indicate that the origin of replication is a major recombination point in the IR, but not the only one. Analyses of the IR also suggest that recombinants may be frequent in the population, and that recombination may have an important role in the generation of CLCuV variability.

J. Mol. Evolution (in press), 1999.

COTTON LEAF CURL VIRUS INDUCED SYMPTOMS AND THEIR RELATION WITH VIRUS TITER AND EPITOPE PROFILE

Leaf curl disease of cotton (*Gossypium hirsutum* L.) is characterized by vein thickening, enation formation, and curling of leaves. The disease is caused by cotton leaf curl virus (CLCuV) of genus begomovirus (*Geminiviridae*, subgroup III), transmitted by whitefly (*Bemisia tabaci*). Generally the virus induces two types of leaf

Khalid, S. & H. Shah

curling i.e. upward (UP) or downward (DW). Both types of symptoms are observed in almost all commercially cultivated cotton varieties in naturally infected as well as artificially inoculated plants. In addition, mixed type (DW and UP) can also be seen in some plants. To find out relationship, if any, between type of symptoms, virus titer and epitope profile of CLCuV infected plants, a study was initiated. Samples of cotton plants showing UP & DW leaf curling symptoms were separately collected during 1996-97 cotton seasons from Multan area. Virus titer and epitope profile of the samples were determined through TAS-ELISA using polyclonal (ACMV-African cassava mosaic virus) and panel of Monoclonal antibodies (Mabs) raised against other whitefly transmitted Geminiviruses (ICMV, Indian cassava mosaic virus and Okra leaf curl virus-OLCV).

Statistical analysis (T-test at alpha=1%) of TAS-ELISA regarding virus concentration and type of symptoms, showed that virus concentration are independent of symptom's type. Similarly no relation could be established between epitope profile of tested samples and type of symptoms. Generally, it has been observed that initially most infected plants develop Up-curling in the field or when artificially inoculated (data not shown) and Dw-curling mostly in ratoon cotton. It appears that time of infection, combination of virus variants, environmental conditions and cottons genotype or combination of these and other unknown factors might have to play some role type of CLCuV induced symptoms.

ICAC-CCRI – Reg. Consult. on Insecticide Resist. Manag. in Cotton. Jun 28-July 1, 1999. CCRI, Multan, (Abs.). p. 33.

DNA VARIANTS AMONG PAKISTAN ISOLATES OF COTTON LEAF CURL VIRUS

Mansoor, S., S.H. Khan, M. Hussain, A. Bashir, M. Saeed, Y. Zafar, J. Stanley, R. Bridon, P. Markham & K.A. Malik

Cotton leaf curl disease remained the most important constraint for cotton production in Pakistan. Four distinct begomoviruses were identified during the cloning of the causative agent. Several full-length and partial clones of these viruses were obtained. These begomoviruses showed high sequence homology to some other members of the genus from the Indian subcontinent. Analysis of the putative Rep-binding motifs in the intergenic region suggest these as distinct begomoviruses and were named as CLCuV-Pak1, CLCuV-Pak2, CLCuV-Pak3 and CLCuV-Pak4, respectively. A full-length or partial dimer of the virus was able to replicate, caused systemic infection produced leaf curl symptoms but the lack of enations suggested that additional viral components are involved in the disease.

During the search for additional virus-like component a novel DNA component, of about 1.38kb which showed homology to plant nanoviruses was found associated with the disease. The novel component was found to be encapsidated in geminate particles and transmitted by whiteflies. The absence of the molecules in tolerant cotton genotypes and the presence in other hosts of the disease strongly suggested a role for the nanovirus in the disease. Interestingly, the component was found associated with all four begomoviruses and was also detected in whiteflies. We propose that the begomoviruses associated with the disease provide encapsidation and transmission of the viral component while the nanovirus has a role in symptom development and movement of the virus. The implication of the hypothesis on the diversity of the geminivirus, symptom variants and control strategies will be discussed.

Begomoviruses associated with the disease were detected specifically by intergenic region

probes and by PCR with primers specific for the viruses. A simple protocol was developed for the isolation of template suitable for PCR and the two of these viruses were detected by multiplex PCR such that the whole procedure of template preparation, PCR and analysis of the PCR products by agarose gel electrophoresis could be completed in a single day. These molecular diagnostic methods were used for the identification of natural hosts of the viruses. Twenty plant species in six plant families were found infected with either one or more of the cotton begomoviruses. The largest number of host plants belonged to the family *Malvaceae* with several hosts in *Solanaceae* and *Cucurbitaceae* families. Multiple infection of cotton begomoviruses was commonly detected in the field. However, no correlation was found between the presence of symptoms and a particular combination.

Cotton genotypes were analyzed for resistance to the disease under natural conditions and its correlation with the level of viral DNA was investigated. It was found that susceptible varieties accumulate several folds higher level of viral DNA as compared to the tolerant varieties.

ICAC-CCRI – Reg. Consult. on Insecticide Resist. Manag. in Cotton. Jun 28-July 1, 1999. CCRI, Multan, (Abs.). p. 35.

GENETIC ENGINEERING FOR LEAF CURL VIRUS RESISTANCE IN COTTON

Asad, S., A. Bashir, W.A.A. Haris, C.P. Lichenstein, Y. Zafar & K.A. Malik

Cotton leaf curl (virus) disease incidence has showed a declined production of cotton in Pakistan for the last six years. To develop resistance cotton against leaf curl virus, genetic engineering was used to manipulate the DNA of CLCuV DNA-A borne AC1 gene along with the antisense DNA of the adjacent AC2 and AC3 genes to construct different vectors for plant transformation. *Agrobacterium* mediated and

Biolistic mediated transformations by using sense and antisense constructs were made and independent transgenic lines of tobacco and cotton were selected on kanamycin. The progenies of tobacco and cotton (T_0 and T_1) grown in containment facility, were analyzed by PCR, Southern, RT-PCR and Northern analysis. The primary transformants showing presence and expression of transgene were challenged to pathogen (CLCuV) by grafting and exposure to viruliferous whiteflies.

2nd Nat. Symp. on Pl. Tissue Culture and Genetic Engg. Jun 1-3,1999. Agri. Biotech. Inst., NARC, Islamabad (Abs.) p: 12.

COTTON LEAF CURL DISEASE IN PAKISTAN: MOLECULAR CHARACTERIZATION, AND GENETICALLY ENGINEERED VIRUS RESISTANCE

Mansoor, S., S.H. Khan, A. Bashir, M. Saeed, Y. Zafar, R.W. Briddon, J. Stanley, P.G. Markham & K.A. Malik

Cotton leaf curl disease in Pakistan was shown to be a novel combination of begomoviruses and a novel DNA component showing homology to plant Nanoviruses. Four distinct begomoviruses were identified during the cloning of the causative agent. Several full length and partial clones of these viruses were obtained. These begomoviruses showed high sequence homology to some other members of the genus from the Indian subcontinent. Analysis of the putative Rep-binding motifs in the intergenic region suggest these as distinct begomoviruses and were named as CLCuV-Pak1, CLCuV-Pak2, CLCuV-Pak3 and CLCuV-Pak4, respectively. A full length or partial dimmer of the virus was able to replicate, caused systemic infection produced leaf curl symptoms but the lack of enations suggested that additional viral component are involved in the disease.

During the search for additional virus-like component a novel DNA component of about 1.38kb that showed homology to plant nanoviruses was found associated with the disease. The novel component was found to be encapsidated in geminate particles and

transmitted by whiteflies. The absence of the molecule in tolerant cotton genotypes and the presence in other hosts of the disease strongly suggested a role for the nanovirus in the disease. Interestingly, the component was found associated with all four begomoviruses and was also detected in whiteflies. We propose that begomoviruses associated with the disease provide role in symptom development and movement of the virus. The implication of the hypothesis on the diversity of the geminivirus, symptom variants and control strategies will be discussed.

Virus-induced expression of ribosome inactivating protein (RIPs), a novel method for the induction of genetically engineered resistance was used to develop transgenic resistance against the disease in an elite local cultivar (S-12) from Pakistan. Transformation of dianthin, an RIP in the S-12 was confirmed by PCR and Southern hybridization and transgenic T1 plants were resistant to the virus when inoculated through whiteflies.

The encapsidation of the nanovirus-like component in geminate particle and transmission by whiteflies is a novel phenomenon. This is the first report of a whitefly transmitted nanovirus-like DNA molecule and the first demonstration of a disease involving two single stranded DNA components which share no sequence homology. The ability to transform a local elite variety of cotton and the fact that transgenic plants show resistance to the disease provides a tool for the use of biotechnology to solve this important problem.

2nd Nat. Symp. on Pl. Tissue Culture and Genetic Engg. Jun 1-3,1999. Agri. Biotech. Inst., NARC, Islamabad, (Abst.) p.82

IDENTIFICATION OF A NOVEL CIRCULAR SINGLE-STRANDED DNA ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

Mansoor, S., S.H. Khan, A. Bashir, M. Saeed, Y. Zafar, K.A. Malik, R. Briddon, J. Stanley, & P.G. Markham

Recent reports have suggested that cotton leaf curl virus (CLCuV), a geminivirus of the genus *Begomovirus*, may be responsible for cotton leaf curl disease in Pakistan. However, the causal agent of the disease remains unclear as CLCuV genomic components resembling begomovirus DNA A are unable to induce typical disease symptoms when reintroduced into plants. All attempts to isolate a genomic component equivalent to begomovirus DNA B have been unsuccessful. Here, we describe the isolation and characterization of a novel circular single-stranded(ss) DNA associated with naturally infected cotton plants. In addition to a component resembling DNA A, purified geminate particles contain a smaller unrelated ssDNA that we refer to as DNA 1. DNA 1 was cloned from double-stranded replicative form of the viral DNA isolated from infected cotton plants. Blot hybridization using probes specific for either CLCuV DNA or DNA 1 was used to demonstrate that both DNAs co-infect naturally infected cotton plants from different geographical locations. DNA 1 was detected in viruliferous *Bemisia tabaci* and in tobacco plants infected under laboratory conditions using *B. tabaci*, indicating that it is transmitted by whiteflies. Sequence analysis showed that DNA 1 is approximately half the size of CLCuV DNA but shares no homology, indicating that it is not a defective geminivirus component. DNA 1 has some homology to a genomic component of members of Nanoviridae, a family of DNA viruses that are normally transmitted by aphids or planthoppers. DNA 1 encodes a homologue of the nanovirus replication-associated protein (Rep) and has the capacity to autonomously replicate in tobacco. The data suggest that a nanovirus-like DNA has become whitefly-transmissible as a result of its association with a geminivirus and that cotton

leaf curl disease may result from a mutually dependent relationship that has developed between members of two distinct DNA virus families that share a similar replication strategy.

Virology, 259:190-199, 1999.

UTILIZATION OF COTTON LEAF CURL VIRUS COAT (AV1) AND REPLICATION ASSOCIATED (AC1) PROTEINS FOR POLYCLONAL ANTIBODY PRODUCTION

Faisal, A.

Serological tests based on the reaction of an antibody with the antigen are important diagnostic tools for the identification of plant viruses but they are limited by the availability of antisera for plant viruses. The present study was carried out to develop polyclonal antibodies against cotton leaf curl virus, to facilitate the acquisition of the antiserum against this particular virus species, make the system accessible to the scientists involved in disease diagnosis and particularly the identification of host plant species for geminiviruses. Antisera are usually raised by injecting purified virus particles in laboratory animals but in case of geminiviruses it is limited by the low concentration of the virus in the plants and its fragility after purification. To combat this problem, viral proteins were expressed in a bacterial system, purified and used for antibody production.

The coat (AV1) and replication associated (AC1) proteins of two isolates of CLCuV (CLCuV-72b and CLCuV-26) were used for antibody production. These proteins were selected based on the amino acid sequence homology with other geminiviruses. So that the antibodies raised against these proteins could differentiate between CLCuV and other geminiviruses. The two genes were cloned in a bacterial expression vector pET32a for the over expression of the proteins. The proteins were purified from crude cellular extract with affinity chromatography. After purification,

concentration and quantification, a 500:g dose of each of these proteins was injected intramuscularly into female albino rabbits. A total of three injections were given with a gap of one week and the antiserum was collected one week after the booster dose.

The antibodies raised against AV1 of CLCuV-72b were of good titre and detected the two isolates in the infected plants. However, the antibodies against AC1 protein of two isolates although indicated a good titre but were unable to detect the virus in the infected plant. This could be due to the low titre of this protein in the infected plants. The present study helped to establish a system for the over expression of proteins in bacteria. The antibodies produced can be used for various serological tests like ELISA or Western blotting for the detection of cotton leaf curl virus in infected plants and would certainly pave way to study the virus movement in the cells to have a better understanding of systemic infection and long distance movement in plants.

M.Phil. Thesis, Quaid-i-Azam Univ., Islamabad, 1999.

MULTIPLE INFECTION, RECOMBINATION AND GENOME RELATIONSHIPS AMONG BEGOMOVIRUS ISOLATES FOUND IN COTTON AND OTHER PLANTS IN PAKISTAN

Sanz, A.I., A. Fraile, F. Garcia-Arenal, X. Zhou, D.J. Robinson, S. Khalid, T. Butt & B.D. Harrison

Begomoviruses are found in many plant species in Pakistan and, since 1985, have caused an increasingly serious epidemic of leaf curl disease in cotton. PCR analysis with primer pairs specific for each of the four already identified types of cotton leaf curl virus (CLCuV-PK types a, 26, 72b and 804a) and for okra yellow vein mosaic virus (OYVMV) showed that many individual naturally infected plants of cotton and other malvaceous species contained two or three begomoviruses. Similarly, comparison of the sequences of overlapping fragments of begomovirus DNA-A, obtained from single naturally infected plants,

revealed much multiple infection in both malvaceous and non-malvaceous species. Some cotton plants contained sequences typical of begomoviruses from non-malvaceous species, and some non-malvaceous plants contained sequences typical of CLCuV-PK. Several putative recombinant sequences contained elements typical of different types of CLCuV-PK, or of different malvaceous and/or non-malvaceous begomoviruses. Often a recombination site occurred at the origin of replication. Sequence elements typical of CLCuV-PK, but not CLCuV-PK itself, were found in begomovirus-infected malvaceous and non-malvaceous species growing in Pakistan outside the area of the cotton leaf curl epidemic. We suggest that recombination among such viruses was a key factor underlying the emergence of CLCuV-PK. Recombination, following multiple infection, could explain the network of relationships found among begomoviruses in the Indian subcontinent, and their coordinated divergence from begomoviruses causing similar diseases in other major geographical regions.

J. Gen. Virology (in press), 1999.

EFFECT OF SOME BIO-PESTICIDES ON COTTON LEAF CURL VIRUS TRANSMISSION THROUGH *BEMISIA TABACI*

M.B.A. Khan

Cotton leaf curl is an important disease of cotton caused by cotton leaf curl virus (CLCuV), transmitted by whitefly (*Bemisia tabaci*) in a persistent manner. In this study the effects of bio-pesticides on the transmission of CLCuV and *B. tabaci*) was investigated in a highly susceptible cotton cultivar (S-12). Foliar application of mixture of surf, sunflower oil and water (1:50:1250 w:v:v), 1% neem oil in acetone and crude extract of neem leaves in water (1:10) resulted in 100% reduction in disease transmission along with an efficient check on whitefly population. Eighty percent plants

remained asymptomatic and the number of whitefly per replication was also significantly reduced after the application of crude extract of neem seed in water (1:10), 1% rape seed oil, 0.5% neem oil in acetone and 3% neem flavonoids in water. On the other hand, nimbokil in water (0.5%) resulted in 40% reduction in disease incidence or disease transmission and the whitefly population was also suppressed. Twenty percent reduction in disease transmission occurred with neem oil (0.1%) in acetone and 3% neem flavonoids in ethanol without any effect on the whitefly population. Severe disease symptoms were developed on control plants (acetone treated and untreated) and whiteflies were also present on all plants in different number. The CLCuV was detected through triple antibody sandwich enzyme-linked immunosorbent assay using polyclonal antisera to african cassava mosaic virus (ACMV) and monoclonal antibody SCR-60 to indian cassava mosaic virus (ICMV). All plants showing CLCuV symptoms were positive to ACMV/ICMV antiserum while asymptomatic plants were negative. In case of soil application 75% reduction of disease transmission was achieved with crushed neem leaves and seeds while 50% with NJC-3. However, NJC-1 and NJC-2 did not inhibit the disease transmission. Severe symptoms were developed on untreated control plants. The treatments used in soil application did not affect the whitefly population.

M.Sc.(Hons.) Thesis. Deptt. Pl. Path., Faculty of Crop Prot. Sci., NWFP Agri. Univ., Peshawar, 1999.

RAPID MULTIPLEX PCR FOR THE SPECIFIC DETECTION OF TWO WHITEFLY-

Cotton leaf curl disease in Pakistan is associated with two whitefly-transmitted geminivirus species named cotton leaf curl virus Pk1 (CLCuV-Pk1) and cotton leaf curl virus Pk2

TRANSMITTED GEMINIVIRUS SPECIES ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

Mansoor, S., A. Bashir, S.H. Khan, M. Hussain, M. Saeed, Y. Zafar, P.G. Markham & K.A. Malik

(CLCuV-Pk2). PCR is a highly specific and reliable technique for the detection of geminiviruses. A protocol has been developed for rapid isolation of a suitable template for PCR. The method is based either on the adsorption of DNA template from cleared lysate on PCR tubes or a rapid minipreparation of total DNA by CTAB method. Similarly, a simplified protocol is used for the isolation of total DNA from individual whitefly which is suitable for PCR amplification. Primers have been designed in such a way that the two geminivirus species are amplified in a single tube by multiplex PCR. In this PCR virus sense primer is common to both viruses in the rep gene whereas the complementary sense primer is specific for either of the two viruses. For CLCuV-Pk1, the reverse primer is designed at the start of C4 ORF gene whereas for CLCuV-Pk2 the primer is designed at the start of rep gene. The two PCR products of about 360 bp (CLCuV-Pk1) and 510 bp (CLCuV-Pk2) are resolved on an agarose gel. A rapid profile for multiplex PCR was used and completed in 2 hr. The whole process of template preparation, PCR and the detection of PCR product by agarose gel electrophoresis is completed in a single day. The protocol has been used reliably for the detection of cotton geminiviruses in plant and whitefly samples collected from the field.

Pak. J. Bot., 31(1):115-123, 1999.

STATUS OF COTTON LEAF CURL VIRUS IN PAKISTAN

Kandhro, M.M., K. Soomro & S.D. Khanzada

Existing and future genotypes including candidate varieties of cotton (*Gossypium hirsutum* L.) were screened against prevailing virulences of CLCuV at adult plant stage under natural epiphytotic conditions at different locations in Sindh, Punjab and NWFP during Kharif 1997-98. Among 16 coded varieties only one candidate variety V8 (VH-53) was found

symptomatologically free from CLCuV at all locations.

Virulence spectrum prevailing in Punjab and NWFP and occurrence of CLCuV in south Pakistan (Sindh) and its threat to cotton crop will also be discussed.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p.63.

RELATIONSHIP OF ENVIRONMENTAL CONDITIONS CONDUCIVE TO COTTON LEAF CURL VIRUS DISEASE DEVELOPMENT

Khan, M.A., J.H. Mirza & S. Ahmad

Weekly air temperature (max/min), rainfall, relative humidity, wind velocity and movement were regressed against percent plant infection by leaf curl virus on eight varieties of cotton. Relationship of weekly air temperature (max/min), relative humidity, wind velocity and movement to cotton leaf curl virus (CLCuV) disease development was explained by linear regression in most of the varieties. Percent plant infection by CLCuV increased on all varieties at maximum and minimum air temperature of 33-45 and 25-30°C, respectively. There was poor correlation of weekly rainfall to CLCuV disease development humidity, 6 km/h wind velocity and 7000 km/h hours total wind movement.

Pak. J. Phytopath., 10 (1): 5-8, 1998.

RAPID MULTIPLEX PCR FOR THE SPECIFIC DETECTION OF TWO WHITEFLY-TRANSMITTED GEMINIVIRUS SPECIES ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

Mansoor, S., A. Bashir, S.H. Khan, M. Hussain, Y. Zafar & K.A. Malik

Cotton leaf curl disease in Pakistan is associated with two whitefly-transmitted geminivirus species named cotton leaf curl virus Pk1 (CLCuV-Pk1) and cotton leaf curl virus Pk2 (CLCuV-Pk2). A protocol has been developed which allows rapid isolation of a suitable template for PCR which is based either on the adsorption of DNA template from cleared lysate on PCR tubes or a rapid minipreparation of total DNA by CTAB method. Similarly, a simplified protocol is used for the isolation of total DNA from individual whiteflies. A multiplex PCR is performed where forward primer is common to

both the viruses in C1 gene whereas the reverse primer is specific for either of two viruses. For CLCuV-Pk1, the reverse primer is designed at the start of C4 gene whereas for CLCuV-Pk2 the primer is designed at the start of C1. The two PCR products of about 360 bp (CLCuV-Pk) and 510 bp (CLCuV-Pk2) are resolved on an agarose gel. A rapid profile for multiplex PCR was used and completed in two hours. The protocol has been used reliably for the detection of cotton geminiviruses in plant and whitefly samples collected from the field.

World Cotton Res. Conf. - 2, Sep. 6-12, 1998, Athens, Greece, (Abst) p. 280.

POLYMERASE CHAIN REACTION-BASED DETECTION OF COTTON LEAF CURL AND OTHER WHITEFLY-TRANSMITTED GEMINIVIRUSES FROM SINDH

Mansoor, S., M. Hussain, S.H. Khan, A. Bashir, A.B. Leghari, G.A. Panwar, W.A. Siddiqui, Y. Zafar & K.A. Malik

Samples of cotton plants showing symptoms of cotton leaf curl disease were collected from cotton fields in Sindh. Samples of some other plants including tomato, chillies, okra and *Hibiscus* suspected for whitely-transmitted geminiviruses were also collected from these areas and total DNA was extracted. Degenerate primers designed to amplify DNA-A of whitefly-transmitted geminiviruses were used in PCR for the amplification of viral DNA. A product of expected (1.4 kb) was obtained from all these samples which confirmed the infection of whitefly-transmitted geminiviruses. PCR primers specific for the two whitefly-transmitted geminiviruses species namely; CLCuV-Pk1 and CLCuV-Pk2 found associated with cotton leaf curl disease in Punjab were also used to confirm the identity of cotton leaf curl virus in Sindh. A product specific for CLCuV-Pk1 was obtained from all four symptomatic cotton samples. The results showed that cotton samples were infected with CLCuV-Pk1 while CLCuV-Pk2 was not detected in these samples. This is the first report of detection of whitefly-transmitted gemini-viruses on these crops from Sindh. Our data not only confirm the presence of a whitefly-

transmitted geminivirus on cotton but also showed that the disease is caused by one of the virus species found in Punjab.

Pak. J. Biol. Sci., 1(1): 39-43, 1998.

PATTERNS OF VARIATION IN DNA-A AND IN DEFECTIVE DNA MOLECULES OF COTTON LEAF CURL VIRUS

Harrison, B.D., X. Zhou, Y. Liu & D.J. Robinson

Complete DNA-A sequences of nine Pakistani begomovirus isolates from leaf curl-affected cotton (CLCuV-PK) or from okra, and the partial sequences of several additional isolates were determined. Of the four main types of sequence found among isolates from cotton, two differ principally in their intergenic region and represent closely related strains. DNA-A of begomovirus isolates from leaf curl-affected okra was essentially the same as one or other of these two types of CLCuV-PK DNA-A, whereas Pakistani isolates of okra yellow vein mosaic virus (OYVMV) had another type of DNA-A which was nevertheless closely allied to DNA-A of the isolates from cotton. The third type of CLCuV-PK DNA-A had *AV1(CP), AV2, AC3* and *AC2* genes very like those of OYVMV but *AC1* and *AC4* genes of unknown origin. In the fourth type of CLCuV-PK these affinities were reversed. These last two types of CLCuV-PK appear to be derived from OYVMV and one or more other unspecified begomoviruses by recombination involving sites at the origin of replication and at a point near the 3' end of the *AC1* gene. Individual cotton plants can contain two or three of these types of CLCuV-PK DNA-A. Moreover, CLCuV-PK can be transmitted experimentally to okra, *Phaseolus vulgaris*, tobacco and tomato, species which are naturally infected in Pakistan with other begomoviruses. Co-infection of such species would provide opportunities for a variety of recombination events to occur. Our evidence suggests that recombination has played a vital part in emergence of the several types of CLCuV-PK that are associated with the current major

epidemic of cotton leaf curl in Pakistan.

When CLCuV-PK was transmitted by *Bemisia tabaci* to tobacco, and the tobacco plants were kept in the glasshouse for two years or more, defective molecules of circular dsDNA accumulated in them in substantial amounts. Eleven plants all contained a different predominant form of defective molecules, each of which was derived from DNA-A by a unique combination of sequence deletion, inversion, duplication and/or rearrangement. A few also contained segments of DNA not derived from CLCuV-PK DNA-A. The defective molecules were mostly of 1300-1400nt and were transmissible between plants, along with full-size DNA-A, by grafting and by *B. tabaci*. They all contained the intergenic region and 5' part of the *AC1* gene. They also contained wholly and partly novel open reading frames, some of which could perhaps have a role in begomoviral evolution.

2nd Int. Workshop on Bemisia and Geminiviral Diseases, Jun. 7-12, 1998, San Juan, Puerto Rico, p. L-90.

MOLECULAR DISSECTION OF COTTON LEAF CURL VIRUS INFECTION

Bashir, A., S. Hashmi, M. Saeed, Y. Zafar & K.A. Malik

A geminivirus was purified from the cotton leaf curl (CLCu) diseased plants. The convalently closed circular DNA molecules (replicative form of the virus) were isolated and restriction enzymes producing a single cut were identified. The viral DNA were cloned and sequenced. Sequence identity indicated two different viral species associated with the disease. Primers were designed in the common region of two species and were used to screen the related viral components. A 1.4 Kb fragment was found to be always associated with the infected plants. Another molecule of a similar size was also detected in the viral genomic preparations. The molecules were cloned and sequenced. The homology search indicated that one of the 1.4

Kb DNA molecule had homology to the CLCuV species but had some deleted genes (a subgenomic particle). The second 1.4 Kb DNA molecule had no similarity to the A component genome of geminiviruses but GenBank search indicated that a part of this molecule had identity to the banana bunchy top virus C1 ORF. Infection of healthy cotton and tobacco with partial dimers of all the four components in different combinations indicated that the CLCuV genomic component A related molecules were able to replicate in both the plant species, while the non-A component could not replicate. Leaf curling could only be observed in tobacco but without enations, while cotton never showed any of the CLCuV disease related symptoms.

World Cotton Res. Conf. - 2, Sep. 6-12, 1998, Athens, Greece, (Abst.) p. 270.

INFECTIOUS CLONES OF COTTON LEAF CURL VIRUS

Briddon, R.W., S. Mansoor, M.S. Pinner & P.G. Markham

Cotton leaf curl disease is a major constraint to cotton production across Pakistan. The present epidemic first began in the vicinity of Multan in 1988 and has since spread throughout the Pakistani Punjab and across the border into the Indian states of Punjab and Rajasthan. The causative agent of cotton leaf curl disease has yet to be conclusively identified. A whitefly-transmitted geminivirus, termed cotton leaf curl virus (CLCuV), has been shown to be associated with diseased cotton plants, and the disease itself can be transmitted by the whitefly *Bemisia tabaci*.

We have obtained full-length clones of CLCuV by both standard cloning techniques and by PCR. These clones are infectious to *Nicotiana benthamiana*, but not to cotton. The complete nucleotide sequence of an infectious clone has been compared to other viruses. The

significance of these findings will be presented.

2nd Int. Workshop on Bemisia and Geminiviral Diseases, Jun. 7-12, 1998, San Juan, Puerto Rico, (Abst.) p. 35.

IMPROVEMENT OF COTTON THROUGH THE USE OF POLLEN IRRADIATION TECHNIQUE

Aslam, M. & M.T. Elahi

Different cross combination (9NIAB-7BxREBA-288, CIM-240Xreba-288, NIAB-78Xreba-279 etc) were made with irradiation (5Gy and 10Gy) male pollen. M_o seed was collected and M_1 was grown. M_2 population was grown from M_1 seed in the field. From M_2 population about 20 mutants were finally selected. These mutants had higher yield, early maturity along with better tolerance to CLCuV than the standard cotton variety CIM-240 and parent NIAB-78. These mutants were evaluated in subsequent segregating generation to achieve uniformity. Out of these five mutants significantly out-yielded the parental and commercial cotton varieties. Moreover these have acceptable fiber quality traits. The results of these research studies are described and discussed.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p. 37.

FOUR DNA-A VARIANTS AMONG PAKISTANI ISOLATES OF COTTON LEAF CURL VIRUS AND THEIR AFFINITIES TO DNA-A OF GEMINIVIRUS ISOLATES FROM OKRA

Zhou, X., Y. Liu, D.J. Robinson & B.D. Harrison

Complete DNA-A sequences of nine Pakistani geminivirus isolates from leaf curl-affected cotton (CLCuV-PK) or from okra, and the partial sequences of several additional isolates were determined. Sequences of isolates from cotton were of four types. Isolates from leaf curl-affected okra had virtually the same sequences as those from cotton. Isolates from yellow vein mosaic-affected okra were of two types (OYVMV types 201 and 301), both distinct from but closely related to the virus isolates from cotton. Of these six types, two types of CLCuV-PK type 72b) is the most distinct. Of the encoded proteins, coat protein (CP) is the most strongly

conserved (92-100% amino acid sequence identity), and *AC4* protein the most variable (41-87%). The 5' and 3' halves of the intergenic region of some isolates had different affinities and occurred in seven combinations, suggesting that recombination had occurred and that the origin of replication was a favored recombination site. Similarly, the first 1520 nt of CLCuV-PK types 804a DNA resembled those of OYVMV type 301 DNA but the remaining 1224 nt were very different. The *AC1 (Rep)* gene and 5' part of the intergenic region of CLCuV-PK type 72b closely resembled those of OYVMV type 301, whereas the rest of the sequence did not. The cotton leaf curl epidemic in Pakistan is caused by several distinct variants, with recombination events involving OYVMV and other unspecified geminiviruses having probably been involved in their evolution.

J. Gen. Virology, 79: 915-923, 1998.

EVALUATION OF TWO COTTON LEAF CURL VIRUS TRANSMISSION TECHNIQUES AND THEIR RESPONSE TO DIFFERENT COTTON CULTIVARS

Faqir, M., A.H. Tariq, J. Ihsan & A. Saleem

Eighteen cotton varieties were evaluated against CLCuV transmitted by tow methods i.e. (i) bottle leaf grafting (ii) large cage methods. All the test cultivars showed more or less susceptible reaction and required different periods for symptoms development. While disease intensity in both the techniques remained same. Nine cultivars viz; CIM-70, BH-36, NIAB-86, FH-682, F-149, NIAB-78, Gohar-87, FH-87 and NIAB-26 exhibited susceptible reaction and displayed 5 and 6 disease intensity, while these cultivars took 18-27 and 16-32 days for symptoms (vein thickening) development in bottle leaf grafting and large cage techniques, respectively. CIM-109, CIM- 240, AC-134, MNH-147, MNH-93, RH-1 and SLH-41 displayed comparatively less disease intensity (3-4) and attained 17-24 days for symptoms development in B.L.G and 17-31

days in large cage methods, respectively. S-12 proved the most susceptible cultivar and showed highest disease intensity (7) and attained minimum days (17) for symptoms development in both the methods. Both the methods produced 10% successful transmission of virus, but bottle leaf grafting methods is bit laborious and cannot be practiced for large scale screening of genetic material.

Pak. J. Phytopath., 10 (1): 18-22, 1998.

EVALUATION OF COTTON GENOTYPES AGAINST COTTON LEAF CURL VIRUS IN CONTROLLED CONDITION

Shah, H. & S. Khalid

Sixteen genotypes of upland cotton (*Gossypium hirsutum* L) of Central Cotton Research Station, Bahawalpur, were screened to cotton leaf curl virus (CLCuV) in controlled conditions through whitefly (*Bemisia tabaci*) and graft inoculation. Inoculated plants were placed in glasshouse at 35-40 °C for observation of symptom development. Of all whitefly inoculated genotypes only one plant of V-7 produced severe vein thickening and upward leaf curling 15 days after inoculation, while other remained symptomless. The susceptible check S-12 produced characteristic symptoms of CLCuV 15-20 days after inoculation.

Graft-inoculated plants of V-5, V-6, V-7, V-8, V-9, V-12 and V-14 showed very mild symptoms. Only a single spot of dark green thickened spot on vein near the leaf margin was hardly visible, while in V-11, V-15 and V-16 such spots were 2-5 in numbers. On the other hand V-1, V-2, V-3, V-4, V-10 and V-13 remained asymptomatic two months after graft inoculation. Generally symptoms were very mild in all lines. Latent infection and virus concentration in plant showing symptoms will be checked through TAS-ELISA.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) pp. 53-54.

EFFECT OF SOME BIO-PESTICIDES ON COTTON LEAF CURL VIRUS TRANSMISSION THROUGH *BEMISIA TABACI*

Khan, M.B.A.
S. Khalid &
H. Shah

The effect of bio-pesticides (mixture of surf, sunflower oil and water, neem oil, crude extract of neem seed and leaves, neem flavonoids, rape seed oil, and Nimbokil-neem based commercial product) on cotton leaf curl virus (CLCuV) transmission through whitefly (*Bemisia tabaci*) was investigated in a highly susceptible cotton variety (S-12). The mixture of surf, sunflower oil and water (1:50:1250), 1% neem oil in acetone and crude extract of neem leaves in water (1:10) resulted in 100% reduction in disease transmission. Eighty percent plants remained symptomless after the application of crude extract of neem seed in water (1:10), 1% rape seed oil and 0.5% neem oil in acetone, and 3% neem flavonoids in water. Nimbokil (0.5%) results in 40% reduction in disease transmission. Only 20% reduction in disease transmission was achieved when neem oil (0.1%) in acetone and 3% neem flavonoids in ethanol were applied. Severe disease symptoms developed on untreated controls of S-12. The presence of CLCuV or otherwise was confirmed through triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using polyclonal antisera to ACMV and monoclonal antibody SCRI-60 to ICMV. All plants showing CLCuV symptoms were ELISA positive and symptomless gave negative values.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p. 54.

DEVELOPMENT OF RESISTANCE IN COTTON AGAINST COTTON LEAF CURL VIRUS (CLCuV) USING VIRAL GENES

Asad, S., A. Bashir,

Cotton leaf curl virus (CLCuV), a geminivirus, is a serious plant pathogen which cause severe losses in cotton production in Pakistan. We used antisense DNA of CLCuV DNA-A borne *AC1* gene alongwith the antisense DNA of the adjacent *AC2* and *AC3* gene for vector construction. The plasmid pSQMW1 containing double CaMV35S promoter and poly A in Blue

W.A.A. Haris, C.P. Lichenstein, Y. Zafar & K.A. Malik

Script was constructed. The PCR products of respective genes were introduced into the polylinker of pSQMW1. Finally the expression cassette was cloned into the plant transformation vector pGA482 and transformed into Agrobacterium strain LBA4404. These constructs were introduced into tobacco and cotton. Independent transgenic lines of tobacco and cotton were selected on Kanamycin. The progeny of tobacco (T0 & T1) and cotton (T0), grown in containment facility, were analyzed by PCR, Southern, RT-PCR and Northern analysis. The transformants indicating presence and expression of transgene were challenged to pathogen by grafting and exposure to viruliferous whiteflies.

World Cotton Res. Conf. - 2, Sep. 6-12, 1998, Athens, Greece, (Abst.) p. 272.

DEVELOPMENT OF POLYCLONAL ANTIBODIES AGAINST PRECOAT (*AV2*) AND REPLICATION ASSOCIATED (*AC1*) PROTEINS OF COTTON LEAF CURL VIRUS

Nazish, A.

Serological diagnosis is based on the specificity of antibodies to an infectious agent. The antibodies are generally raised against a pathogen by direct inoculation of laboratory animals. Such techniques are reliable if the pathogen is obtained in sufficient amount for eliciting immune response. Geminiviruses impose a major constraint on the development of antibodies due to their low titre in the plant tissues and difficulty in obtaining sufficient concentration for eliciting immune response in laboratory animals. Current developments in genetic engineering have made it possible to express the viral proteins *in vitro* and to raise specific antibodies against the engineered proteins.

In this study polyclonal antibodies were raised against pre-coat (*AV2*) and replication associated (*AC1*) proteins of the two species of cotton leaf curl virus (CLCuV), a geminivirus. These proteins were selected due to their little homology

(50-60%) to the similar proteins coded by other geminivirus species.

The *AV2* and *AC1* genes were cloned in an expression vector (pET 32a) and induced in *E. coli* host. The expressed *AV2* and *AC1* proteins were purified from the crude cellular extract by affinity chromatography utilizing the histidine tag placed at N-terminal region of these proteins. The purified proteins (antigens) were injected subcutaneously in three subsequent doses of 0.1 mg each on every 10th day to the female albino rabbits. The obtained antisera were cross reacted with respective purified proteins and with the crude cellular extracts of infected cotton and tobacco leave samples.

This study helped to establish a protein expression, purification and antibody raising system which could be utilized to further fine tune the antibody production of similar proteins related to CLCuV or some other pathogens.

M.Phil. Thesis Dept. Biol. Sci., Quaid-i-Azam Univ., Islamabad, 1998.

DEVELOPMENT OF LEAF CURL VIRUS RESISTANT VARIETIES OF COTTON THROUGH THE USE OF INDUCED MUTATIONS AND RELATED TECHNIQUES

Awan, M.A., M.S.I. Khan, M. Aslam & M. Hussain

Development of cotton leaf curl virus resistant germplasm through the use of induced mutations and related techniques was undertaken at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. Seed of commercial cotton varieties namely CIM-240, NIAB-78, CIM-109 and SLS-1 was irradiated with appropriate dose of ^{60}Co gamma rays, and selection for desirable plants was made in the subsequent M_2 generation. F_1 seed resulting from the interaspecific crosses i.e., NIAB-78 × Reba P-288, NIAB-78 × Reba-279 as well as interspecific cross between *G. hirsutum* and *G. barbadense* were also irradiated and desirable plants were isolated in F_2/M_2 generation. DNA of the donor parents

(*G. barbadense* and *G. arboreum*) was injected into the styles/ovaries of the recipient (*G. hirsutum*). A large number of CLCuV resistant mutants/variants were isolated form segregating generations resulting from gamma rays irradiation, intra and interspecific hybridization and DNA macroinjection. These mutants/ variants were evaluated in subsequent generations and several plant progenies showing CLCuV resistant coupled with high yield potential and desirable fibre quality have been selected. A couple of the selected plant progenies hold the promise of direct release as CLCuV resistant varieties.

Pak. J. Phytopath., 10 (1): 1-4, 1998.

DEVELOPMENT OF IMPROVED GERMPLASM OF COTTON THROUGH RADIATION AND DNA-MEDIATED EMBRYO TRANSFORMATION TECHNIQUE

Aslam, M., M.T. Elahi & N. Iqbal

The research studies were carried out to enhance the incorporation of *G. arboreum* and *G. barbadense* genes for disease resistant and quality traits respectively into *G. hirsutum* through DNA-mediated embryo transformation technique. The self fertilized flower/ovaries of the recipient were injected with the donors DNA solutions irradiated at low doses i.e. 2.5 Gy of gamma rays. The results of D_1 and D_2 generation revealed the enhanced incorporation and preservation of the donor parent traits into the recipient. Higher percentage of CLCuV resistant plants and plants with increased quality traits were obtained from the irradiated DNA treatments. The transformed genotypes had higher yield and other economic traits better as compared to recipient. Moreover, the expression of qualitative traits of the donor i.e. petal spot, pollen color and flower colors were observed in D2 generation.

Pak. J. Biol. Sc. 1(4): 291-294, 1998.

DEFECTIVE FORMS OF COTTON LEAF CURL VIRUS DNA-A THAT

Tobacco and tomato plants inoculated at least 9 months previously with a Pakistani isolate of cotton leaf curl virus (CLCuV-PK), a whitefly-

HAVE DIFFERENT COMBINATIONS OF SEQUENCE DELETION, DUPLICATION, INVERSION AND REARRANGEMENT

Liu, Y., D.J. Robinson & B.D. Harrison

transmitted geminivirus, contained substantial amounts of circular dsDNA molecules that were mostly about half the size of CLCuV-PK dsDNA-A. They appeared to be derived from CLCuV-PK DNA-A by various combinations of sequence deletion, duplication, inversion and rearrangement and, in a few instances, insertion of sequences of unknown origin. Each of ten tobacco plants contained a different predominant form of such a defective molecules; however, all the forms contained the intergenic region and part of the *AC1 (Rep)* gene. Some of the forms contained novel open reading frames and might have a role in the evolution of variant geminiviruses. The defective components were not detected at 3 months after the original culture of CLCuV-PK was transmitted by whiteflies *(Bemisia tabaci)* from cotton to tomato but were present after a further 6 months. They were transmitted, along with full-length DNA-A, between tobacco and tomato plants by grafting and by *B. tabaci*.

J. Gen. Virology, 79: 1-8, 1998.

CONSTRUCTION OF RARTIAL DIMERS FOR COTTON LEAF CURL DISEASE RELATED COMPONENTS AND EVALUATION FOR THEIR INFECTIVITY TO COTTON AND TOBACCO PLANTS

Saima, H.

Total genomic DNA was isolated form the cotton leaf curl virus (CLCuV) infected cotton plants for isolating and cloning of disease related DNA components. The covalently closed circular DNA molecules (cccDNA, replicative form of the CLCuV) were isolated through density gradient centrifugation from the total genomic DNA of infected plants. Restriction enzymes producing a single cut were identified and the viral DNA was cloned and sequenced. Sequence identity indicated that two different CLCuV species are associated with the disease and each of the species possesses 2750 bases. Several primers were designed in the common region of the two species and were used to screen the related viral components. A 1.4 Kb fragment was found to be always associated with the CLCuV infected plants. Another molecule of a similar size was

also detected in the cccDNA preparations. Both the molecules were cloned, sequenced and homology search indicated that one of the 1.4Kb molecule had homology to the CLCuV species but had some deleted genes. This molecule was found to be a subgenomic form of viral genomic component A. The second 1.4Kb DNA molecule had no similarity to the A component genome of geminiviruses but the GeneBank search indicated that a part of this molecule had identity to the banana bunchy top virus *C1* ORF. Infection of healthy cotton and tobacco with partial dimers of all the four components in different combinations indicated that the CLCuV genomic component A related molecules were able to replicate in both the plant species, while the non-A component could not replicate. However, leaf curling could only be observed in tobacco without enations, while cotton never indicated any of the CLCu disease related symptoms.

M.Phil. Thesis Dept. Biol. Sci., Quaid-i-Azam Univ., Islamabad, 1998.

GLOBAL ASSESSMENT OF COTTON VIRUS DISEASES

Nelson, M.R., A. Nadeem, W. Ahmed & T. V. Orum

Virus diseases of cotton have only been of sporadic importance to global cotton production. Recent devastating epidemics in Pakistan and other areas have brought new awareness to the potential for disaster of a pathogen once considered to be of minor importance. However, under changing conditions the pathogen emerges as a serious problem.

Beltwide Cotton Conf. Nat. Cotton Council of America, Jan 5-9, 1998, San Diego, CA, USA.

SURVEY OF COTTON GROWING AREAS OF PAKISTAN FOR THE DETECTION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES BY

Viral disease suspected or associated with whitefly-transmitted geminiviruses are causing heavy losses in many crops in Pakistan. Besides crops there are many ornamental plants and weeds which show symptoms typical of whitefly-transmitted geminiviruses. A full length clone of cotton leaf curl virus was used

DOT-BLOT HYBRIDIZATION AND CHAIN REACTION

Mansoor, S., S.H. Khan, Y. Zafar, P.G. Markham & K.A. Malik

as general probe for the screening of plants suspected for the presence of whitefly-transmitted geminiviruses by dot-blot hybridization. The presence of whitefly-transmitted geminiviruses was confirmed by PCR using degenerate primers designed to amplify all these viruses. For the design of degenerate primers complete nucleotide sequences of 17 whitefly-transmitted geminiviruses were aligned. Conserved sequences in replication associated protein (*AC1*) in the virus sense and coat protein sequences in the complementary sense were used to design degenerate primers. These primers amplified about half of the DNA-A which is more variable among whitefly-transmitted geminiviruses. More than 40 plant species suspected for the presence of geminiviruses and some asymptomatic weeds were tested for the presence of virus. Whitefly-transmitted geminiviruses were detected in 36 plant species, belonging to different families. Some plant species were found to be host for more than one geminivirus as defined by symptom phenotype. The presence of geminiviruses on most of these crops, weeds and ornamental plants has not been recorded previously from Pakistan.

1st Nat. Conf. on Biotech. for Sustainable Dev. Nov. 24-25, 1997, BSP-Govt. College, Lahore, (Abst.) p. 69.

RESPONSE OF COTTON GERMPLASM TO COTTON LEAF CURL VIRUS

Shah, H. & S. Khalid

Twenty upland cotton (*Gossypium hirsutum* L.) cultivars/lines were screened for their response to cotton leaf curl virus (CLCuV) in controlled conditions. Seedlings were raised in sterilized soil in insect-proof cages at 30°C under artificial lights. Whiteflies culture used in this study was maintained and reared on cotton. Prior to inoculation, whiteflies were allowed for acquisition feeding period of 72 hours on infected CLCuV cotton plant in a cage. Twelve plants of each cultivar/line were exposed to a large number

of adult viruliferous whiteflies in a cage. After a period of 72 hours they were killed mechanically, and treated with Aldicarb and transferred to glasshouse. Highly susceptible variety (S-12) was included as positive control. The appearance of disease symptoms were recorded, till 60 days after inoculation. Presence and concentration of the virus was determined after 30, 40 and 50 days after inoculation with the help of triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using polyclonal antiserum to african cassava mosaic virus (ACMV) and monoclonal antibody SCRI-60 to indian cassava mosaic virus (ICMV). Most of the cultivars/lines showed typical CLCuV symptoms i.e vein thickening, different size and types of enation on the underside of the leaves, and upward or downward curling of leaves, some produced mild symptoms, while other (CIM-436, 443, 446 and 448) remained symptomless. Virus concentration ranged from 0.368 to above 2. It indicated that some cotton genotypes were easily infected and produced more virus, others were harder to infect/slow to develop systemic infection and produced moderate amount of virus and the rest were highly resistant/immune to infection. The cultivars/lines in which no viral symptoms were recorded nor the presence of virus was detected through TAS-ELISA are being graft inoculated for their response to CLCuV.

Int. Conf. on Integrated Pl. Dis. Manag. for Sustainable Agric., Nov. 10-15, 1997, New Delhi, India, (Abst.) PIC-010, p. 224.

OCCURRENCE OF COTTON LEAF CURL VIRUS (CLCuV) IN SINDH

Khalid, S., M.H. Soomro & I. Ahmad

The leaf curl disease of cotton (*Gossypium hirsutum* L.) was first observed in Pakistan during 1967 from Multan area. The disease remained in low intensities or was ignored until 1987 when it erupted as an epidemic. The cause of the disease was established in 1992 as a whitefly-transmitted geminivirus with the properties of cotton leaf curl virus (CLCuV). On the basis of departmental

reports, the disease was found to be restricted only in the Punjab area and the cotton crop in Sindh was considered disease free. Keeping in view the magnitude of the disease in Punjab and whitefly (*Bemisia tabaci*) as its vector, a program of monitoring virus diseases with emphasis on geminiviruses including CLCuV in Sindh was initiated in late 1996. Hyderabad, Sanghar and Nawabshah districts were surveyed during September, 1996 and the districts of Ghotki, Sukkur, Khairpur and Naushehro Feroze during January, 1997. CLCuV-like symptoms showing curling, cupping upward/downward and shortening of leaves, thickening of veins and development of enations on underside of leaves were observed in cotton fields around Ubaro in district Ghotki, the area not far from Punjab border, where the disease incidence in two fields was about 10%. According to local farmers, such symptoms in cotton were also observed during the 1995 season also. Disease symptoms were also observed on other wild and ornamental plants around Mirpur Mathelo and Ghotki.

The presence of CLCuV was confirmed through triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using panel of monoclonal antibodies against whitefly-transmitted geminiviruses (WTGs), polymerase chain reaction (PCR) using CLCuV-specific primers and by reproducing CLCuV symptoms in cotton (cv. S-12) through whitefly inoculation under controlled conditions. This confirms that the disease is present in Sindh as well. At present only a limited area near the Punjab border has been found infected. But there is every likelihood that the disease may further spread southwards. It is interesting to note that in 1990-91, the disease was found only in a small area in the Punjab, but it spread to almost all cotton growing areas of the province in a span of 2-3 years.

DETECTION OF TWO COTTON GEMINIVIRUSES IN ALTERNATE HOSTS OF COTTON LEAF CURL VIRUS

Mansoor, S., S.H. Khan, M. Hussain, A. Bashir, P.G. Markham & K.A. Malik

Leaf curl disease of cotton is the major cause of decline in cotton production in Pakistan. The disease is associated with two whitefly-transmitted geminivirus species which can cause the same disease independently but are often found co-infecting the same plant in the field. Survey of cotton growing areas were conducted and plants which were previously known for the presence of geminiviruses were tested for the presence of two cotton geminiviruses by a rapid multiplex PCR. Ten plant species were found to be infected with either one or both of cotton geminiviruses and exhibit symptoms similar to cotton leaf curl disease. The host plants were mostly members of the family *Malvaceae*. The disease was transmitted from one of these host plants *Hibiscus rosa-sinensis* to cotton by grafting and developed symptoms typical of the disease. The disease was also transmitted to tobacco by whiteflies from *Hibiscus rosa-sinensis* and developed identical symptoms. Several partial and full length clones of virus were obtained from this host plants. These clones were analyzed by RFLP and sequence determined through variable region show that *Hibiscus* is infected with cotton leaf curl virus. The alternate hosts identified in this study may play a significant role in disease epidemic and thus could be an important target for the control of disease.

1st Nat. Conf. on Biotech. for Sustainable Dev. Nov. 24-25, 1997, BSP-Govt. College, Lahore, (Abst.) p.68

DETECTION AND RELATIONSHIPS OF COTTON LEAF CURL VIRUS AND ALLIED WHITEFLY-

A stock culture of cotton leaf curl virus from Pakistan (CLCuV-PK), was transmitted by whiteflies (*Bemisia tabaci*) to seven plant species, including french bean, okra, tobacco and tomato, and caused vein thickening and leaf curl

TRANSMITTED GEMINIVIRUSES OCCURRING IN PAKISTAN

Harrison, B.D., Y. Liu, S. Khalid, S. Hameed, G.W. Otim-Nape & D.J. Robinson

symptoms. It was readily detected in triple antibody sandwich ELISA (TAS-ELISA) by 11 out of 31 monoclonal antibodies raised against the particles of three other geminiviruses: african cassava mosaic, indian cassava mosaic and okra leaf curl viruses. Reaction strength was enhanced when the tissue extraction fluid contained sodium sulphite. Minor variations in epitope profile were found among virus isolates from cotton (*Gossypium hirsutum*) collected from different districts in Pakistan over a 5-year period. These epitope profiles were distinguishable from that of cotton leaf curl virus from *G. barbadense* in southern India but indistinguishable from the profiles of viruses causing yellow vein disease of okra in India or Pakistan, or leaf curl of okra (*Abelmoschus esculentus*), *Hibiscus tiliaceus*, radish or sunflower in Pakistan, suggesting that these plants are putative natural hosts of CLCuV-PK. The viruses in cotton, and in okra with leaf curl or yellow vein symptoms, were also detected by PCR with three pairs of CLCuV-PK-specific primers.

Five additional whitefly-transmitted geminiviruses were found among isolates from 11 other naturally-infected species in Pakistan, and were distinguished by their epitope profiles. These viruses were associated, respectively, with tobacco leaf curl, squash yellow blotch, tomato yellow leaf curl, watermelon leaf crinkle and soybean yellow mosaic diseases. The first four of these viruses were detected readily by PCR with geminivirus general primers but only weakly, if at all, with two pairs of CLCuV-PK-specific primers. Pakistani crops are infected with a range of distinguishable but relatively closely related whitefly-transmitted geminiviruses, some of which resemble those found in India.

Ann. Appl. Biol., 130: 61-75, 1997.

COTTON LEAF CURL VIRUS EPIDEMIC IN PAKISTAN: VIRUS CHARACTERIZATION, DIAGNOSIS AND DEVELOPMENT OF VIRUS RESISTANT COTTON THROUGH GENETIC ENGINEERING

Zafar, Y., A. Bashir, S. Mansoor, M. Saeed, S. Asad, N.A. Saeed, R. Briddon, P.G. Markham, C.M. Fauquet & K.A. Malik

The cotton leaf curl virus (CLCuV), a whitefly-transmitted geminivirus has caused heavy losses to the cotton crop and still remains the most important constraint for the development of the cotton sector in the country.

We at the NIBGE initiated the research program with the following objectives:

- Biological and molecular characterization of CLCuV which includes virus purification, cloning and sequencing of the genomic components and generation of infectious clones.
- Development of PCR/DNA probe-based diagnostic test for the detection of virus in insects and plants and use of this diagnostic test for the identification of alternate hosts of CLCuV.
- Development of virus-resistant cotton through genetic engineering.
- Molecular diversity and distribution of virus in cotton growing areas of Pakistan.
- The cotton group of NIBGE extensively studied leaf curl disease and made vital contributions to solve this problem.

56th Plenary Meeting of the Int. Cotton Advisory Committee, Oct., 1997, Asuncion, Paraguay, pp. 33-39.

COTTON LEAF CRUMPLE VIRUS AND COTTON LEAF CURL VIRUS ARE TWO DISTANTLY RELATED GEMINIVIRUSES

Nadeem, A., Z. Weng & M.R. Nelson

Cotton leaf crumple bigeminivirus (CLCrV) and cotton leaf curl bigeminivirus (CLCuV) are whitefly-transmitted. Using a PCR-based technique, the complete DNA A component of each virus was amplified as 2 DNA fragments from total nucleic acids extracted from infected cotton leaves, and was subsequently cloned. Two pairs of PCR primers were designed according to sequences. Electrophoretic analysis of PCR products suggested that the DNA A of CLCuV was approx. 2.6 and 2.7 Kb. respectively.

Southern hybridization analyses of the cloned PCR fragments showed that CLCrV DNA and CLCuV DNA did not hybridize with each other under high stringency conditions, whereas they hybridized weakly with each other under low stringency conditions. Riboprobes prepared from cloned DNA fragments hybridized with their respective single-stranded virion DNA and the double-stranded replicative form extracted a subgenomic DNA, was detected in CLCuV-infected cotton, but not in CLCrV-infected cotton leaves. A smaller-than-genomic virtual DNA, presumed to be a subgenomic DNA, was detected in CLCuV-infected cotton, but not in CLCrV-infected cotton by the hybridization. The hybridization data were consistent with partial sequence analysis of the ACI gene and the capsid protein gene. Although both viruses shared a high degree of nucleic acid identity, CLCrV was more closely related to the New World geminiviruses such as abutilon mosaic bigeminivirus, sida golden mosaic virus, bean dwarf mosaic virus and tomato mottle bigeminivirus; whereas CLCuV was more closely related leaf curl virus, cassava african mosaic bigeminivirus and tomato yellow leaf curl bigeminivirus. It is suggested that CLCrV and CLCuV are 2 distinct and distantly related bigeminiviruses.

Mol.-Pl.-Path-On-line. 1997, 0612nadeem; http.www.bspp.org. uk-mppol.

BREEDING OF COTTON VARIETIES FOR RESISTANCE TO COTTON LEAF CURL VIRUS

Ali, M.

The paper describes the procedure followed in Central Cotton Research Institute, Multan, for breeding cotton varieties resistant to the disease of leaf curl virus. It was possible to grade the existing varieties for degree of tolerance to the virus after screening on a natural hot spot. Apparently, healthy plants existed in these varieties, but their selection did not result in producing virus resistant types nor did it help in

improving the tolerance/resistance of these varieties. This was against the experience in Sudan where the resistance was built-up by such selection of plants and systematic hybridization between susceptible and resistant cotton was not considered necessary. It has been possible to evolve varieties for complete virus resistance by hybridization between the existing cultivars and the resistant strains picked up for this character from the germplasm collection of the institute. It has been suggested that the resistance is controlled by a single dominant gene and can be transferred to any cultivar by back-cross technique.

Existing source of resistance has been indicated which may be exploited by the breeders and biotechnologists for incorporating varied sources of resistance and for creating multigenic resistance for this disease.

Pak. J. Phytopath., 9(1): 1-7, 1997.

BIOLOGICAL AND MOLECULAR PROPERTIES OF COTTON LEAF CURL VIRUS, A NEW MEMBER OF SUBGROUP III OF GEMINIVIRIDEAE FROM PAKISTAN

Mansoor, S., I.D. Bedford, M. Pinner, R. Briddon, A. Bashir, Y. Zafar, P.G. Markham & K.A. Malik

The record cotton production achieved in 1992 could not be sustained in Pakistan, mainly due to the attack of cotton leaf curl virus. In the present study biological and molecular properties of this virus have been investigated. The transmission efficiency of this virus was affected by temperature, light intensity and insect biotype. Gemini particles were observed by electronmicroscopy and coat protein detected by Western blotting. The virus was transmissible by whiteflies or grafting to several laboratory hosts such as tobacco, tomato, *Datura* and *N. benthamiana* while natural hosts of this virus were found to be mostly member of *Malvaceae* family.

Molecular cloning of CLCuV was carried out

from replicative form of viral DNA isolated from infected cotton plants and sequence of some clones was determined (EMBL acc No. X 98995). The genome A has typical arrangement of whitefly-transmitted geminiviruses with two ORFs in sense and five ORFs in complementary sense orientation. The comparison of complete sequence and ORF shows that CLCuV is related with whitefly-transmitted geminivirus from Old World especially those found in Indian subcontinent. The use of cloned DNA as probe detected CLCuV in cotton plants and whiteflies collected from different cotton growing districts and severity of symptoms correlated with the level of viral DNA in infected plants. Sequencing of several clones identified two variable strains called CLCuV Pak-1 and CLCuV Pak-2. Primers specific for Pak-1 and Pak-2 were used in PCR to find out the distribution of these viruses in cotton growing districts. In most of the samples both viruses could be amplified from the same plant. We conclude that CLCuV is new whitefly-transmitted geminivirus with two strains, which are related with other geminiviruses found in the region.

5th Int. Cong. of Pl. Mol. Biol. Sep., 21-27, 1997, Singapore,(Abst. 1050).

VARIATION, RELATIONSHIPS AND IDENTIFICATION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES

Harrison, B.D.

Different whitefly-transmitted geminiviruses (WTGs) share nucleotide sequences in parts of their DNA-A molecules. Probes for DNA-A, primers based on conserved sequences in DNA-A, and antisera to the DNA-A-encoded coat protein therefore can be used to detect many heterologous WTGs in Southern blots, PCR and ELISA, respectively. Conversely, WTGs can be distinguished by tests based on their DNA-B sequences, by the nucleotide sequences of their large intergenic region, by their pattern of reactions in TAS-ELISA with panels of

heterologous monoclonal antibodies and by their reactivity with selected homologous monoclonal antibodies.

Relationships among WTGs are closest for viruses from the same geographical region, irrespective of host range. WTGs causing similar diseases (e.g. in cassava or tomato) in different regions tend to be more distantly related. This can be explained by: 1. Evolution of WTG coat protein along three main pathways represented by viruses from the America, Africa/Mediterranean area, and Asia/Australia, respectively; 2. Occurrence of different biotypes of the vector whitefly *Bemisia tabaci (sensu latuo)* in different areas; 3. The key role of WTG coat protein in transmission and the apparent adaptation of individual WTGs (and presumably their coat proteins) for enhanced transmission by a sympatric *B. tabaci* biotype; and 4. A hypothesized ability of WTGs to adopt to new plant hosts.

Recent research on individual WTGs for example Pakistani cotton leaf curl virus, confirms that although the viral coat protein differs little among isolates, the large intergenic region can vary appreciably. A range of molecules, derived from genomic DNA by deletions and rearrangements perhaps also contribute to WTG evolution. Current major epidemics of WTGs in Pakistan, Uganda and USA/Central America provide excellent opportunities to study WTG evolution in progress.

Proc. Bellagio Conf. on Whiteflies and Viruses: Menace to World Agri., Aug. 12-16, 1996, Bellagio, Italy, p.24.

STUDY ON VARIATION IN WHITEFLY BASED ON

The importance of the sweet potato whitefly, *Bemisia tabaci* as a serious pest in vegetables and

BIOLOGICAL CHARACTERISTICS AND ISOZYME ANALYSIS

Hameed, S., S.M.S. Naqvi & S. Khalid

fiber crops has increased worldwide in the past decade. It transmits at least 19 virus diseases. Presently, in Pakistan, cotton production is being threatened by cotton leaf curl virus vectored by whitefly, resulting in record losses. Measures adopted presently for pest management have shown limited success. On the other hand whitefly is under tremendous pressure due to frequent exposure to a number of pesticides, which is expected to result in genetic variation. This necessitated study of whitefly population for its characterization. In present study, whitefly associated-symptoms were developed on squash which showed characteristic pattern "silver squash leaf" indicating the presence of biotype-B. Biological symptoms are further supported by esterase banding of single whitefly using polyacrylamide gel electrophoresis which revealed difference in two major bands, in addition to few minor bands. Based on these findings we report here the occurrence of biotype-B whitefly for the first time in Pakistan. Any future strategy for control of whitefly should take into account this study.

Crop Prot. Conf., Apr. 20-22, 1996. NWFP Agric. Univ. Peshawar, (Abst.) pp.38-39.

OCCURRENCE OF B-BIOTYPE OF *BEMISIA TABACI* IN PAKISTAN

Hameed, S., S. Khalid & S.M.S. Naqvi

The presence of B-type of *Bemisia tabaci* in Pakistan was established through biological (squash silverleaf-SSL symptoms) and bio-chemical (esterase banding patterns) studies. Distinct and definitive SSL symptoms were produced in squash (*Cucurbita pepo)* on which whiteflies obtained from cotton growing area of Multan, Pakistan were allowed to feed. Temperature had an effect on duration of symptoms development. Parent population yielded two types of esterase banding patterns, one with two densely stained bands and the other

with only one major band. Whiteflies from SSL exhibiting plants produced only single band pattern. This is the first substantiated evidence for the presence of B-type of *B. tabaci* in Pakistan.

Brighton Crop Prot. Conf. – Pests & Diseases, 1996. 2B-4, pp. 81-85.

MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF COTTON LEAF CURL VIRUS AND DEVELOPMENT OF VIRUS-RESISTANT COTTON THROUGH GENETIC ENGINEERING

Zafar, Y., A. Bashir, S. Mansoor, M. Saeed, S. Asad, N.A. Saeed, S. Shabnam, M.J. Iqbal & K.A. Malik

Cotton leaf curl virus, a whitefly-transmitted bipartite geminivirus has caused heavy losses to cotton crop and still remained the most important constraint for the development of cotton sector in the country. The symptoms produced on cotton plant are leaf curling, thickening of veins, enations and stunt plant growth. Cotton leaf curl disease was recorded as early as 1967 from Pakistan. Since 1991-92 cotton leaf curl disease that was only curiosity previously, is now major threat to the stability of this crop. The epidemic of CLCuV in Pakistan is one of the best examples of this dramatic shift in importance what was an unimportant endemic disease in the past. The yield losses due to cotton leaf curl virus disease during the past five years and consequently severe setback to monocrop based national economy are well known.

The recent advances in molecular biology and genetic engineering have opened new avenues in understanding and control of disease epidemics. Genetic engineering of crop species such as cotton allows introduction of a specific character such as disease resistance to be incorporated in existing varieties without compromising other agronomic characters. This technology is superior to conventional plant breeding, as breeding for disease resistance using resistant germplasm may result in some undesirable characters contributed by resistant germplasm. Work on development of a cotton

variety resistant to CLCuV, using molecular techniques, have been initiated at NIBGE, Faisalabad.

Proc. 1st Biotech. Symp., Jun., 1996, CABB, Univ. Agric., Faisalabad, pp. 40-51.

ISOLATION, PURIFICATION AND MOLECULAR CHARACTERIZATION OF COTTON LEAF CURL VIRUS

Bashir, A., S. Mansoor, M. Saeed, S. Shabnam, R. Tanvir, Y. Zafar & K.A. Malik

Cotton leaf curl virus (CLCuV) has proved to be a devastating disease of cotton in Pakistan since 1992. It brought down the cotton production from 12 million bales to 8 million bales and greatly disturbed the economy of this major cotton exporting country.

In order to determine the nature of the virus and devise appropriate measures, the viral DNA was identified through PCR amplification using universal primers against geminiviruses. The total genomic DNA from the infected plants was Southern hybridized to the two available probes of geminivirus DNA, african cassava mosaic virus (ACMV) and indian cassava mosaic virus (ICMV) which further confirmed that CLCuV is a geminivirus. CLCuV was purified from the field infected cotton plants through sucrose gradient centrifugation and observed under the electronmicroscope. The viral particles were identified based on their typical bipartite morphology that is characteristic for geminiviruses. The DNA was isolated from the purified virus particles and was characterized to be ssDNA based on RNAse, DNAse and nuclease S1 digestion. The viral DNA was amplified using geminivirus universal primers against the genome-A component of the geminivirus group. A 2.8 Kb amplification product corresponding to the reported size of genome-A component was obtained. The amplified product was cloned into the pGEM-T vector. When used as probe, this clone was able to differentiate the CLCuV infected plants from healthy plants. This clone was named pSI/pYASI

and was used to identify other viral host in combination with the PCR diagnosis based on universal primers. The viral DNA insert was sequenced and compared with the 24 available geminiviral DNA sequences through DNA BLAST gene data bank search facility. The CLCuV DNA showed 50-70% homology to the other available geminivirus group not reported earlier. The DNA sequence of CLCuV is a new strain of geminivirus group not reported earlier. The DNA sequence of CLCuV was fed into the computer and the putative coding sequences were identified using DNASIS program. The efforts are underway to design vectors for plant transformation based on the antisence constructs of the viral replicase protein or the defective interfering particles.

1st Int. Conf. & Symp., Mar. 6-7, 1996, Univ. Agric., Faisalabad, (Abst.) pp 37-38.

ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF COTTON LEAF CURL VIRUS IN PAKISTAN

Bashir, A., S. Shabnam, S. Aftab, M. Saeed, N.A. Saeed, S. Mansoor, Y. Zafar & K.A. Malik

In 1992 severe outbreaks of cotton leaf curl virus (CLCuV) disease in Pakistan resulted in reducing the cotton production by 30%. The country which was among the top 3 countries of cotton exporter turned into a net importer. Thus for the first time in over fifty years, cotton growers are faced with widespread virus epidemics for which no promising defenses are available.

A method for the preparative scale isolation of the cotton leaf curl virus from infected cotton plants has been established. This is the first report of its kind in the world. A universal primers set for geminivirus A genome was used to clone genome-A component of the CLCuV. Caseium chloride density gradient of the total genomic DNA preparations was used to obtain replicative form (RF) of CLCuV. The restriction digest of the viral DNA was used to clone A and the non-A genome clones. One of the clones (pSAB5) showing differences in hybridization pattern was

partly sequenced and 87% identity was observed in the BLI region of ToLCV-Indian. The full length inserts of CLCuV genome A and B range between 2.7-2.8 Kb. Our data clearly indicated that more than one strain/subtypes of CLCuV are prevalent in the field.

Molecular characterization of CLCuV made it possible to construct sense and antisense plant expression vectors for generating virus resistant tobacco plants. None of the 19 local cultivars evaluated in tissue culture found to be responsive except Coker 312 and Siokra 1-3 model cotton varieties. A complete protocol was developed to culture meristem tip of 10 local cultivars of cotton (*Gossypium hirsutum*). These meristem tips were transformed with GUS marker gene by using biolistic gun and this technology will be exploited for the development of viral resistance in local elite cultivars of cotton.

Proc. Bellagio Conf. on Whiteflies and Viruses, Menace to World Agri., Aug. 12-16, 1996, Bellagio, Italy, p. 27.

GENETIC VARIATION AMONG WHITEFLY POPULATION

Hameed, S., S.M.S. Naqvi & S. Khalid

More than 500 plants species are listed as host of whitefly which transmit at least 19 viral diseases. In Pakistan whitefly (*Bemisia tabaci*) is becoming increasingly important since the outbreak of cotton leaf curl virus in cotton crop. Based on virus transmission efficiency and biological characteristic of whitefly colonies reared at Plant Virology Lab., NARC on cotton plants, it was suspected that perhaps more than one population of whitefly are present. This raised our interest in genetic variation existing among whitefly population.

Above 30 random samples were taken from whitefly colony maintained on cotton. Genetic variation among these samples was observed using isozyme electrophoresis patterns of adult

whiteflies. Differences in esterase banding patterns have been observed indicating that more than one populations exist.

2nd Int. Cong. Entomol. Sci., Mar. 19-21, 1996, NARC, Islamabad, BIOL-2(Abst.) p. 7.

GENETIC VARIABILITY AND CORRELATION STUDIES IN SOME PROMISING COTTON STRAINS UNDER COTTON LEAF CURL VIRUS EPIDEMIC

Altaf, M., H.A. Sadaquat, M.A. Khan & M. Zubair

High genetic variability was found for fibre sugar contents, seed cotton yield and number of monopodial branches while number of bolls per plant, number of nectaries per leaf and plant height were observed moderately variable. Genotypic, phenotypic and environmental correlations were positive and significant between seed cotton yield and number of bolls per plant, plant height and boll weight under the natural prevalence of cotton leaf curl virus epidemic. It is concluded that selection of taller plants bearing large number of heavier bolls under leaf curl conditions would be rewarding in improving the tolerance to disease.

The Pak. Cotton, 40 (3-4): 80-90, 1996.

STUDY ON COTTON LEAF CURL VIRUS IN THE PUNJAB

Tanveer, M., T. Mehmood & Z. Ahmed

Cotton is the back bone of the economy of Pakistan. Since 1988, cotton leaf curl virus transmitted by whitefly decimated cotton crop of the country resulting in heavy production losses. Studies on the prevalence, assessment of losses and varietal resistance of cotton varieties were made. It was noted that the production loss increased progressively since 1988-89 to 1992-93. Thereafter, with a change of varieties although disease spread to more wider areas yet the production loss was comparatively less.

Commercial varieties recommended during 1993-94 showed more tolerance to the disease. Cultivars and lines of cotton differ in their response to infection by cotton leaf curl virus. The most widely grown cultivars like S-12 and

CIM-70 proved highly susceptible which were discouraged for planting during 1993-94. Cultivars LRA- 5116 and advance lines CIM-1100 and CIM-1098 developed at Central Cotton Research Institute, Multan, were highly resistant or immune to the disease. Many lines from breeding material showed highly variable response. Six lines showing resistant or immune reaction like that of LRA-5166 and CIM-100.

5th Nat. Conf. Pl. Scientists, Mar. 28-30,1995, NARC, Islamabad, (Abst.) p. 119.

MOLECULAR PROPERTIES AND PHYLOGENETIC ANALYSIS OF COTTON LEAF CURL VIRUS, A NEW WHITEFLY-TRANSMITTED GEMINIVIRUS FROM PAKISTAN

Mansoor, S., P. Markham, J. Stanley, Y. Zafar & K.A. Malik

For the last three years cotton leaf curl disease has devastated cotton crop in Pakistan and has resulted in about 40% decrease in cotton yield. We have shown that cotton leaf curl virus (CLCuV) is a whitefly transmitted geminivirus. In the present communication molecular and some biological properties of CLCuV will be discussed.

Overlapping primers were designed on the basis of available sequence of CLCuV. The complete genome of CLCuV amplified by these primers was cloned in appropriate vector. A combination of primer extension and subcloning was used for the determination of complete sequence of CLCuV. The data was assembled and compared with known dicot-infecting geminiviruses. The sequence was more similar to whitefly-transmitted geminiviruses as compared to leafhopper-transmitted geminiviruses. Among whitefly transmitted geminivirus CLCuV was more related to old world geminiviruses, especially those found in Indian subcontinent. Phylogenetic analysis of CLCuV for each open reading frame will also be presented.

A partial dimer of full length clone of CLCuV

was cloned in T-DNA borders of an *Agrobacterium* vector. The resulting dimer was used for virus replication studies both in suspension cell culture and leaf discs. The same clone was also used for agro-inoculation studies on certain indicator hosts and cotton plants.

The evaluation of diversity of CLCuV in field conditions is essential for effective management and development of virus-resistant varieties, either through conventional methods or genetic engineering. A strategy for the determination of field diversity has been developed which include southern hybridization under stringent conditions, RFLP analysis of replicative form of virus or PCR amplified DNA and limited sequence analysis. For this purpose a number of virus isolates from different parts of cotton growing areas of Pakistan affected by CLCuV has been collected and CLCuV has been detected by southern hybridization. Correlation of biological and molecular properties of CLCuV with the management of cotton virus under field condition and development of virus-resistant varieties through genetic engineering will be discussed.

4th Int. Symp. - Workshop on Applications of Mol. Biol. Res. in Agric., Health and Environ., Apr. 9-11, 1995, CEMB, Lahore, (Abst) pp. 19-33.

MOLECULAR CHARACTERIZATION OF TWO COTTON GEMINIVIRUSES

Nadeem, A.

Two whitefly transmitted cotton geminiviruses that differ in symptoms, but have similar ecological properties, were characterized and compared at the molecular level. The viruses, cotton leaf crumple (CLCrV) and cotton leaf curl (CLCuV) are found opposite sides of the world, CLCrV in the Western Hemisphere, including southwestern US, Mexico and Central America and CLCuV in Pakistan, other Asian countries and Africa. During recent years leaf curl has been

a catastrophic disease being responsible for converting Pakistan to net importer rather than exporter of cotton.

DNA-A component of each virus was cloned with a polymerase chain reaction-based technique. The complete sequence of CLCrV and CLCuV DNA-A were determined to be 2630 and 2725 nucleotides, respectively. Sequence analysis show that CLCrV and CLCuV were most related to geminiviruses found in that part of the world from which they originated. Thus CLCrV was most closely related to 'New World' viruses such as bean dwarf mosaic and squash leaf curl viruses, while CLCuV was most closely related to 'Old World' viruses such as indian cassava mosaic and tomato yellow leaf curl. CLCrV and CLCuV are least related to each other among the whitefly-transmitted geminiviruses. Genome organizations predicted from the nucleic acid sequences and phylogenetic analysis of whitefly-transmitted geminiviruses also reflect these relationship.

Ph.D. Thesis, Graduate College, Univ. Arizona, USA, 1995.

EFFECT OF COTTON LEAF CURL VIRUS ON YIELD AND YIELD COMPONENTS OF COTTON

Khan, S., M. Aslam & M. Bashir

During 1992-93 cotton production was drastically decreased by 3.7 million bales against the target of 8 million bales, and this loss was substantially increased upto 4 million bales during 1993-94, mainly due to appearance of cotton leaf curl virus (CLCuV) in epidemic form. The disease has been spread in all cotton growing areas of Punjab. On the basis of observations during the last two years it has been noticed that all the cotton cultivars are prone to virus. To determine the response of various cotton cultivars to CLCuV with respect to yield, an experiment was conducted under field conditions during 1993-94 at Adaptive Research Farm, Karor, district Bhakkar.

Fourteen cotton cultivars; NIAB-92, NIAB-26, NIAB-78, RH-1, FH-682, CIM-243, CIM-109, FH-87, MNH-93, GR-156, MNH-147, VIM-240, SLH-41 and Gohar-87 were planted in a randomized complete block design with four replications. The disease appeared in severe form particularly on S-12, which was planted around the experimental plot. Losses inflicted by CLCuV were assessed by comparing yield and yield components (plant height, number of bolls per plant and yield per plant), 10 plants from each treatment in each replication were compared at random. The disease significantly decreased the plant height, number of bolls per plant and cotton seed yield when compared with healthy plants (visually free from virus symptoms). A great variability was observed among cotton cultivars in exhibiting disease effect on yield and yield components. The disease reduced plant height 7.9% (MNH-93) to 36.6% (Gohar-87), bolls per plant 42.2% (NIAB-92) to 80.9% (Gohar-87) and cotton seed yield 58.8% (MNH-93) to 96.8% (RH-1). On the average all the 14 cultivars exhibited yield depression more than 50% with highest 96.8% by RH-1.

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, (Abst.) p. 72.

VARIETAL RESPONSE TO LEAF CURL VIRUS ON EARLY SOWN CULTIVARS OF COTTON *(GOSSYPIUM HIRSUTUM* L.)

Tahir, M., M. Naveed & T. Mahmood

Incidence of cotton leaf curl in eighteen early sown cultivars/strains of upland cotton was investigated. The cultivar CIM-434 was found to be immune and GH-3 highly susceptible to leaf curl virus. The differences in whitefly population on tested cultivars were non-significant indicating that whitefly number has no relation with the CLCuV incidence.

Pak. J. Phytopath., 6 (2): 107-109, 1994.

THE DETECTION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES INFECTING COTTON AND OTHER PLANTS IN PAKISTAN BY PCR AMPLIFICATION

Mansoor S., J.A. Qureshi, J. Stanley, P.G. Markham & K.A. Malik

Geminiviruses have emerged as an important threat to fibre, vegetable and legume crops in Pakistan. Cotton has been the worst affected crop. In the last few years cotton leaf curl disease which is associated with a whitefly transmitted geminivirus has acquired epidemic proportions. It is estimated that in 1993-94 about one million acres of land was affected by cotton leaf curl disease and has resulted in the loss of about five million bales of cotton.

In the present study we have used polymerase chain reaction (PCR) for rapid detection of geminiviruses in plants showing symptoms of geminivirus infection. Two sets of primers were used for amplification of geminivirus DNA. One set of primers designed previously for amplification of DNA A of all whitefly transmitted geminiviruses was used. This set of primers is based on conserved sequences in geminiviruses. Another set of primers based on cotton leaf curl virus (CLCuV) sequence was also used. Five plants belonging to family *Malvaceae* (*Gossypium hirsutum, Hibiscus rosasinensis, H. esculentis, H. tiliaceus* and *H. cannabinus*) showing leaf curl symptoms were found to be infected with geminivirus under field conditions. Geminivirus was also detected in tomato, chillies, tobacco, zinnia and nightshade plants, all showing leaf curl symptoms. Ageratum plant showing yellow veins and mosaic symptoms was also found to be infected with geminivirus. Same strategy is being used for the detection of geminivirus infection in some other plants.

On the basis of symptoms (leaf curl with thick, dark veins and enations) and PCR amplification with CLCuV sequence-based primers, viruses infecting plants of *malvaceae* have been grouped together and seem to be closely related. Our data

suggest that many plants showing leaf curl symptoms are infected with geminiviruses and PCR- based diagnostic test can be used for their rapid identification and classification.

I[st] Int. Symp. geminivirus, Sep. 14-17, 1994. Elejido Almeria, Spain, (Abst. 8.3) p. 74.

STUDIES ON THE IDENTIFICATION, TRANSMISSION AND HOST RANGE OF COTTON LEAF CURL DISEASE IN PUNJAB WITH SPECIAL REFERENCE TO ITS CONTROL

Mirza, J.H., W. Ahmed, M.A. Ayyub, O. Khan & S. Ahmed

Disease screening studies of cotton cultivars against natural infection by CLCuV, carried out in the fields at Mamunkanjan, district Faisalabad, for findings sources of resistance against the disease, revealed that of the ten cultivars screened, none was immune or resistant or even moderately resistant. Unlike the common belief, cultivars S-12 was found to be susceptible whereas the rest of the cultivars were highly susceptible under the natural climatic conditions prevailing during the growing year 1993 under local natural conditions.

Studies on growth response of ten genetically different cotton cultivars to CLCuV infection revealed that both the vegetative and productive components were adversely affected due to infection by the virus. However, the effected cultivars varied in their response to virus infection and suffered to variable extent probably due to their different genotype.

The response of CLCuV infection of productive components also varied depending upon the cultivars. There was 2.26 to 47.83 (mild-medium reaction), 46.43 to 77.07 (mild-severe reaction) and 15.32 to 56.44 (medium-severe reaction) percent decrease in number of bolls set per plant. For the size of bolls, there was 10.91 to 16.94 (mild-medium reaction), 12.50 to 22.69 (mild-severe reaction) and 2.80 to 16.47 (medium-severe reaction) percent reduction. The percent reduction in the cotton yield was 21.03 to 49.57 (mild-medium •reaction), 45.83 to 75.98 (mild-

severe reaction) and 31.40 to 58.25 (medium-severe reaction) percent per plant.

For the control of CLCuV infection by controlling the possible vector (whitefly) two seed-dressing insecticides viz., Confidor and Promit were used along with different spray insecticides. As concerned the seed-dressing insecticide, the disease appeared after three weeks of sowing. So these seed-dressing insecticides are unable to check the disease for long time. However, the seed dressing insecticide Confidor proved to be better as compared to other seed-dressing insecticide Promit.

Similarly, the spray insecticides Nuvacron, Confidor, Master, Tamaron and Heptokil are unable to check the disease after each application. However, the whitefly population is reduced. Four sprays of Nuvacron, Polo, Confidor and Tamaron were applied at one week interval in that order. The insecticides were found to be effective against whitefly population, but did not control the disease.

Final Res. Report, Dept. Plant Path., Univ. Agric., Faisalabad, pp. 44-45, 1994.

STATE OF COTTON LEAF CURL DISEASE IN PAKISTAN

Hameed, S., S. Khalid & B.D. Harrison

Pakistan produces about 13 million bales of cotton annually and it's fibre based economy (60% of foreign exchange come from cotton and cotton related commodities) is dependent on cotton production. Cotton was grown successfully until 1987-88 when a virus like disease, known to occur in patches since 1967, caused considerable losses. An epidemic developed in 1991-92 and again in 1992-93 when about 300,000 acres were affected. Symptoms resembled those caused by cotton leaf curl virus (CLCuV). These include upward or downward cupping and shortening of leaves, distortion and thickening of veins, and development of enations on the underside of

leaves. Geminiviral etiology of the disease was confirmed by reproduction of symptoms from diseased to healthy cotton and to a number of other hosts by whitefly and graft transmission, electronmicroscopic observations of geminivirus-like particles and through serology (TAS-ELISA) using polyclonal and monoclonal antibodies to african cassava mosaic virus and indian cassava mosaic virus, respectively.

Symptom based surveys during 1991-92 in key cotton growing districts in Punjab province indicated that the disease is well distributed and its vector *Bemisia tabaci* is present in abundance. The situation during 1993-94 is comparatively better than in 1992-93. It seems that the change in varietal spectrum (by not sowing susceptible varieties), weeding in and around cotton field and frequent rains early in the season have checked vector population growth and might have played an important role. On the other hand, areas which were comparatively free during 1992-93 were badly affected in 1993-94, the reasons for which are not well understood. The situation is complicated and needs systematic studies on all aspects of the disease. Priority must be given to the development of a specific and superior detection method for CLCuV to be used in epidemiological and other studies .Work on these lines is in progress.

World Cotton Res. Conf. 1, Feb. 14-17, 1994, Brisbane, Australia, (Abst. 152) p.95.

RESPONSE OF UPLAND COTTON GENOTYPES TO LEAF CURL VIRUS INFECTION

Mahmood, T.,
M. Arshad,

Incidence of cotton leaf curl disease and yield of seed cotton in six varieties/strains of upland cotton viz; CIM-109, CIM-240, CIM-243, CIM-262, MS-84 and S-12 were investigated at nine locations. Leaf curl infection was minimum and yield of seed cotton was highest in CIM-240 followed by CIM-109. The differences between

M. Afzal & M. Tahir

the varieties and locations were highly significant. Interaction between varieties × locations were also significant.

Pak. J. Phytopath., 6(2): 147-151, 1994.

RESPONSE OF SOME COTTON VARIETIES TO COTTON LEAF CURL VIRUS

Mahmood, T., M. Arshad, M. Tahir & M. Afzal

Incidence of cotton leaf curl bigeminivirus and yield of seed cotton in six varieties/strains of upland cotton viz; CIM-109, CIM-240, CIM-243, CIM-262, MS-84 and S-12 were recorded at nine locations. Leaf curl infestation was minimum in CIM-240 followed by CIM-109. Based on average at 9 locations, CIM-240 gave the highest yield closely followed by CIM-109.

Pak. J. Sci. Indust. Res., 38(1): 30-32, 1995.

INSECTICIDE RESISTANCE IN *BEMISIA TABACI* FROM PAKISTAN

Cahill, M., D. Johnston, K. Gorman & I. Denholm

Cotton production in Pakistan has declined markedly over the last two seasons. This was as a consequence of several factors, including unprecedented infestations by cotton leaf curl virus (CLCuV) and its vector the cotton whitefly (*Bemisia tabaci*). Research at Rothamsted and in Pakistan has disclosed moderate to very strong resistance in *B. tabaci* to all of the commonly used insecticides, supporting frequent claims that these chemicals are losing their effectiveness under field conditions. These findings reinforce the urgency of identifying and implementing sustainable pest management practices for the cotton cropping system.

Brighton Crop Prot. Conf. - Pests and Dis. 1994, Brighton, U.K., 4C-5, pp. 431-436.

DEVELOPMENT OF A HIGH YIELDING COTTON MUTANT, NIAB-92 THROUGH THE USE OF INDUCED MUTATIONS

Soaked seeds of an exotic variety Stoneville-231 (*Gossypium hirsutum* L.) were treated with gamma-rays at 30 kR from ^{60}CO source having dose rate of 40 kR per hour and planted in the field as M_1 generation during 1984. From the segregating generations, a high yielding mutant

Iqbal, R.M.S., M.B. Chaudhry, M. Aslam & A.A. Bandesha

was selected and named as NIAB-92. The plant of NIAB-92 is a semi-hairy, compact sympodial type of medium stature. It has 0-2 fruit bearing monopodial branches and more number of shorter sympodial branches as compared to the parent, Stoneville-231 and commercial cotton variety NIAB-78. The mutant is early maturing and matures twenty days earlier than the parent Stoneville-231. It has higher yield potential alongwith desirable fibre properties such as G.O.T.(%), fibre length, fibre fineness and fibre strength. In the comparative yield trials the mutant NIAB-92 significantly outyielded both the prevalent commercial cotton varieties i.e., NIAB-78 and S-12. At NIAB it gave 15.3% and 19.1% higher yield than NIAB-78 and S-12, respectively, while at farmers fields the increase in yield was 8.8% and 14.1% as compared with NIAB-78 and S-12. The mutant has also shown resistance against leaf curl virus disease.

Pak. J. Bot., 26(1): 99-104, 1994.

COTTON LEAF CURL DISEASE IN PAKISTAN CAUSED BY A WHITEFLY - TRANSMITTED GEMINIVIRUS

Hameed, S., S. Khalid, E. Haq & A.A. Hashmi

In Pakistan, cotton leaf curl disease of cotton (*Gossypium hirsutum* L.) was first observed in 1967 in Multan, Pakistan. In 1987, the disease reached epidemic proportions in most cotton-growing areas. Symptoms resembling those caused by cotton leaf curl virus (CLCuV) which is transmitted by the whitefly *Bemisia tabaci* (Gennadius) included upward or downward cupping and shortening of leaves, distortion and thickening of veins, and development of enations on the underside. In a 1992 survey of key districts, the disease was well established in Vehari, Multan, Khanewal, Bahawalpur, and Sahiwal. Incidence of symptomatic plants ranged from 30 to 80% among districts, and *B. tabaci* occurred in abundance. An infectious agent was isolated by whiteflies transmission from field-infected cotton and was subsequently transmitted

by grafting and whiteflies to cotton, cowpea, okra and soybean. Symptoms characteristic of CLCuV developed in these experimental hosts. Electron-microscopic examination confirmed the presence of geminivirus-like particles (monomers, dimers and paired dimers) in extracts from symptomatic experimental hosts. Dimers were 15-18nm in diameter. Triple antibody sandwich (TAS) ELISA was conducted with antibodies specific to whitefly transmitted geminiviruses. Polyclonal antibodies to african cassava mosaic virus (ex SCRI) and monoclonal antibodies to indian cassava mosaic virus (2HI 2 SCR-60) were used to test inoculated experimental hosts and naturally infected cotton (antibodies provided by B.D. Harrison, Dundee, Scotland). Extracts of these infected plants reacted positively with these antibodies in TAS-ELISA. We conclude that cotton leaf curl disease in Pakistan is caused by a whitefly-transmitted geminivirus with the properties of CLCuV.

Pl. Disease, 78(5): 529, 1994.

WHITEFLY, *BEMISIA TABACI*, AS A VECTOR OF COTTON LEAF CURL VIRUS

Hashmi, A.A., E. Haq, S. Khalid & S. Hameed

The whitefly, *Bemisia tabaci* has been a source of regular damage to cotton every year in Pakistan, with population fluctuations from year to year. However, during the last two years leaf curl virus has assumed an epidemic form, at a time when whitefly is also present in the field, thus posing big threat to the economy of Pakistan. Since whitefly is known to be vector of several viral diseases, it was apprehended that whitefly could be transmitting this virus disease in cotton. In retrospect cotton leaf curl virus first reported in 1967 from Multan area remained a minor disease until 1987 after which it flared up. Whitefly was therefore collected from cotton fields in Sahiwal area and its culture was established to carry out disease transmission studies. Two years field and laboratory studies have confirmed that leaf curl disease is transmitted by whitefly. Whitefly also

acts as vector for transmission of this disease from cotton to cotton, cotton to cowpea, french beans, okra, tomato and soybean; but has failed to transmit it to tobacco and sunflower. The disease was also transmitted on desi cotton. Through experiment it is also confirmed that a single specimen of *B. tabaci* could transmit leaf curl disease from cotton to cotton.

Proc. 13th Pak. Cong. Zool, Mar. 31-Apr. 1, 1993, UGC, Islamabad, (Abst.) pp. 76-77.

USE OF POLYMERASE CHAIN REACTION FOR THE IDENTIFICATION OF ALTERNATE HOSTS FOR COTTON LEAF CURL VIRUS

Mansoor, S., J.A. Qureshi, J. Stanley, P.G. Markham & K.A. Malik

Identification of alternate hosts for cotton leaf curl virus (CLCuV) which serve as virus reservoir is important for the management of cotton leaf curl disease. We have previously shown that CLCuV is a whitefly-transmitted geminivirus. geminiviruses are single stranded DNA virus with circular genome and can be detected by polymerase chain reaction (PCR). Use of universal primers for geminiviruses detects all geminiviruses. Another set of primers based on CLCuV sequence has been designed. These primers amplify CLCuV DNA but did not amplify ACMV and ICMV under identical conditions and suggest that these primers may be used to distinguish CLCuV from other geminiviruses. In the present communication universal primers were used for the detection of all geminiviruses where as CLCuV based primers were used for specific detection of CLCuV. Based on this strategy *Hibiscus esculentis*, *H. rosasinensis*, *H. cannabinis* and another *Hibiscus* sp. were found to be alternate hosts for CLCuV. These results suggest that PCR based diagnostic test can be used for rapid identification of alternate hosts of CLCuV.

Biotech. for Sustainable Dev., Dec.15-20, 1993, NIBGE, Faisalabad, (Abst.) p. 117.

MOLECULAR CHARACTERIZATION OF A GEMINIVIRUS ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

Mansoor, S., J. Stanley, K.A. Malik & P.G. Markham

Cotton leaf curl disease has acquired epidemic proportions in the last few years in Pakistan. It has been shown previously that the disease is associated with a whitefly-transmitted geminivirus. Present work describes the characterization of the virus. Universal primers for DNA-A of geminiviruses were used to amplify viral DNA by polymerase chain reaction (PCR). DNA-A primers amplified viral DNA from infective tobacco plant as well as cotton plants collected from fields. Degenerate primers for DNA-B were also used in PCR and were unable to amplify viral DNA. DNA amplified by DNA-A primers was cloned into Bluescript vector and was used as a probe for the detection of various DNA forms associated with geminiviruses. Conditions were optimized for purification of total and viral DNA forms associated with geminiviruses. Conditions were optimized for purification of total and viral DNA from cotton leaves. Purified DNA from infected cotton plants hybridized with Cotton Leaf Curl Virus (CLCuV) DNA probe. Partial sequencing of cloned DNA revealed the presence of conserved nanomer sequence but did not match with any well characterized geminivirus. These results suggest that CLCuV is a new geminivirus, which has not been characterized previously.

Proc. Int. Symp. on Biotech. Sustainable Dev., Dec. 15-20, 1993. NIBGE, Faisalabad, Pakistan, pp. 123-128.

GROWTH RESPONSE OF COTTON PLANTS AGAINST COTTON LEAF CURL VIRUS INFECTION

Bukhari, S.S.A.

Disease screening studies of cotton cultivars against natural infection by CLCuV, carried in the demonstration plot of Punjab Seed Corporation (Peruwal) Khanewal, for finding resistant sources against the disease revealed that of the 25 cultivars screened, none was found to be

completely free from disease or highly resistant. However, 4 cultivars CIM-109, CIM-240, MNH-147 and NIAB-92 were found to be resistant. Three cultivars such as BH-36, SL-100, SLH-41 behaved as moderately resistant. The remaining cultivars were moderately to highly susceptible.

Studies on grown responses of ten genetically different cotton cultivars to CLCuV revealed that both the vegetative and reproductive components were reduced on infection to virus. However, the affected cultivars varied in their response to virus infection and were suffered to variable extents probably due to their different genotype.

In response to CLCuV infection the cultivars exhibited 9.20 to 45.65% decrease in plant height, 34.01 to 72.30% decrease in number of branches, 22.68 to 55.59% decrease in number of leaves, 19.48 to 57.19% decrease in size of branches and 16.77 to 82.11% decrease in dry stem weight.

The response of CLCuV on reproductive components also varied and depending upon the cultivars there was 54.88 to 80.26% decrease in number of bolls set per plant, 19.14 to 36.87% decrease in boll size, 9.69 to 42.11% decrease in boll weight, 13.52 to 69.52% decrease in number of seeds per plant, 7.40 to 12.28% decrease in 100-seed weight and 12.45 to 78.85% decrease in plant yield of cotton.

M.Sc.(Hons.) Thesis Dept. Pl. Path., Univ. Agric., Faisalabad. 1993.

A WHITEFLY-TRANSMITTED GEMINIVIRUS ASSOCIATED WITH COTTON LEAF CURL DISEASE IN PAKISTAN

Cotton (*Gossypium hirsutum* L.) is one of the most important crop of Pakistan which accounts for 60% of the export product of the country. In the last few years cotton leaf curl disease has acquired epidemic proportions in Pakistan and has seriously threatened cotton production. The

Mansoor, S., I. Bedford, M.S. Pinner, J. Stanley & P.G. Markham

characteristic symptoms of the disease are severe leaf curling, thick dark veins and enations which sometimes differentiate into cup shaped leaf-like structures on the underside of the leaf.

Whitefly *(Bemisia tabaci)* was suspected as the insect vector of cotton leaf curl disease. Whiteflies maintained in the controlled conditions were used for insect transmission of the disease from cotton to cotton and from cotton to tobacco (*Nicotiana tabacum*). The symptoms developed on cotton and tobacco were similar to leaf curling in cotton plant with thick dark veins and development of cup shaped structures on underside of leaf.

Pak. J. Bot., 25(1): 105-107, 1993.

A RESEARCH COMPENDIUM ON COTTON LEAF CURL VIRAL DISEASE AND ITS VECTOR - WHITEFLY

Hashmi, A.A., E. Haq, M.A. Rana, R. Masih,S. Hameed, M. Aftab & S. Khalid

During 1990-92 the cotton crop was badly hit in the Punjab by leaf curl viral disease. It has caused a loss of 5-8% in the number of bolls, and 25-30% in the boll weight. The overall yield loss has been estimated at 30-35%. The consecutive field surveys have indicated that although the disease is well established, its incidence varied from district to district and from variety to variety. The scientists of Pakistan Agricultural Research Council have confirmed the identity of the disease as geminivirus with electronmicroscope and have established the cotton whitefly as vector of the disease through experimental procedures.

In order to keep the other researchers, planners, policy makers, extension workers, farmers and donor agencies well informed, all research work accomplished so far is being documented. It is hoped that this synthetic information will reduce duplication of efforts at other institutions and will push forward the novel approaches

meant to help solve the upcoming leaf curl disease problems.

Pak. Agric. Res. Council (PARC), Islamabad, 1993, pp. 1-62.

COTTON LEAF CURL DISEASE: A REVIEW

Hussain, T., M. Tahir & T. Mahmood

Topics briefly discussed in this review of the disease caused by cotton leaf curl geminivirus includes the economic importance in Pakistan, symptoms, transmission, alternate hosts, losses caused by CLCuV and varietal resistance.

Pak. J. Phytopath., 3 (1-2): 57-61,1991.

A NOTE ON LEAF CURL DISEASE OF COTTON

Hussain, T. & T. Mahmood

Leaf curl disease of cotton was recorded from Multan area in 1967 on a few individual plants. Almost the same position of the disease persisted till 1986. During 1987, its incidence increased substantially and its spread in most of the growers fields. This disease had already been reported from the Sudan, Nigeria, Western and Central Africa as one of the most important diseases of cotton crop. Some preliminary observations and studies have been given in this paper for the benefit of the growers and others associated with cotton crop.

The disease causes either upward or downward curling of the leaves. The veins of the leaves become thick which are more pronounced on the lower surface. Thick veins may become 'enations' (finlike outgrowths). In young leaves, the thickening first appears on the lower surface of small veins, beginning at separate foci and gradually linking together. This process makes the leaves curl. From the underside, affected veins appear abnormally dark green and opaque. New leaves developed, after appearing of the first symptoms, are usually small and much distorted by curling.

Survey conducted in third week of September and first week of October, 1987 to record incidence of this disease growers' fields and at Cotton Research Institute, Multan, indicated that the incidence of the disease was very high up to 80% in some of growers' fields. All the varieties/ strains were found to be susceptible to this disease but commercial variety, NIAB-78 was least affected.

The virus is not mechanically transmitted nor carried through soil or seed. It is transmitted by the feeding of the whitefly *Bemisia tabaci* (Genn.), Which can complete the entire cycle from acquisition of the virus and infection of a host plant, within 6.5 hours. The experiments on the transmission of this virus among different species of cotton through grafting and whitefly were carried out in caged plants and it was found that this virus can be transmitted from *Gossypium hirsutum* to *G. raimondii* and *G. arboreum*. However, further studies need to be carried out.

The leaf curl symptoms have been recorded on *Hibiscus esculentus, H. rosasinensis, G. raimondii, G. Davidsoni, Poinsettia* sp., *Althaea rosea* and *Zinnia elegans* in the vicinity of Multan.

The Pak. Cotton, 32(4): 248-251, 1988.

COWPEA

COWPEA

SOURCES OF GENETIC RESISTANCE IN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.) TO COWPEA APHID-BORNE MOSAIC POTYVIRUS

Bashir, M. & R.O. Hampton

Fifty-one cowpea (*Vigna unguiculata* (L.) Walp.) genotypes were tested by mechanical inoculation with seven geographically diverse isolates of cowpea aphid-borne mosaic potyvirus to identify resistant sources. Of 51 genotypes three; TVu-401, TVu-1582 and Tvu-1593 were found immune to all the seven isolates. Forty-five genotypes gave different reactions to individual isolates. Several immune, resistant and tolerant genotypes against each isolate were identified. A considerable evidence of pathogenic variability among the virus isolates was also observed.

European J. Pl. Path., 102: 411-419, 1996.

SEROLOGICAL AND BIOLOGICAL COMPARISONS OF BLACKEYE COWPEA MOSAIC AND COWPEA APHID-BORNE MOSAIC POTYVIRUS ISOLATES SEED- BORNE IN *VIGNA UNGUICULATA* (L.) WALP. GERMPLASM

Bashir, M. & R.O. Hampton

Serological and biological comparisons were made among 45 seed-borne isolates of blackeye cowpea mosaic (BlCMV) and 54 seed-borne isolates of cowpea aphid-borne mosaic (CAbMV) potyviruses derived from cowpea seed-lots or young nursery-grown seedlings comprising 2112 germplasm accessions or pre-introduction seed-lots of *Vigna unguiculata*. Isolates were identified by DAS-ELISA using polyclonal immunoglobulin G specific for these viruses. Twenty isolates of BlCMV and 32 isolates of CAbMV were also compared by ACP-ELISA with selected monoclonal antibodies and by SDS- immunodiffusion. By all approaches, isolates of BlCMV were clearly distinguished from CabMV isolates. Isolate comparisons on selected cowpea genotypes (TVu-401, TVu-1582, TVu-2657 and TVu-3433) partitioned most isolates into two distinct groups. A few isolates seed-borne in Indian cv., Pusa Phalguni, however, were clearly BlCMV by all serological tests, but behaved as CAbMV

in definitive cowpea genotypes. Although BlCMV is generally considered to be 'a New World virus', both BlCMV and CAbMV occurred in *V. unguiculata* seed-lots originating in 'old–world regions', including Afghanistan, Botswana, Nigeria, Senegal and South Africa. Seed-borne CAbMV was isolated from 6 of 155 tested US *V. unguiculata* germplasm accessions originating respectively, in Afghanistan (2), Botswana (2), India (1) and Pakistan (1).

J. Phytopath., 144: 257-263, 1996.

IDENTIFICATION OF COWPEA (*VIGNA UNGUICULATA*) CULTIVARS AND LINES IMMUNE TO VARIANTS OF BLACKEYE COWPEA MOSAIC POTYVIRUS

Bashir, M. & R.O. Hampton

Fifty-one cowpea (*Vigna unguiculata*) cultivars and lines were tested by mechanical inoculation against seven geographically and pathogenically diverse isolates of blackeye cowpea mosaic potyvirus (BlCMV), to identify genetic resources with comprehensive BlCMV resistance. Five genotypes, IT 80S 2049, Big Boy, Corona, Serido and Tennessee Cream#8 were immune from all seven isolates except PU-7B, and aberrant BlCMV isolate. The diversity among BlCMV isolates was illustrated by the range of responses to inoculation among cowpea genotypes, many of which were either immune from or tolerant of individual BlCMV isolates.

Pl. Pathology, 45: 984-989, 1996.

DETECTION AND IDENTIFICATION OF SEED-BORNE VIRUSES FROM COWPEA(*VIGNA UNGUICULATA* (L.) WALP.) GERMPLASM

Bashir, M. & R.O. Hampton

Seedlings from 182 cowpea (*Vigna unguiculata*) pre-introductions/germplasm accessions from 12 countries were tested under greenhouse conditions for six seed-borne viruses. Twenty-one (13.3%) accessions from eight countries were found to be seed-infected with one of the three following viruses: blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses and cucumber mosaic cucumovirus (CMV). Natural seed

transmission incidence of 0-6.9%, 0-13.3% and 0-2.0% were determined for BlCMV, CABMV and CMV, respectively.

Another set of 2930 cowpea germplasm accessions, mostly from Botswana and Senegal (Africa), were examined under field conditions for detection and identification of seed-borne viruses. Only CABMV was detected in this material. Most of the lines were free from other viruses reported in cowpea seed. Eight isolates of BlCMV and 28 isolates of CABMV were derived from cowpea pre-introductions/ germplasm accessions evaluated under green-house and field conditions.

Pl. Pathology, 45: 54-58, 1996.

SOURCES OF IMMUNITY IN COWPEA AGAINST BLACKEYE COWPEA MOSAIC POTYVIRUS

Bashir, M., Z. Ahmed, Z. Riaz & B.A. Malik

Fifty cowpea genotypes were screened for resistance against a seed-borne isolate of blackeye cowpea mosaic potyvirus (BlCMV) by mechanical inoculation. Ten genotypes viz., IT86F-2089-5, IT86D-880, IT86F-2062-5, IT92 KD-226-2-1, IT90K-284-2, IT90K-76, IT86D-1010, IT87D-611-3, TVU-7676 and PAK45443 were found immune against BlCMV isolate "PU-7B". No virus was detected from inoculated plants of immune genotypes when the leaf samples were tested by double antibody sandwich enzyme-linked immunosorbent assay in repeated tests.

Pak. J. Phytopath., 7 (2): 94-97, 1995.

PURIFICATION AND ELECTRON- MICRO-SCOPY OF SOME ISOLATES OF BLACK-EYE COWPEA MOSAIC AND COWPEA APHID-BORNE MOSAIC POTYVIRUSES

Two isolate of blackeye cowpea mosaic virus (BlCMV) and two of cowpea aphid borne mosaic virus (CABMV) potyviruses were purified by three purification procedures. Variable virus yields of each isolate of each virus were obtained with different virus-host combinations and purification procedures. Highest average virus yields of 4.6 µg and 3.4 µg per gm of infected

Bashir, M. & R.O. Hampton

tobacco leaf tissue were obtained from CABMV isolate RN-7C and BICMV isolate PU-7B. Method-3 was most effective for purification of both viruses.

Carbon tetrachloride when used in combination with chloroform a clarifying agent improved purification of the BICMV isolates, but was harmful to CABMV isolates. Borate buffer was superior than phosphate for purification of both viruses. Addition of EDTA and Triton X-100 in extraction and resuspension buffers circumvented virion aggregation.

Leaf dip or purified virus preparations of BICMV or CABMV isolates, examined under the electron microscope, contained elongated flexious particles of modal length of 742 nm and 725 nm for BICMV and CABMV, respectively, and confirmed particle morphology of potyvirus group.

Pak. J. Bot., 27(1): 243-249, 1995.

ANTISERUM PRODUCTION AGAINST COWPEA APHID-BORNE MOSAIC VIRUS AND STRANDARDIZATION FOR ENZYME-LINKED IMMUNOSORBENT ASSAYS

Bashir, M. & R.O. Hampton

A reasonable yield of cowpea aphid-borne mosaic virus (CABMV) was obtained from systemically infected cowpea leaves and was injected into a male white New Zealand rabbit to produce antiserum. The titre of the antiserum was determined to be 1/64 by SDS-immunodiffusion tests. The antiserum produced reacted positively with homologous and heterologous antigens producing ELISA A_{405} nm values that distinguished between extracts from CABMV-infected vs. healthy plants. Both direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA; using antiserum) and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) [using purified immuno-gammaglobulin (IgG)] were standardized to determine optimum concentration of ELISA reagents. The crude antiserum in

DAC-ELISA or purified IgG in DAS-ELISA, when diluted to 1:1000 were found to be capable of detecting virus in fresh or desiccated infected tissue that was also diluted 1:1000. Protocols for DAC-ELISA (indirect) and DAS-ELISA (direct) assays have been discussed.

Sarhad J. Agri., 11(4): 505-512, 1995.

SEED AND APHID TRANSMISSION OF SOME ISOLATES OF BLACKEYE COWPEA MOSAIC AND COWPEA APHID-BORNE MOSAIC POTYVIRUSES

Bashir, M. & R.O. Hampton

Rate of seed-transmission was investigated for 10 isolates of blackeye cowpea mosaic (BlCMV) and 12 isolates of cowpea aphid-borne mosaic (CABMV) potyviruses in some cowpea genotypes. Seed-transmission rates varied between viruses, and significantly different among isolates, and among cowpea genotypes. The highest seed transmission rate demonstrated for BlCMV (55%) occurred in seeds from plants of 'Pusa Phalguni' that had been inoculated with isolate PI-25Bl. BlCMV isolates PI-22B and PI-3B also were seed-transmitted in this genotype at rates of >40%. Likewise CABMV isolate RN-27C was seed-transmitted at a rate of 55% in cowpea genotype '58-57', from which the isolate was initially obtained. CABMV isolates RN-28C and PI-44C were also seed-transmitted at rates of 30% in this genotype. Approximately one-third of the isolate-genotype combinations for both BlCMV and CABMV potyviruses resulted in non-measurable seed-transmission of virus.

Non-persistent aphid-transmission was found for three selected isolates each of BlCMV and CABMV, using three aphids (*Aphid craccivora* Kock) per cowpea plant. Average transmission rates of the two viruses ranged from 24 to 55% and from 18 to 57% for BlCMV and CABMV, respectively.

Pak. J. Phytopath., 6(2): 140-146, 1994.

SEROLOGICAL DISTINCTION AND BIOLOGICAL COMPARISON OF BLACKEYE COWPEA MOSAIC AND COWPEA APHID-BORNE MOSAIC POTYVIRUSES

Bashir, M. & R.O. Hampton

Blackeye cowpea mosaic virus (BICMV) isolates (forty-two) and fifty-four of cowpea aphid-borne mosaic (CABMV) were derived from cowpea germplasm accession, seed lots and field-grown plants to compare their serological and biological properties to distinguish between these two viruses. Identity of each isolate of BICMV and CABMV was determined by double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) and verified by electron microscopy and host reactions. Thirty isolates of BICMV and 37 of CABMV were compared by DAS-ELISA, monoclonal antibody (Mab) and SDS-immunodiffusion tests. DAS-ELISA, monoclonal antibody and SDS-immunodiffusion tests clearly distinguished BICMV isolates from isolates of CABMV. Based on DAS-ELISA, monoclonal antibody, SDS-immunodiffusion tests, and biological behavior of several isolates of BICMV and CABMV on selected cowpea genotypes (TVU-401, TVU-1582, TVU-2657 and TVU-3433), it was concluded that BICMV and CABMV are two distinct potyviral entities.

Proc. Biotech. Sustainable Dev., Dec. 15-20, 1993. NIBGE, Faisalabad, pp. 129-136.

NATURAL OCCURRENCE OF FIVE SEEDBORNE COWPEA VIRUSES IN PAKISTAN

Bashir, M. & R.O. Hampton

A total of 151 cowpea leaf samples with virus-like symptoms were collected from 13 districts of Punjab and North West Frontier Province (NWFP) of Pakistan during the summers of 1990 and 1991. Desiccated samples were tested by direct antigen coating (DAC) or double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) for the presence of seven viruses known to be seedborne in cowpea: blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses, cucumber mosaic (CMV) cucumovirus, cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV) comoviruses, cowpea

mottle carmovirus (CPMoV), and southern bean mosaic sobemovirus (SBMV). One or more seedbrone viruses were detected in 47% (71 of 151) of the symptomatic plant samples. The viruses detected and the percent incidence were: CABMV, 29%; SBMV, 21%; CSMV, 17%; BlCMV, 8%; and CPMoV, 4%. Neither CMV nor CPMV was detected. Seven cowpea seed lots representing two commercial seed types and seven locations in Punjab and NWFP were collected for tests of the same seven seedborne viruses. Only CABMV was seed-transmitted, and only in four of seven seed lots, at frequencies of <1 to 7%. Likewise, although leaf samples collected from plants of a local Punjab cultivar contained four ELISA-detectable seedborne viruses (BlCMV, CABMV, CSMV and SBMV), only CABMV was transmitted through seeds (7% frequency) from these plants. None of the seven seedborne viruses tested for were detected in 80 of 151 virus-symptomatic samples, suggesting infection with other viruses. This is believed to be the first record of the natural occurrence of BlCMV, CABMV, CPMoV, CSMV and SBMV in Pakistan and the first report of CPMoV outside of Africa.

Pl. Disease, 77(9): 948-951, 1993.

NATURAL OCCURRENCE OF FIVE COWPEA VIRUSES IN PAKISTAN

Bashir, M. & R.O. Hampton

Ninety-one cowpea field samples were collected in July 1990 from two northern Pakistan provinces, Punjab and NWFP. Desiccated samples were tested by DAS-ELISA for the possible presence of seven viruses known to be seed-borne in cowpea: blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CAMV) potyviruses, cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV) comoviruses, cowpea mottle carmovirus (CMoV), cucumber

mosaic cucumovirus (CMV), and southern bean mosaic sobemovirus (SBMV). The following viruses previously unreported in Pakistan-grown cowpeas were detected in one or more field samples: BlCMV (6), CAMV (22), CMoV (11), CSMV (5), and SBMV (9). Eleven of 91 samples contained a complex of two or more viruses, with CAMV occurring most frequently. Field collected seeds from a virus-infected local cultivar were tested for seed borne viruses. Whereas the parent plants were infected with BlCMV, CAMV, CSMV, and SBMV, only CAMV was seed-transmitted (7% frequency).

Phytopath., 81 (10): 1166 (Abst. 241), 1991.

BLACKEYE COWPEA MOSAIC (BlCMV) AND COWPEA APHID-BORNE MOSAIC (CAMV) POTYVIRUSES: BIOLOGICAL COMPARISONS AND SEROLOGICAL DISTINCTIONS

Bashir, M. &
R.O. Hampton

Some investigators have proposed that these two potyviruses are indistinguishable and should be called BlCMV. We report biological and serological (ELISA) comparisons of 22 BlCMV isolates and 44 CAMV isolates, each seed-borne in selected *Vigna unguiculata* seed lots. Antiserum to a tentative CAMV isolate (9-7c, intermediate virulence) yielded IgG that reacted homologously with the Morocco CAMV type isolate and was completely non-reactive with all BlCMV isolates including the Georgia BlCMV type isolate. This distinction was confirmed against the potyvirus monoclonal panel of R. Jordan. BlCMV and CAMV isolates were not delineated reliably by infectivity/symptomatology on eleven *V. unguiculata* genotypes previously reported as differential hosts. BlCMV seed-borne isolates tended to be more virulent than CAMV seed-borne isolates. BlCMV and CAMV type isolates were highly virulent.

Phytopath., 81(10): 1166-1167 (Abst.241), 1991.

TESTING OF COWPEA SEEDS FOR SEED-BORNE VIRUSES

Azeemuddin, S.

Multiplication seeds of cowpea (*Vigna sinensis* Endl.) cvs. white and red were checked for the presence of seed-borne viruses, under controlled conditions. Distinct symptoms such as crinkling, mottling, slight enations, necrotic lesions and yellowing of tips appeared on the seedlings. Where necrotic lesions were present on the primary leaves of both the cultivars, the infection eventually become systemic and the trifoliate leaves showed typical mottling. Of the 400 cowpea seeds var. white tested, 20% were found infected. Work on the thermal inactivation, dilution end point retention of infectivity and host range including differential hosts of viruses present in both the cultivars of cowpea is under study.

Pak. J. Bot., 14: 46 (Abst. 104), 1982.

WHITEFLY (*BEMISIA TABACI*) TRANSMISSION OF A YELLOW MOSAIC DISEASE OF COWPEA (*VIGNA UNGUICULATA*)

Ahmed, M.

A yellow mosaic disease, presumably caused by a virus, was recorded on cowpea (*Vigna unguiculata*). It is characterized by irregular, bright yellow patches over the laminae of leaflets. It was transmitted by grafting and the whitefly, *Bemisia tabaci*. No transmission, however, was obtained by sap, seed, dodder, or soil. The disease appears to be distinct from yellow mosaic of urd and mung beans.

Pl. Dis. Reporter. 62(3): 224-226, 1978.

EGG-PLANT

DETECTION OF CUCUMBER MOSAIC CUCUMOVIRUS IN EGGPLANT (*SOLANUM MELONGENA* L.) BY ENZYME-LINKED IMMUNOSORBENT ASSAY

Bashir, M.,
S.M. Iqbal,
T. Mahmood &
C.A. Ozair

During December, 1995 plants of eggplant (*Solanum melongena* L.) variety Hari grown in plastic tunnel at National Agricultural Research Centre, Islamabad, showed symptoms of some virus-like disease. The leaves of the infected plants were showing mosaic and mild yellowing with whole plant stunting. A total of 20 samples, 16 from symptomatic and 4 from asymptomatic plants were collected and tested to detect virus by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA). The samples were tested against agdia potyvirus monoclonal antibody and polyclonal antibodies to cucumber mosaic cucumovirus (CMV) supplied by Dr. R.O. Hampton. None of the samples reacted with agdia potyvirus monoclonal antibody, which excluded the possibility of occurrence of any Potyvirus. The samples from asymptomatic plants were also negative in ELISA. However, sixteen (100%) samples from infected plants reacted positively with polyclonal antiserum to CMV indicating the presence of CMV in these samples. The virus was also successfully transmitted mechanically on eggplant, and *Datura stramonium*. On *Chenopodium amaranticolor* virus produced local lesions by sap inoculation. The occurrence of CMV on eggplant in Sindh, Pakistan has been reported previously on the basis of symptomatology, host plant reaction and physical properties without conducting any serological test. The study confirms the previous report about the occurrence of CMV on eggplant in Pakistan not only on the basis of host reaction but also by serological test (DAC-ELISA).

Crop Prot. Conf., Apr. 20-22, 1996,
NWFP Agri. Univ., Peshawar, (Abst.) pp. 25-26.

OCCURRENCE OF CUCUMBER MOSAIC VIRUS (CMV) ON EGG PLANT IN SINDH

Rana, N.H. & M.B. Jilani

Survey of egg plants (*Solanum melongena* L.) in six localities of Sindh indicated that 7 to 28% of the plants were infected with CMV. The virus was identified on the basis of symptomatology, host reactions, physical properties and transmission by the vectors-*Myzus persicae* and *Aphis gossypii*, of which the former was more effective. The virus lost its infectivity in plant sap when heated for 10 min. at 60°C, stored for 24-30 hrs. at room temperature or diluted to 10^{-4}.

Pak. Agric., 7(5): 7-8, 1985.

FABABEAN

REACTION OF FABA BEAN GENOTYPES TO NATURAL INCIDENCE OF BROAD BEAN MOSAIC VIRUS IN PAKISTAN

Iqbal, S.M., A. Ghafoor, M. Bashir & M. Aftab

Disease reaction of 18 faba bean genotypes to broad bean mosaic virus was studied under field conditions in Islamabad, Pakistan. The genotypes varied significantly in their reaction to the disease. Of the 18 genotypes tested, only one genotype (ILB 2785) rated resistant. 11 were moderately resistant, and 5 were susceptible to the disease. The study indicated the feasibility of developing resistant lines of faba bean to the disease.

FABIS Newsletter, 24: 27-29, 1989.

OCCURRENCE AND IDENTIFICATION OF BEAN YELLOW MOSAIC VIRUS FROM FABA BEAN IN PAKISTAN

Aftab, M., S.M. Mughal & A. Ghafoor

An elongated virus was isolated from broad bean cultivars introduced from ICARDA. The virus was characterized on the basis of biological, serological and physical properties and identified as bean yellow mosaic virus (Pea strain). Its host range was limited to species of *Chenopodiaceae* and *Leguminoseae*. The virus was seed-borne in broad beans to an extent of 2.6%. The purified virus preparations absorbed UV light typically of nucleoprotein, contained large number of flexuous and unaggregated particles which had a modal length of 750 nm.

Indian J. Virol., 5(1-2): 88-93, 1989.

LENTIL

DETECTION OF LENTIL STRAIN OF PEA SEED-BORNE MOSAIC POTYVIRUS AND CUCUMBER MOSAIC CUCUMOVIRUS FROM EXOTIC LENTIL GERMPLASM BY ENZYME-LINKED IMMUNOSORBENT ASSAY

Bashir, M., H. Shah, S.M. Iqbal, A. Bakhsh & B.A. Malik

During winter of 1993-94 one thousand and two hundred lentil (*Lens culinaris* L.) germplasm accessions from International Centre for Agricultural Research in Dry Areas (ICARDA) were planted in experimental fields under pulses program at National Agricultural Research Centre, Islamabad. One or more than one plants of one hundred-thirty two accessions were observed with virus-like symptoms. The symptoms were in the form of reddening and size reduction of the leaves, reduced internodes, mild yellowing or mottling of leaflets and stunting of plants. Fresh leaf samples from the virus-infected plants were collected and processed by direct antigen coating enzyme-linked immunosorbent assay (DAS-ELISA) against immunoglobulin (IgG) for pea seed-borne mosaic (PSbMV) potyvirus and cucumber mosaic (CMV) cucumovirus. The source of antisera was from Dr. R. O. Hampton. Of 130 samples tested 45 (34.6%) samples reacted positively either with IgG for PSbMV, or CMV. 33 samples reacted exclusively with IgG for PSbMV. Whereas nine samples reacted with IgG for CMV in repeated tests. Three samples were found with mixed infection of both PSbMV, three samples produced high absorbence ELISA values (A_{405} nm) ranging from 1.002 to 1.206 indicating high virus titre.

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, p. 75.

NATURAL OCCURRENCE OF CUCUMBER MOSAIC VIRUS IN LENTIL IN PAKISTAN

Bashir, M.,

Lentil (*Lens culinaris* Medik.) is one of the most important legume crops in Pakistan. It is grown on an area of 76,000 ha with annual production of 33,000 tonnes. Lentil suffers a number of diseases caused by fungi, bacteria, viruses and nematodes. These diseases reduce vigor of the plant and ultimately minimize grain yield.

M. Tahir &
B.A. Malik

During *rabi* (winter) of 1992-93, a few plants from lentil breeding material planted in experimental plots at the National Agricultural Research Centre, Islamabad, Pakistan, were found with virus-like symptoms. The infected plants showed stunting with mild mosaic on leaves and reduced size of the terminal buds and branches. Leaf samples from virus-infected plants were tested by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) with antisera to bean yellow mosaic virus, bean common mosaic virus, alfalfa mosaic virus, pea enation mosaic virus, bean leaf roll virus, cowpea aphid-borne mosaic virus and cucumber mosaic virus (CMV). Of 25 samples tested, 16 (64%) reacted positively with CMV antiserum in repeated DAC-ELISA assays. None of the samples reacted with any of the other antisera.

The presence of CMV in infected leaf samples was also confirmed by mechanical inoculation on tobacco (Samsun and Xanthi) with systemic infection and on *Chenopodium amaranticolor* with local lesions. Occurrence of CMV in lentil has previously been reported in Iran, but this is believed to be first report of natural occurrence of CMV in lentil in Pakistan.

LENS Newsletter, 21(1): 44, 1994.

EFFECT OF LENTIL STRAIN OF PEA SEEDBORNE MOSAIC VIRUS ON LENTIL

Aftab, M.,
S.M. Iqbal &
C.A. Rauf

Pea seedborne mosaic virus was isolated from naturally infected lentil variety Precoz showing serious mosaic symptoms. Leaves demonstrated chlorotic pale spots and shortening of internodes, and plants were stunted. Infected plants bore fewer flowers and pods. In growth and yield components decrease in plant height, number of pods, number of seeds and yield/plant was 51.2, 58.4, 65.6 and 72.0%, respectively. An electron micrograph from leaf dip preparation showed flexuous, rod-shaped virus particles.

LENS Newsletter, 19(2): 51-53, 1992.

MASH & MUNG

MASH

SCREENING OF MASH (*VIGNA MUNGO* L.) GERMPLASM AND ADVANCED BREEDING LINES FOR VIRUS DISEASES UNDER FIELD CONDITIONS

Bashir, M., A. Ghafoor, M. Zubair & B.A. Malik

In order to identify sources of resistance against MYMV and ULCV in mash for breeding purpose eighty germplasm/advanced lines were evaluated under field conditions at National Agricultural Research Centre, Islamabad, Pakistan.

Each test line was planted in a four meter row length 30 cm apart during last week of July, 1994. After every five test lines one row of susceptible check (mixture of two mash varieties susceptible to MYMV and ULCV) was also planted to favor disease development. Data regarding MYMV and ULCV severity was recorded following 0-6 scale (0-2 resistant, 3-4 tolerant, 5-6 susceptible) at 15 days interval. Due to favorable weather conditions both the viral diseases developed in high intensity on check and the screening purpose was achieved. Of 80 lines the following ten were found completely free of MYMV and ULCV symptoms: 9011, 92011, 92013, 92014, 92020, 92048, 92050, 92054, 92055 and 9080. Thirty and 60 lines were resistant to MYMV and ULCV respectively. The rest of the lines were either tolerant or susceptible to MYMV or ULCV.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agri. Univ. Peshawar, (Abstr.) p. 26.

SCREENING FOR RESISTANCE IN MASH AGAINST YELLOW MOSAIC DISEASE

Screening of mash (*Vigna mungo* L.) germplasm against yellow mosaic virus under natural field conditions indicated the presence of genetic resistance against the disease. Out of 89 lines tested, 20 lines were immune, 15 resistant,

Ghafoor, A., C.A. Rauf & S.M. Iqbal

7 moderately resistant and 43 susceptible. The study also showed that mash lines with hairiness character were resistant to YMV disease.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agri. Univ. Peshawar, (Abstr.) p. 36.

SCREENING OF MASH AGAINST YELLOW MOSAIC DISEASE

Iqbal, S.M., M. Zubair & A. Ghafoor

Field screening of mash lines against yellow mosaic virus indicated the presence of genetic resistance against the disease. Of the 160 lines/cultivars tested, 22 lines were immune, 102 resistant, 10 moderately susceptible and 26 susceptible. The study also showed that mash lines/cultivars with hairiness character were resistant to YMV disease.

Sarhad J. Agric., 6(4): 403-405, 1990.

MUNGBEAN

INHERITANCE OF YELLOW MOSAIC VIRUS IN MUNGBEAN (*VIGNA RADIATA* L. WILCZEK)

Saleem, M., W.A.A. Haris & I.A. Malik

Six elite lines of mungbean, two local NM-92 & NM-93 and four exotic VC-1973A, VC-2254A, VC-2771A, VC-3726A, and their crosses were studied for the inheritance of mungbean yellow mosaic virus (MYMV). The results showed that local parents had marked resistance to the disease infection while exotic parents appeared to be highly susceptible to yellow mosaic disease. The F_2 populations segregated into three susceptible and one resistant lines thereby indicating that susceptibility and resistance were controlled by a single genetic factor. The data proved to be good fit to 3:1 ratio showing the dominance of susceptibility over resistance.

Pak. J. Phytopath. 10(1): 30-32, 1998.

RELATIVE RESISTANCE OF MUNGBEAN VARIETIES TO WHITEFLY, *BEMESIA TABACI* GENN. AND YELLOW MOSAIC VIRUS

Naqvi, S.M.S., T. Hussain, M.A. Rustamani & M.A. Talpur

The studies on the relative resistance of different mungbean cultivars to whitefly, *Bemesia tabaci* Genn. and yellow mosaic virus were conducted under field conditions at Agriculture Research Institute, Tandojam. Ten mutant strains/varieties viz. M-20-21, M-121-25, Pak-22, RC-71-27, M-7-40, M-8-20, M-32-30, M-40-30, M-19-19 and C-23 were sown in randomized complete block design with four replicates. The observations on whitefly population and disease intensity were recorded at weekly intervals till maturity. The grains yield was also recorded at harvest. The results on disease intensity and whitefly population indicated that none of the cultivars were immune to the disease and whitefly infection. Mutants, M-8-20, M-20-21 and M-40-30 were found comparatively more resistant to whitefly and yellow mosaic virus than other mutants/ varieties. There is positive significant correlation between whitefly population and disease

intensity. The mutants/varieties which exhibited better tolerance to whitefly and yellow mosaic virus yielded significantly higher than susceptible ones. The correlation between whitefly and grain yield was negative but non-significant.

15th Pak. Cong. Zool, Apr. 15-17, 1995,NARC, Islamabad, (Abst.) p. 41.

EVALUATION OF SELECTED GERMPLASM OF MUNGBEAN (*VIGNA RADIATA* (L.) WILCZEK)

Ghafoor, A., M. Zubair, B.A. Malik & S.M. Iqbal

A total of 112 *Vigna radiata* genotypes selected from local and exotic germplasm over 3 years, were evaluated for agronomic characters and incidence of mung bean yellow mosaic bigeminivirus (YMV) and [urd bean] leaf crinkle virus (UBLCV) under natural infection conditions. Twenty eight genotypes were superior on the basis of yield potential and resistance to YMV and UBLCV. A high yielding line (NCM 201) was resistant to both viruses. A strong correlation was observed between yield and pods/plant and biological yield/plant. It is suggested that large scale testing of selected lines and their utilization in breeding programs will be of great value in *V. radiata* improvement.

Pak. J. Bot. 24(1): 112-118. 1992.

BIOLOGY AND CONTROL OF MUNGBEAN YELLOW MOSAIC VIRUS DISEASE - A REVIEW

Ayub, M.A. & M.B. Ilyas

Mungbean (*Vigna radiata* L.) is an important pulse crop of Pakistan. The crop may be vulnerable to a number of fungal, bacterial and viral diseases, but the most disastrous disease is the mungbean yellow mosaic virus disease. This paper reviews the impact of the disease on the host plant, its biology and possible control measures.

Proc. Nat. Sem. on the Role of Plant Health and Care in Agri. Prod., Dec. 28-29, 1988, Dept. Pl. Path., Univ. Agric., Faisalabad, pp. 123-129.

STUDIES ON THE SCREENING OF MUNGBEAN GERMPLASM AND GROWTH RESPONSES OF MUNGBEAN PLANT TO MUNGBEAN YELLOW MOSAIC VIRUS INFECTION

Ayyub, A.

Two disease screening nurseries were planted in the field areas of the Department of Plant Pathology, University of Agriculture, Faisalabad for screening of mungbean germplasm for the sources of resistance against mung bean yellow mosaic virus disease. Of the two disease screening nurseries, one included 32 test lines and the other included 91 entries of mungbean. None of the cultivars evaluated was found to be immune or highly resistant to MYMV disease. However, in nursery No. 1, six cultivars (NCM-68, V-6601, M-13-1, NCM-5, NCM-69 and E-321) were found to be moderately resistant or tolerant. The remaining cultivars were moderately to fairly susceptible. Of the 91 test entries evaluated in disease screening nursery No. 2, seven cultivars found to resistant, 32 were moderately resistant, thirty seven were tolerant and fifteen were susceptible to MBYMV disease.

Studies on growth responses of fourteen mung-bean cultivars to MBYMV infection revealed, that the virus may affect both vegetative components (plant height, number of branches, number of leaves dry stem weight) and yield component (number of pods, pod size, number of seeds and seed weight) of the mungbean plant. The degree of effect, however varied with the cultivars and probably depended on the genetic make of the affected cultivars. Thus various mungbean cultivars suffered form 16.25 to 46.95% decrease in plant height, 28.35 to 67.72% decrease in number of branches, 26.72 to 59.74% decrease in leaves/plant and 48.71 to 81.74% decrease in dry stem weight.

The affect of MBYMV on yield component also varied greatly and depended upon the cultivar infected. Various mungbean cultivars suffered 52.43 to 91.37% reduction in pod number, 15.77 to 24.74% reduction in pod size 18.07 to 56.55%

decrease in number of seed/plant and 43.13 to 80.17% decrease in plant yield.

M.Sc.(Hons.) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad, 1987, pp. 67-68.

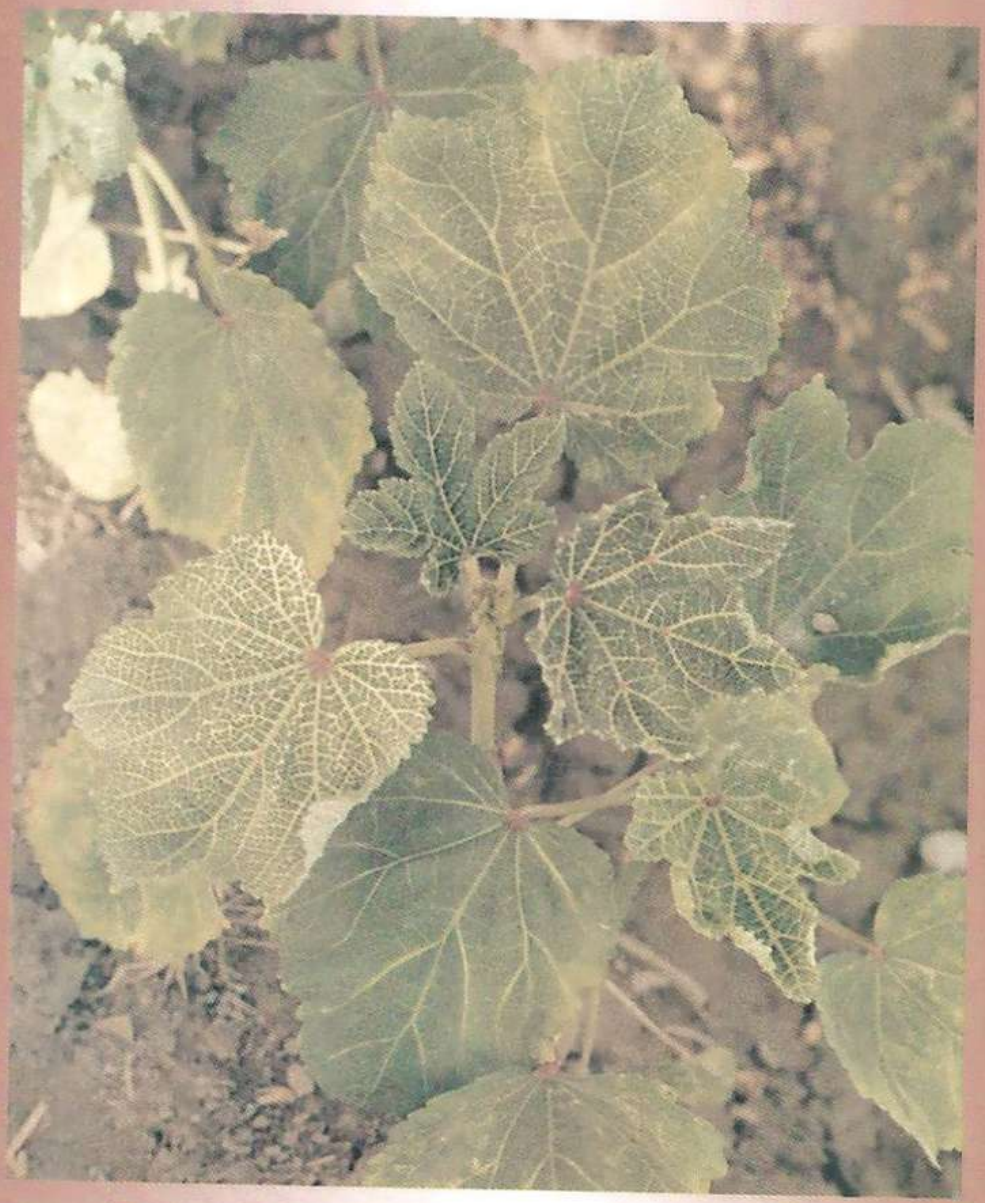
SUSCEPTIBILITY OF MUNGBEAN VARIETIES TO WHITEFLY (*BEMISIA TABACI* GENN.) AND YELLOW MOSAIC

Murtaza, M.A., M.A. Bhatti, & H.A. Qayyum.

Eighteen varieties of mung bean, M-13-1, M-19-19, M-20-21, M-94-73, M-121-25, M-131-37 and M-131-98 (NIAB mutants) RC-71-17, 6601, Pak-22, RC-71-27 (Department of Agriculture, Punjab) and AUM-233 (University of Agriculture, Faisalabad) and V-1381, V-2010, V-3092, VC-1560 D, VC-1973 and VC-2778 A (Exotic) were screened against whitefly and yellow mosaic disease. Varieties M-20-21, M-19-19, M-121-25, M-13-1 and M-131-37 were found more resistant to whitefly and yellow mosaic disease than other varieties. The yield of these varieties was also significantly higher than that of all other varieties. The correlation coefficients of yield and whitefly population (-0.8671): yield and intensity of yellow mosaic (-0.8542) were obtained.

Pak. Entomologist. 5(1-2): 51-56, 1983.

OKRA



OKRA

SCREENING OF DIFFERENT OKRA VARIETIES/LINES AGAINST VEIN YELLOWING MOSAIC VIRUS

Rehman, A. & W. Ahmed

Twenty six Okra varieties/lines were screened against vein yellowing mosaic virus under the natural conditions in a completes randomized block design (CRBD) at Ayub Agricultural Research Institute, Faisalabad during the year, 1994-95. Both the percentage of the disease incidence and severity of the disease were recorded. For recording severity of the disease, 0-5 scale was evolved. Out of these 26 tested varieties/lines, eleven (11) varieties/lines i.e N.E, PK (Parent), PK x CHR, GV x DLPG, CHR, CHR x DLPG, CHR x PK, PK x PG, PK x Amred, Green Emerland and chines Red showed complete resistance against vein yellowing mosaic virus. Where as three varieties/lines i.e. DLPG, PG and PG x PK showed highly susceptible reaction to this viral disease. However, twelve varieties i.e. PK x DLPG, GV x PK, GV x CHR, CHR x GV, DLPG x PK, DLPG x CHR, CHR and PK x GV, G. Velvet, DLPG x GV, P. Spineless, No.8 CS (Asgrow) showed moderately resistant to moderately susceptible reaction, respectively.

1st Int. Conf. & Symp. of Pak. Phytopath. Society, Mar. 6-7, 1996. Univ. Agric., Faisalabad, Abst. p. 19

SCREENING OF DIFFERENT OKRA VARIETIES/LINES AGAINST OKRA LEAF CURL VIRUS

Rehman A. & W. Ahmed

Twenty six Okra varieties/lines were screened against Okra leaf curl virus under the natural conditions in a complete randomized block design (CRBD) at Ayub Agricultural Research Institute, Faisalabad during the year, 1994-95. Both the percentage of the disease incidence and severity of the disease were recorded, for recording severity of the disease, 0-5 scale was evolved. Out of these 26 tested varieties/lines, sixteen (16) varieties/lines i.e. N.E, PK (Parent), PK x CHR, PK x DLPG, DLPG x CHR, PK x

PG, PK x Amred, PG x PK, Green Emerland and Chines Red showed complete existence against Okra leaf curl virus. Where as six varieties/line i.e, Gv x DLPG, CHR x GV, DLPG x GV, P. Spineless, No.8 and CS (Asgrow) showed highly susceptible reaction to this viral disease. However four varieties i.e. CHR, PG and GV, DLPG showed moderately resistant to moderately susceptible reaction respectively.

1st Int. Conf. & Symp. of Pak. Phytopath. Society, Mar. 6-7, 1996. Univ. Agric., Faisalabad, Abst. p. 18

PAPAYA

PAPAYA

FIRST REPORT OF PAPAYA LEAF CURL DISEASE IN PAKISTAN

Nadeem, A.,
T. Mahmood,
M. Tahir,
S. Khalid &
Z. Xiong

Papaya plants with virus-disease-like symptoms were observed in back yards and commercial groves in Multan, Pakistan. Leaves of the diseased plants displayed downward curling and thickened, dark green veins. Leaf-like enations grew from the base of the diseased leaves. These symptoms are similar to those of cotton leaf curl disease. In addition, diseased papayas were stunted and distorted. Leaf extracts from 3 diseased and 2 healthy papayas were tested in enzyme-linked immunosorbent assay (ELISA) against antibodies to geminiviruses. SCRI-52 and SCRI-60, two monoclonal antibodies to indian cassava mosaic virus, reacted positively (more than 7 × healthy background) with the diseased samples but not with the healthy ones. Total nucleic acids from the papaya samples were used as templates in polymerase chain reaction with primers F500 and R1800, which are capable of amplifying a region of DNA A component of the whitefly-transmitted geminiviruses. A DNA fragment of approx. 1.4 kb was amplified from the nucleic acids of the diseased but not the healthy papayas. Under high stringency conditions, cloned DNA A fragments of both cotton leaf curl virus and cotton leaf crumple virus cross-hybridized with the amplified DNA fragment, but the hybridization signals were much weaker than those of the homologous hybridization. This is the first report of the papaya leaf curl disease in Pakistan. These data demonstrated that a geminivirus may be the causative agent of this papaya disease. We are currently determining the relationship between the geminivirus infecting papaya and cotton leaf curl virus.

Pl. Disease, 81(11): 1333, 1997.

NATURAL OCCURRENCE OF PAPAYA RINGSPOT VIRUS

Hameed., S., T. Gillani & S. Khalid

Papaya ringspot virus (PaRSV) has posed a serous threat to the papaya (*Carica papaya* L.) cultivation throughout the tropical and subtropical areas. After mechanical inoculation on indicator plants; (*C. quinoa* and *C. amaranticolor*) local lesions and the systemic symptoms like mottling malformation and distortion of leaves were observed on *C. papaya*. Our isolate did not infects *Nicotiana benthimiana* confirming the early reports. The DAC-ELISA carried out, showed strong reactions using antiserum obtained from USA. Electronmicroscopic studies of grids prepared by leaf dip method revealed flexious particles measuring 750 x 18 nm.

On the basis of serological reaction, host range, and particle morphology, the virus isolated from papaya plants was identified as papaya ring spot virus PaRSV

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, p. 166.

VIRUS INFECTION COMMON TO PAPAYA AND TOMATO PLANTS

Mahdi H.S.

A wide range of viral symptoms in papaya, designated as shredded disease of the papaya tree and in tomato curled leaf are described. Effect on plant growth and general appearance is also mentioned.

Pak. J. Sci. Indust. Res., 28(2): 134, 1985

PEA

INCIDENCE OF PEA SEED-BORNE MOSAIC VIRUS IN PAKISTAN

Mehmood, M.K., M. Bashir & Z. Ahmed

Farmers' pea fields in three districts of Punjab (Lahore, Kasur and Rawalpindi) and one of NWFP (Haripur Hazara) and experimental plots at four research stations were surveyed during winter season of 1995. A total of 984 samples were collected at random from farmer's fields and tested against polyclonal antiserum to pea seed-borne mosaic virus (PSbMV) by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA). Based on ELISA results and field observations, it was concluded that PSbMV was not present in farmers' fields, but was present at four experimental stations.

Pak. J. Phytopath. 9(2): 152-155, 1997.

DETECTION AND IDENTIFICATION OF PEA SEED-BORNE MOSAIC VIRUS FROM SEEDS OF *PISUM SATIVUM* L.

Mehmood, M.K., M. Bashir & A. Ayub

Of 38 cultivars/lines tested by grow-out test, 50 % found infected with pea seed-borne mosaic virus (PSbMV). The natural seed-transmission rate was determined to be 2.8-25.8 %. When the seed extracts of the same 38 seed-lots of pea cultivars/lines were tested directly by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA), 60 % seed-lots were found contaminated with PSbMV, and the rate of seed-transmission varied from 2-15 %.

Pak. J. Phytopath., 8 (2): 127-131, 1996.

DETECTION AND IDENTIFICATION OF PEA SEED-BORNE MOSAIC VIRUS FROM SEED LOTS OF PEA (*PISUM SATIVUM* L.) AND SCREENING FOR RESISTANCE

Pea (*Pisum sativum* L.) is grown all over the world. It is an important source of vegetable protein in human diet and is consumed in several different forms. In nature the crop is vulnerable to a large number of economically important diseases, of which the diseases caused by viruses are considered more important. More than 20 viruses are reported to naturally infect peas, but among the economically important viruses, pea

seed-borne mosaic virus (PSbMV) has recently gained significant importance due to its world-wide occurrence and heavy economic losses.

In Pakistan PSbMV was first reported in 1993, naturally infecting pea plants at experimental stations. Therefore, keeping in view the importance of PSbMV and potential threat to pea crop in Pakistan, this study was conducted with the objectives to detect PSbMV from seed of different genotypes from various sources, screening of pea genotypes for- resistance, survey of commercial fields and experimental stations to know the occurrence and distribution of PSbMV and to establish rapid detection techniques for screening programmes.

Thirty eight pea genotypes obtained form different sources were evaluated to detect and identify PSbMV by grow-out test or direct antigen enzyme-linked immunosorbent assay (DAC-ELISA) methods. The presence of virus in systemically infected pea plants was confirmed by serological specific electronmicroscopy (SSEM), when flexuous rod shaped PSbMV particles were observed under the electron-microscope. Of 38 genotypes tested by grow-out test 50% were found infected with PSbMV and the natural seed-transmission rate was determined to be 2.8% to 25.8%. Of 30 seed-lots when tested directly by DAC-ELISA, 60% seed lots were found contaminated with PSbMV at the rate of 2% to 15%.

Sixty five pea lines/cultivars were also screened against PSbMV by sap inoculation method under green house conditions. Of 65 genotypes tested, the following 12 were found immune to PSbMV; 90-19, 93-9, Bonneville, Dual, 90-12, PMR-80, 88P22-3-9, 88P022-6-29, 88P038-10-13, 89P117-5, 89P134-2 and 89P166-5. These

genotypes can be used in breeding program to develop PSbMV resistant cultivars with desirable agronomic attributes.

Based on survey results of some districts of Punjab (Lahore, Kasur and Rawalpindi) and NWFP (Haripur Hazara) and testing of 984 samples by ELISA, we concluded that PSbMV is not yet present at farmers field in these districts. However, we detected PSbMV in samples collected from experimental station such as National Agriculture Research Centre (NARC), Islamabad, Ayub Agricultural Research Institute (AARI), Faisalabad, Barani Agriculture Research Institute (BARI), Chakwal, and University of Agriculture (UA), Faisalabad. This suggested that the introduction of PSbMV in Pakistan has occurred through infected-seed from exotic sources.

DAC-ELISA test and SSEM are being recommend for large scale screening of pea cultivars for resistance and for certification programmes to avoid PSbMV introduction in commercial field and for future study on epidemiology of PSbMV. Recommendations have been made for local quarantine measures to check the further spread of PSbMV in the areas, where the virus is not yet present.

M.Sc.(Hons.) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad, 1995.

FIRST REPORT OF PEA SEED-BORNE MOSAIC VIRUS IN PAKISTAN

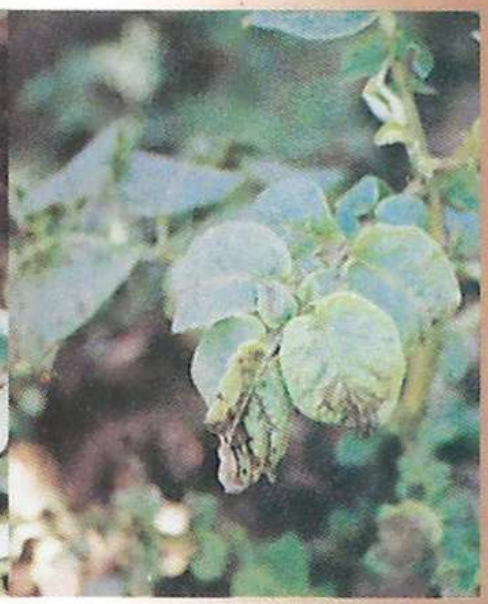
Bashir, M., S. Khalid, M. Asif & M. Banaras

During last week of January, 1993 it was observed that pea plants of the following pea varieties: Harzerin, Bordi, Gloria (from Germany), Metore, Green Feast and PF-400 (local varieties) planted at National Agricultural Research Centre, Islamabad, Pakistan, were showing virus-like symptoms. The infected plants were slightly stunted, with slight curling of

leaflets, mild yellowing and in some cases with vein chlorosis. Forty-two leaf samples taken form plants showing virus-like symptoms were tested by direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) against the following six antisera of pea viruses: pea seed-borne mosaic virus, pea mosaic virus, pea enation mosaic virus, pea early browning virus, pea leaf roll virus and bean common mosaic virus. Thirty-one (72%) samples out of forty three reacted positively against pea seed-borne mosaic virus (the source of antiserum of PSbMV was from Dr. R.O. Hampton whereas no reactions of the test samples were obtained with rest of the antisera tested. Mechanical sap-transmission of the virus was confirmed on pea plants in form of systemic infection and the virus produced local lesions on *Chenopodium quinoa* and *C. amaranticolor*. Elongated flexous rod-shaped virus particles were noticed by leaf-dip preparation under electronmicroscope. On the basis of test plant reaction, particle morphology, and serology (DAC-ELISA), this virus was identified as pea seed-borne mosaic (PSbMV). This is the first report of pea seed-borne mosaic virus naturally occurring in pea form Pakistan.

Int. Working Group on Legume Viruses, Jul. 25-27, 1993, Montreal, Canada, p. 25.

POTATO



POTATO

ROLE OF READ-THROUGH PROTEIN GENE OF POTATO MOP-TOP FUROVIRUS IN FUNGUS TRANSMISSION BY *SPONGOSPORA SUBTERRANEA* f.sp. SUBTERRANEA

Arif, M., B. Reavy & L. Torrance

Potato mop-top (PMTV), fungally-transmitted rod-shaped virus, contains three different species of single-stranded RNAs in its tripartite genome. Read-through protein gene is encoded on RNA 3 of the virus genome. To assess the role of the read-through protein gene of PMTV in fungus transmission, comparative transmission experiments were conducted by using a full-length read-through gene isolate (PMTV-S) and an isolate (PMTV-T) having 700 nucleotides deletion in 5'-half of read through gene domain of RNA 3. A mono-fungal *Songospora subterranea* f. sp. Subterranea was used which could not transmit PMTV-T isolate, while PMTV-S isolate was efficiently acquired and transmitted by the same aviruliferous fungal culture. The read-through gene encoded of RNA 3 thus played a vital role in acquisition and transmission PMTV by its fungal vector. Different hypothetical steps are proposed to understand the transmission mechanism of PMTV. This is the first direct report of its kind that PMTV read-through protein gene is involved in fungus transmission. Pp.51-52.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) pp. 51-52.

SOURCES OF POTATO SEED I N RELATION TO PRODUCTION AND POATO LEAF ROLL VIRUS IN PAKHAL PLAIN AND KAGHAN VALLEY OF HAZARA DIVISION

Hassan, A., H. Jan & A. Muhammad

Studies conducted during Autumn, 1992 and summer, 1993, identified that in Pakhal plain, farmers own, Lahore and Daska market were the three main seed sources of potato for autumn crop. Most of the farmers brought seed from Lahore. The seed from two cold stores at Lahore expressed 44 and 72%, farmers, own seed carried 60% and Daska seed contained 2% PLRV while the seed of one cold store at Lahore was free of virus. The yield difference did not reflect the different seed sources of

PLRV incidence because of variation in soil type and time of sowing due to rains. In upper Kaghan valley, farmers own years old seed, Punjab seed and Battakundi Farm seed appeared to be the three major seed sources. Majority of the farmers used their own seed. A high incidence of PLRV (48-72%) was observed in the farmers own and Punjab seed. Battakundi Farm seed had no PLRV and gave highest average yield of 27 t/ha as compared to other two sources (9-12t/ha).

Pak. J. Phytopath., 9(2): 148-151, 1997.

EFFECTIVENESS OF COAT PROTEIN-MEDIATED RESISTANCE TO FUNGUS TRANSMISSION OF POTATO MOP-TOP FUROVIRUS

Arif, M., B. Reavy & H. Barker

Coat protein-mediated resistance (CP-MR) to potato mop-top furovirus was created by transforming *Nicotiana benthamiana*, a test species for PMTV with sequences encoding the coat protein (CP) gene of the virus. The transgenic plants of *N. benthamiana* expressing the PMTV gene were challenged with two prevalent isolates of PMTV (isolate T and isolate S) by mechanical, graft and fungal inoculation. CP-MR against PMTV is extremely effective in *N. benthamiana* and three of transgenic lines were immune to the infection following mechanical, graft and to natural fungal vector. This type of resistance on potato crop will be very valuable because there is no other sources of resistance or tolerance to PMTV.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agri. Univ., Peshawar, (Abst.) p. 34.

DISEASES OF POTATO IN MIANDAM VALLEY, SWAT, PAKISTAN

Ahmad, I., M.H. Soomro,

The Miandam valley falls in potato production zone 5 having very small terraced fields. Farmers are mainly dependent on potato for their livelihood. Understanding of disease problems is vital for obtaining higher yields. Although surveys for potato diseases in hilly

S. Iftikhar, A. Munir, S. Khalid & H. Jan

areas including Swat district have been done in the past, the Miandam area was not surveyed. In 1993 a systematic survey was done by Crop Diseases Research Institute with the support of Pak-Swiss Potato Development Program, PARC. A total of twenty fields belonging to 20 different farmers were examined, diseases assessed using standard rating scales and samples taken for laboratory analysis, where necessary. Among the bacterial and fungal diseases, common scab, black scurf, late blight, early blight, and *Verticillium* wilt were the major diseases, while ring rot, *sclerotinia* rot and black leg were emerging problems. In case of virus diseases, data based on visual observations in the field did not fit well with the results based on ELISA. Visually, PLRV appeared to be the most prevalent virus followed by PVX, whereas no characteristic symptoms of PVS and PVY were observed in the field. However, ELISA analysis showed that PVX was the most prevalent virus followed by PVS, PVY and PLRV being equally prevalent but in low frequency. A number of plant parasitic nematodes were isolated from soil samples. Among these, only *Ditylenchus* and *Pratylenchus* species are important for potato. Potato cyst nematode *(Globodera* sp), the most important and destructive nematode was not found in Miandam.

Crop Prot. Conf., Apr. 20-22l, 1996, NWFP Agric. Univ., Peshawar, (Abst.) p.33.

SOME BIOLOGICAL PHYSICAL AND SEROLOGICAL PROPERTIES OF POTATO LEAF ROLL VIRUS (PLRV) IN PAKISTAN

Mean incidence of potato leaf roll virus (PLRV) in the plains of Pakistan was recorded as 44% in general, 53% in the market or uncertified seed and 11% in certified or improved seed. The virus was successfully transmitted by side cleft grafting of infected potato to *Datura stramonium* and *Physalis floridana* which proved useful hosts for the maintenance and propagation of the virus.

Arif, M., S.M. Mughal, S. Khalid & S. Hassan

The virus was purified through extraction in phosphate or citrate buffer and precipitation with polyethylene glycol followed by 2-3 cycles of differential centrifugation. The average yield of virus was 0.265 mg per kg of source tissue. The virus sedimented as single component in sucrose density gradients, reacted positively against potato leaf roll virus (PLRV) antiserum in gel immunodiffusion tests and absorbed UV light with a 260-280 ratio of 1.7. Isometric particles of 25 nm in diameter were observed under electron-microscope. The virus identity was also confirmed by enzyme-linked immunosorbent assay (ELISA) and serological specific electron microscopy (SSEM).

Pak. J. Bot., 27(1): 233-241, 1995.

SCREENING OF POTATO GERMPLASM FOR RESISTANCE AGAINST MAJOR POTATO VIRUSES UNDER FIELD CONDITIONS

Ahmed, M. & W. Ahmed

One hundred and forty eight potato clones/ germplasm were screened at Murray and Faisalabad for the detection of potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX) by using enzyme-linked immunosorbent assay (ELISA). The potato leaf samples collected from Murree were found infected with PLRV at the rate of 3.7% in the material produced through local crosses, 28.56% in the imported material other than International Potato Centre (CIP) material. CIP material and commercial varieties were found free from PLRV. PVY was present at the rate of 28.56% in CIP material, 14.28% in the imported material other than CIP. Material produced through local crosses and commercial varieties were found free from PVY. PVX infection was detected in CIP material (64.26%), imported material other than CIP (14.28%), local crosses (18.50%) but all the commercial varieties were found free from this virus.

Similar studies were conducted at Faisalabad

during autumn season 1994. CIP material, local crosses and commercial varieties were found free from PLRV. However, PLRV was detected at the rate of 3.84% from imported material other than CIP. PVY was detected in CIP material (29.87%), local crosses (40%), commercial varieties (40%) and imported material other than CIP (15.38%). PVX was detected in 18.18% material from CIP, 50% in local crosses, 20% in commercial varieties and 11.53% in imported material other than CIP.

Pak. J. Phytopath. 7(2): 177-183, 1995.

RECENT DISTRIBUTIONAL TRENDS OF POTATO DISEASES IN PAKISTAN

Ahmad, I., M.H. Soomro, S. Khalid, S. Iftikhar, A. Munir & K. Burney

Extensive surveys of potato diseases in different potato growing areas of Pakistan indicated that fourteen diseases are of common occurrence. Soil and seed borne diseases are most widespread and are likely to become major threat to potato production in Pakistan. Among these stem canker and black scurf, *Verticillium* wilt, *Fusarium* wilt, common scab, and powdery scab (cause controversial) are major diseases and present in almost all production zones. Among the foliar diseases late blight is increasing in its distribution. The disease has been found in areas where macro climate is generally unsuitable for its development such as valleys of Baluchistan and Scared. Bacterial wilt and potato cyst nematode are increasing in distribution in their specific areas of occurrence. Among virus diseases, potato leaf roll virus remains the major disease. However, virus S and A are increasing. Mop top virus has been also found in localized areas. A new disease with symptoms of mycoplasma is spreading fast and has become serious in areas of Punjab.

Nat. Sem. Res. & Dev. Potato Prod. in Pakistan, Apr. 23-25, 1995, PSPDP PARC, Islamabad, pp. 117-125

PRODUCTION OF DISEASE FREE SEED POTATO, PRESENT STATUS AND FUTURE AREAS OF RESEARCH AND DEVELOPMENT IN PAKISTAN

Ahmad, S.I. & A.R. Bhutta

At practical application of disease free seed potato technology developed by Federal Seed Certification Department has been presented. A total of 38328 potato leaf samples were tested during 1985-86 to 1993-94 against five viruses i.e. PLRV, PVX, PVY, PVS, PVA, using "ELISA technique" on commercial scale. Percentages infection of leaf samples came down for 23.90 to 9.43. The impact of work along with present status and suggestions for further research and development has also been given.

Nat. Sem. Res. & Dev. Potato Prod. in Pakistan, Apr. 23-25, 1995, PSPDP/PARC, Islamabad, pp. 78-86.

MYCOPLASMA RESEARCH STUDIES

Shafiq, M., M.H. Chaudhry & M.S. Chaudhry

A trial comprising of four potato varieties viz; Diamont, Patrones, Cardinal and Desiree with three treatment of each variety was designed to study the effect of mycoplasma infection on different yield factors. The crop raised with the infected seed and apparently healthy seed yielded 48% and 27% less tubers yield as compared to the healthy seed respectively. Mycoplasma infection also affected adversely the emergence. Infected seed gave 73% emergence while apparently healthy seed gave 77% emergence as compared to healthy seed which recorded 83% emergence. The infected seed reproduced the infection upto 98% as compared to the healthy seed which got only 1.25% mycoplasma infection while apparently healthy seed gave 20% infection. The mycoplasma also reduced the size and number of tubers/plant in case of Diamont and Patrones while small sized tubers increased in case of Cardinal and Desiree.

Nat. Sem. Res. & Dev. Potato Prod. in Pakistan, Apr. 23-25, 1995, PSPDP PARC, Islamabad, pp. 162-168.

INCIDENCE OF MYCOPLASMA INFECTION OF POTATO IN PUNJAB

Shafiq, M., M. Ahmed, M.S. Chaudhry & M.H. Chaudhry

A survey of Mycoplasma infection of potato crop was conducted during autumn 1994-95. A total number of two hundred fields of different potato growing localities of the Punjab i.e. Okara, Sahiwal, Jhang, T.T. Singh, Chiniot, Gujranwala and Sialkot were surveyed to find out the mycoplasma infection. Two types of infections were recorded. Type A (long and thin stems, having swollen nodes, lush green foliage with slight effect of late blight and frost, numerous number of tubers with deep eyes), was recorded maximum in Sahiwal area ranging from 0-80% with an average infection of 8.4% followed by Gujranwala 6.4%, Sialkot 6.0%, Chiniot 5.0%, T.T. Singh 3.2%, Okara 1.5% and Jhang 0.4%. On the other hand type B (stunted plant growth with purple discoloration of leaves with numerous small sized tubers) was recorded maximum in Okara ranging from 0-95% with an average infection of 21.3% closely followed by Sahiwal with an average of 9.5%. It was 5.9% in Depalpur, 2.1% in T.T. Singh and 0.2% in Chiniot area. No infection of type B was recorded in Jhang, Gujranwala and Sialkot areas.

Nat. Sem. Res. & Dev. Potato Prod. in Pakistan, Apr. 23-25, 1995, PSPDP/PARC, Islamabad, pp. 317-323.

INCIDENCE AND DIRTRIBUTION OF POTATO VIRUSES IN THE UPPER KAGHAN VALLEY OF PAKISTAN

Jan, H. & S.B. Khan

Potato leaf roll virus (PLRV), potato virus S (PVS), potato virus M (PVM), potato virus Y (PVY) and potato virus X (PVX) were found infecting commercially grown potatoes in the upper Kaghan valley of Pakistan. The virus incidence varied widely among the twelve location surveyed i.e. Sharan, Puldran, Naran, Soch, Agli-Battakundi, Seed Farm Battakundi, Maidan, Dranda, Khora, Dunga, Shellibela and Lalazar. The viruses of PLRV, PVS and PVM were widespread while PVY existed at Puldran,

Agli-Battakundi, Maidan and Dranda. PVX was less common in the area and was found infecting potatoes at Agli-Battakundi, Naran, Soch and Miadan. The virus diseases were present in all the commercial potato varieties grown in the area i.e. Ultimus, Cardinal and Desiree. The high incidence of PLRV, PVS and PVM in potatoes in the valley may be attributed to infections derived from the seed brought by the farmers from outside places.

Pak. J. Phytopath., 7(1): 13-16, 1995.

DETECTION OF MAJOR POTATO VIRUSES FROM DIFFERENT POTATO GROWING LOCALITIES OF PUNJAB

Ahmed, M. & W. Ahmad

Different potato growing areas of Punjab viz; Jhang, Toba Tek Singh, Chiniot, Okara, Sahiwal and Gujranwala were surveyed during the autumn season of 1994 for the detection of major potato viruses i.e. potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX) by the use of enzyme-linked immunosorbent assay (ELISA). In total 169 fields from seven different localities were visited and 1227 samples were collected which were assayed for the presence of PLRV, PVY and PVX. All three tested viruses were detected from all localities surveyed in the Punjab but in different percentage. Percentage of PLRV detected was 12.82 (Jhang), 11.34 (Sialkot), 5.55 (Toba Tek Singh), 5.00 (Gujranwala), 4.41 (Chiniot), 3.63 (Okara) and 3.03 (Sahiwal). PVY was detected in the percentage of 52.77 (Toba Tek Singh), 28.20 (Jhang), 27.83 (Sialkot), 18.72 (Chiniot), 14.37 (Gujranwala), 12.72 (Okara) and 6.81 (Sahiwal). PVX was also detected in the percentage of 20.62 (Gujranwala), 17.94 (Jhang), 16.13 (Okara), 12.87 (Sahiwal), 12.12 (Chiniot), 9.79 (Sialkot) and 2.77 (Toba Tek Singh).

Nat. Sem. Res. & Dev. Potato Prod. in Pakistan, Apr. 23-25, 1995, PSPDP/PARC, Islamabad, pp. 175-179.

STUDIES ON THE SCREENING OF POTATO GERMPLASM AND ITS RESPONSE TO POTATO VIRUS Y

Hussain, F.

Sixteen potato cultivars were planted in the field area of the Department of plant Pathology, University of Agriculture, Faisalabad, to visualize the transmission of potato virus Y and the screening of different potato cultivars. For the transmission of virus, some sucking insects, viz, white fly and aphids were used. Transmission by mechanical inoculation, by grafting and by dodder plants was also tried. The maximum disease was found to be transmitted by aphids which was 73.33% while the transmission by mechanical inoculation by grafting and by dodder plants was 66.66, 60 and 20% respectively. The percent plant infection in different cultivars varied with the cultivar sown. The extent of virus Y infection varied from 2.5 to 67.5%. No cultivar remain 100% free from potato virus Y infection. The most affected cultivars, in descending order were Altamush, Petranus, Clone No. 9 and Diamont which exhibited 67.5, 65.0, 52.5 and 47.5% plant infection respectively. Seven cultivars exhibited intermediate percent plant infection and these displayed 25 to 37.5%. The least affected cultivars were 8222-5, No.70B, N0.70A, DHL_2 and FB 9469-9 and they exhibited 20, 15, 12.5, 10 and 2.5% plant infection, respectively.

Studies on growth responses of sixteen potato cultivars to potato virus Y infection revealed that the virus may affect both vegetative components (number of leaves per plant, height of plants, and dry plants weight) and yield components (number of tubers, total weight of tubers and average size of tubers per plant) of potato plant. The degree of effect, however varied with the cultivars and probably depended on genetic make up of the cultivars affected. Thus various potato cultivars suffered from 81.565 to 98.08% decrease in number of leaves. Various cultivars suffered from 31.99 to 88.32% decrease in plant height and 47.07 to 95.58% decrease in dry plant weight.

The affect of PVY on yield components also varied greatly and depended upon the cultivars infected various potato cultivars suffered from 11.90 to 82.61% reduction in number of tubers, 64.23 to 92.53% decrease in total weight of tubers per plant, and 27 to 84.92% decrease in average size of tubers per plant.

MSc.(Hons.) Thesis. Dept. Pl. Path., Univ. Agric., Faisalabad, 1994.

OCCURRENCE AND DISTRIBUTION OF POTATO VIRUSES IN THE UPPER KAGHAN VALLEY OF PAKISTAN

Jan, H., S.B. Khan & A. Mohammad

Potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS) and potato virus M (PVM) were found infecting commercially grown potatoes in the upper Kaghan valley of Pakistan. Composition of virus epidemics varied widely among the ten locations i.e. Naran, Douni, Bun, Agli-Battakundi, Banglan, Seed Farm Battakundi, Maidan, Dranda, Khora and Lalazar. The distribution of PVS and PVY was widespread while PLRV existed at Naran, Douni, Bun and Agli-Batakundi only. PVX was far less common in the area while PVM was present only at Naran. The virus diseases were present in all the commercial potato varieties grown by farmers. The high incidence of PVS, PVY and PLRV in potatoes in the valley may be attributed to infections derived from the seed brought by farmers from outside places.

Sarhad J. Agric., 10(6): 691-696, 1994.

PURIFICATION AND SERODIAGNOSIS OF POTATO LEAFROLL VIRUS IN PAKISTAN

Arif, M., S. Khalid & S. Hassan

Potato leaf roll virus (PLRV) was purified from infected *Datura stramonium* L. and *Physalis floridana* Rydb. with a yield of 0.405 mg/kg tissue. The virus was transmitted by cleft grafting from infected potato to *D. stramonium* and *P. floridana*. It was purified through extraction in 0.1 M citrate buffer, pH 6.0 containing 0.01% disodium ethylene-diamine-tetra-acetate (EDTA), 0.3% sodium diethyl-dithiocarbamate (Na-

DIECA), 2.5% (v/v) 2-mercaptoethanol, clarified by partitioning in chloroform: butanol mixture and precipitated with polyethylene glycol (M.wt. 8,000) followed by 2-3 cycles of differential centrifugation. The virus sedimented as a single component in sucrose density gradients and absorbed UV-light with 260/280 ratio of 1.78. Isometric particles 24.5nm in diameter were observed in negatively stained preparation with 2% potassium phosphotungstic acid (PTA), pH 6.8, under the electronmicroscope. Enzyme-linked immunosorbent assay (ELISA) and serologically specific electronmicroscopy (SSEM) were compared for PLRV- detection. SSEM was more sensitive than ELISA.

Sarhad J. Agric., 8(2): 209-215, 1992.

PESTS AND DISEASES OF AUTUMN POTATOES IN NORTHERN BUNER AND MALAKAND AGENCY

Defoer, T. & S.S. Hussain

The occurrence of aphids, potato leaf roll luteovirus (PLRV) and early and late blight [Alternaria solani and Phytophthora infestans, respectively] on potatoes in demonstration plots in Northern Buner and Malakand Agency, Pakistan, during 1989, was investigated. Aphids occurred in 48% of the plots, and fields infested by aphids were also affected by PLRV. Both were predominant in Northern Buner. Symptoms of early and late blight appeared simultaneously in more than half of the fields in Northern Buner and Malakand Agency, with the degree of infection greater in the former region. Early blight was less severe than late blight. It is concluded that aphids are the main vector of PLRV and that aphid control will also reduce the incidence of this virus, while the main factors influencing the occurrence of late blight were the presence of Solanaceae in nearby fields, and the continuous planting of potatoes. Disease control is considered.

Sarhad J. Agric., 8(1)): 87-94, 1992.

PERFORMANCE OF SEED POTATOES PRODUCED IN THE HIGH AND MID HILLS OF KAGHAN IN PAKISTAN

Chaudhri, J. A., S. M. A. Shah & A. Hussain

In field trials conducted in Islamabad, Pakistan, during the autumn and spring seasons of 1984-86, seed potatoes cv. Cardinal produced on high hills (2400 m alt.) and mid hills (1500 m alt.) were compared with imported (Netherlands) and market (unspecified) seed tubers. Averaged over the years, autumn cropping (Oct.-Jan.) resulted in 18.37, 17.66, 20.70 and 10.74 t tuber yield/ha with high hill, mid hill, imported and market seeds, respectively. Corresponding yields of crops grown during the spring seasons (Feb.-May) were 21.21, 20.13, 22.26 and 13.93 t/ha. Market seeds were heavily infected (on average 48 and 52% during autumn and spring seasons, respectively) with virus diseases, mainly potato virus Y [potato Y potyvirus] and leaf roll [potato leaf roll luteovirus]. Suitable potato rotations are discussed. Seed tubers produced at high altitudes may not be usable in autumn seasons due to tuber dormancy. Seed tubers produced in mid hills for autumn harvesting may avoid the problems associated with seed tuber multiplication in spring and would be available at lower cost than imported seed tubers.

Sarhad J. Agric., 8(1)): 29-32, *1992.*

STUDIES ON VIRAL DISEASES OF POTATO CROP IN NWFP

Mohammad, R.

Three major potato growing areas of the NWFP Pabbi, Bunair and Swabi were surveyed to determine the incidence of potato viruses in autumn crop. Potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS) and potato virus A (PVA) occurred predominantly and were identified on the basis of symptomatology, host range, serology and transmission properties. The mean incidence of PLRV, PVY, PVX, PVS and PVA was recorded as 4.29, 5.97, 13.26 , 36.19 and 0.47%, respectively. Enzyme-linked immuno-sorbent assay (ELISA) was standardized for routine identification of these viruses. Causal

viruses were readily transmitted mechanically except PLRV and symptoms were reproduced on different assay hosts. Best diagnostic hosts for PVY and PVX were *Nicotiana rustica, N. glutinosa* and *Chenopodium quinoa* whereas *N. tabacum* cv. White Burley and *N. tabacum* cv. Samsun were considered the best assay hosts for PVA and *N. rustica* was the best for PVS.

M.Sc.(Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1990.

PRODUCTION OF VIRUS FREE SEED POTATO THROUGH TISSUE CULTURE TECHNIQUES

Hassan, S., M.J. Turangzai & I. Khan

The Meristem tip of two potato varieties, Ultimus and Cardinal were excised under aseptic conditions and cultured on MS medium containing different concentrations and combinations of hormones for regenerating pathogen free plant from infected stocks. The best regeneration of meristem tip was obtained when MS medium was supplemented with either 1 or 2 mg/l pantothenic acid + 0.5 mg/l gibberellic acid. The plantlets developed were tested against various potato viruses.

Sarhad J. Agric., 6(4): 365-370, 1990.

IMPACT OF IMPROVED SEED SOURCES ON GROWTH, CHARACTERISTICS AND YIELD OF POTATO

Chaudhry, J.A., S.M. Mughal & N.A. Khan

Improved seed sources i.e. Swat and Kaghan were compared with the market seed on the farmers field. Improved seed sources were higher in tuber weight and soil coverage with low incidence of virus and produced higher yields. The results of nine different locations were uniform.

Sarhad J. Agric., 6(3): 249-253, 1990.

MAJOR DISEASES OF POTATO AND THEIR CONTROL

Ahmed, S.I. & A.R. Bhutta.

The article describes important bacterial, fungus and viral diseases of potato in Pakistan. Among the viral PVY, PVX and PLRV are the major viral diseases occurring in Pakistan.

Progressive Farming, 9(3): 20-25, 1989.

DEVELOPMENT OF ENZYME LINKED IMMUNOSORBENT ASSAY FOR POTATO VIRUS X IN PAKISTAN

Khalid, S.,
S.M. Mughal &
S. Hameed

Potato virus X (PVX) was purified in substantial quantities and used for the production of antiserum with a homologous titre of 1:256 in double immunodiffusion test. Immunoglobulins (IgG) were separated from the antiserum, conjugated with alkaline phosphatase and ELISA kit was prepared locally at a much cheaper cost (<50%) than the imported one. Appropriate conditions for specific reactions i.e. dilutions, incubation time and precipitation were determined. The results were highly comparable and large number of potato samples tested against PVX with great rapidity and sensitivity.

Pak. J. Bot., 21(2): 331-338, 1989.

DETECTION OF POTATO VIRUSES IN PAKISTAN

Mughal, S.M.,
S. Khalid,
T.S. Gillani &
A. Devaux

Biological and serological tests, particularly the enzyme-linked immunosorbent assay (ELISA) enabled the detection of 8 potato viruses from Pakistan viz; AMV, PVX, PVM and PMTV (in traces) PVX and PVA (2-15%), PVY (5-25%) and PLRV (15-65%). These viruses were also confirmed by infectivity assays, transmission and electronmicroscopy. A 100-fold increase in sensitivity was obtained for PVY and PLRV in ELISA and immuno electronmicroscopy (IEM) than other tests, as positive reactions were observed with low virus concentration (nano-gram/ml).

Proc. 2nd Triennial Conf., Jun. 12-26, 1988, Kuming, China, pp. 189-190.

CHARACTERIZATION OF POTATO LEAFROLL VIRUS (PLRV)

Arif, M.

Potato leaf roll virus is a widely distributed and serious disease of potato in Pakistan. The disease incidence in field surveys ranged between 8.93 to 53.73% and its intensity was higher in areas where uncertified seed was planted. Virus was successfully transmitted through side cleft graft-ing from infected potato to *Datura stramonium* L.

and *Physalis floridana* Rydb., the latter appeared to be suitable indicator and propagative host for PLRV. The virus was purified by extraction in citrate buffer, precipitation with polyethylene glycol and two to three cycles of differential centrifugation, which gave a virus yield of 0.527 mg/kg of fresh infected tissue. Electron-microscopic examination of purified virus preparation revealed the presence of spherical particles with a mean diameter of 25 nm. Virus was serologically identified by enzyme-linked immunosorbent assay (ELISA) and serologically specific electronmicroscopy (SSEM), the latter technique being more sensitive. The concentration of virus was greater in heel than rose-ends of infected tubers.

M.Sc.(Hons.) Thesis, Dept. Plant Path., NWFP Agric. Univ. Peshawar, 1988.

THERMOTHERAPY AND MERISTEM TIP CULTURE OF *SOLANUM TUBEROSUM* FOR ELIMINATION OF POTATO VIRUSES X, S AND Y

Sajid, G.M., A. Quraishi & M. Salim

Meristem tips of 0.4 mm excised form *Solanum tuberosum* cultivars cardinal and desiree having undergone thermotherapy at 34°C for 11-60 days regenerated into rooted plantlets. The effect of duration of thermotherapy on regeneration potential of meristems was insignificant ($P > 0.95$). Thermotherapy for 60 days in cv. Cardinal resulted in 8-35 and 46% elimination of potato viruses S, X and Y respectively, and 9, 48 and 59% from cv. Desiree.

Pak. J. Bot., 18(2): 249-253, 1986.

VIRUS DISEASES IN RELATION TO POTATO PRODUCTION IN PAKISTAN

Mughul, S.M. & S. Khalid

At least six virus diseases (AMV, PVX, PVS, PVA, and PLRV) are usually prevalent in potato growing areas of Pakistan. These have been partially characterized through studies on host range, symptomatology, serology as well as morphological and physico-chemical properties of the particles. Of present six viruses, three i.e. PVX, PVY and PLRV are the most important and cause yield reduction and degeneration of seed

potato by 10-70% and 30-70%, respectively. The most damaging potato viruses are transmitted by aphids, mainly *Myzus persicae*. Some of the important aspects of viral diseases, extent of yield losses, degeneration of seed potatoes population dynamics of the aphids and possible control measures have been discussed in this paper.

Nat. Seminar on Potato in Pak: The Present Situation of Res. Prod. and Marketing Program, Apr. 2-4, 1985, PARC, Islamabad, pp. 154-165.

VIRUS DISEASES OF POTATO IN THE PUNJAB, PAKISTAN

Anwar, M.S. & M.S. Mirza

Surveys were made to record the virus diseases of potatoes in the Punjab. Suspected diseased samples were collected and identified by visual observations, sap inoculations on indicator plants and serological means. The most important diseases recorded were potato leaf roll virus, potato virus X, potato virus Y and potato virus A. Potato virus S and calico virus were also detected in varieties Norland and Red Bed, respectively.

J. Agric. Res. 22(1): 85-87, 1984.

MYZUS PERSICAE FLIGHTS AND SEED POTATO PRODUCTION IN PAKISTAN

Mirza, M.S., M. Ahmed & S.M.I. Wasim

Attempts were made by trapping winged *M. persicae* with Moericke water traps at number of location in Punjab Province of Pakistan to find out location or pin point season suitable for seed potato production. Observations for 4 years (1977-78, 1979-90 & 1980-81) indicated that *M. persicae* started appearing in traps in the last week of October in 1977, second week of January in 1978, second week of December in 1979 and first week of January in 1981. There was no aphid during July to September, flights during October to December, the period which corresponds with Autumn crop in Punjab, were very low and provide favorable condition for seed production. Possibilities of seed production in Pakistan are discussed.

J. Agric. Res., 20(4): 191-200, 1982.

THE ROLE OF APHIDS IN SPREADING POTATO VIRUS DISEASES IN THE PLAINS OF PAKISTAN

Mirza, M.S.

The province of Punjab constitutes a major potato growing region of Pakistan with an annual area of 40,000 acres and production of 200,000 tonnes. The average yield is 5 tonnes per acre which is very low. One of the major causes for the low yield is the prevalence of a number of potato virus diseases. The aphid-borne viruses, potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus A (PVA) have been isolated from potatoes in Punjab. Very little is known about the aphids infesting potato in Pakistan. Study to ascertain role of aphids in spread of potato viruses and to study aphid flight has been initiated.

Potato. Res. Pak., PARC, Islamabad, pp. 29-32, 1978.

EFFECT OF ANTIVIRAL CHEMICALS ON PORDUCTION OF VIRUS X FREE POTATO TUBERS

Vasti, S. M.

Growing tips, nodal segments with rudimentary buds and sprout discs excised from PVX infected tubers and seedlings were grown in modified and enriched medium. Thiouracil and Malachite green were used as antiviral chemicals in different concentrations. Thiouracil was found to be more effective and better than malachite green for antiviral properties. Chemicals either arrested virus multiplication or rendered it inactive. A combination of the antiviral chemicals incorporated in medium did not result in synergistic action as presumed. Its was further observed that potato extract did not support root initiation or development, as claimed by others.

Pak. J. Bot., 5(2): 139-142, 1973.

SOYBEAN

SCREENING OF SOYBEAN GERMPLASM AGAINST SOYBEAN MOSAIC VIRUS

Asad Ali

Soybean mosaic is destructive viral disease of soybean, caused by soybean mosaic potyvirus (SbMV). Field infected plants were characteristically stunted, showing predominant mosaic and mottling symptoms on leaves. Virus was readily transmissible through infected sap with 0.01M potassium phosphate buffer (pH 7.0) when inoculated experimentally. Among the twelve varieties and five soybean lines tested, seed transmission was observed in six soybean varieties/lines in an experiment carried out in screenhouse. The percentage of seed transmission of the virus in soybean germplasm was ranged between 22% and 47.8%, with an average of 31.5% in selected soybean varieties/lines studied. The natural resistance of soybean germplasm was evaluated against soybean mosaic potyvirus-Peshawar valley isolate (SbMV-P) and soybean mosaic potyvirus-Swat valley isolates (SbMV-S). Among twelve soybean varieties and five lines tested against both virus isolates, only one soybean variety i.e 80-B-4007 remained symptomless and did not produce productive virus after repeated back inoculation on young seedling of diagnostic plant species. Soybean varieties i.e. Weber-84, Winchester and Lugan produced rapid severe characteristic virus symptoms and evaluated as most susceptible against both virus isolates whereas Ags-297 and Wahab-93 developed slow virus symptoms. Weber-84 produced highest virus infection (74%), while Wahab-93 showed minimum virus infection (30%) against SbMV-P and SbMV-S in experiment carried out in glasshouse. Percent infection in soybean germplasm and severity of symptoms indicated that SbMV-S is more virulent than SbMV-P.

M.Sc. (Hons.) Thesis, Dept. Pl. Path. NWFP Agric. Univ., Peshawar, 1997.

OCCURRENCE AND IDENTIFICATION OF SEED-BORNE VIRUSES ON SOYBEAN

Khaskheli, M.N.

Field surveys conducted to determine the incidence and occurrence of viral diseases in the soybean seeds in the fields at Tando jam, Tajpur and Badin and laboratory of Plant Pathology department, Sindh Agriculture University, Tando jam during 1997.

The results indicated that infection of virus ranged from 15 to 45% with an average of 28.75% from different soybean growing locations at Tando jam, Tajpur and Badin. Amongst viral isolates, a few corresponded to Soybean mosaic virus (SMV). The viruses produced local as well as systemic symptoms on different indicator plants. The viruses lost infectivity in vitro with the advancement of time at room temperature. Likewise SMV was infective upto 4 days however, three isolates lost infectivity after 3-days. SMV could tolerate pH 5 or pH 8. SMV was found to be the most stable virus which could be inactivated above 70°C. Furthermore, SMV lost infectivity at 10^{-6}, however most of the isolates were inactivated when diluted for 10^{-5}.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., Sindh Agric. Univ., Tando jam, 1997.

RESISTANCE TO YELLOW MOSAIC VIRUS DISEASE IN SOYBEAN

Mirza, M.S. & M. Aslam

Twenty soybean varieties were evaluated for their resistance to yellow mosaic virus transmitted through whitefly (*Bemisia tabaci* Genn.) under natural field conditions during autumn season at the National Agricultural Research Centre, Islamabad. Only, Ware variety was found resistant, 4 varieties, Bay, Desoto, Gail and Williams-82 were moderately resistant, 9 moderately susceptible and 6 susceptible. Resistant varieties expressed significant (P = 0.05) differences in disease severity from the susceptible varieties, Alamo

(19.04%) and V-I (21.55% plants infected).

2nd Int. Cong. Entomol. Sc., Mar. 19-21, 1996, NARC, Islamabad. (Abst. PM-9) pp. 47.

ECOLOGY AND EPIDEMIOLOGY OF SOYBEAN MOSAIC VIRUS IN THE NORTH WEST FRONTIER PROVINCE OF PAKISTAN

Asad Ali, S. Hassan & Akhtar Ali

During May to September 1989, the incidence of soybean mosaic potyvirus (SoyMV) in varieties of soybean from Mingora Swat, Peshawar and Taiwan was studied at 15 locations. Field infected plants were stunted, with shortened petioles and internodes; they showed predominantly mosaic and mottling symptoms on leaves. The incidence of the disease ranged between 7.74 and 72.14%, with an average of 34.4%. Aphids, weeds and alternative hosts did not play a significant role in the transfer of SoyMV due to adverse environmental conditions. Thirty commercial soybean varieties and 20 lines were evaluated for SoyMV transmission through seed. The average virus transmission was 35.13%, with the highest in line 00131-99-6 (95.23%) and the lowest in the variety Hack (12.5%).

Toward enhanced and sustainable agricultural productivity in the 2000's: breeding research and biotechnology.

Proc. SABRAO 7th Int. Cong. & WSAA Symp., Academia Sinica, Nankang, Taipei, Taiwan, Nov. 16-20, 1993. Special-Publication TDAIS, 1(35): 339-347, 1994.

ECOLOGY AND EPIDEMIOLOGY OF SOYBEAN MOSAIC VIRUS IN THE NORTH WEST FRONTIER PROVINCE OF PAKISTAN

Akhtar Ali & S. Hassan

Soybean mosaic is a destructive viral disease of soybean caused by soybean mosaic virus (SMV). Field infected plants were characteristically stunted, with shortened petioles and internodes, showing predominant mosaic and mottling symptom on leaves that turned downward at the margins. The incidence of the disease in NWFP ranged between 7.74-72.14% with an average of 34.40% at 15 different locations surveyed. Insect

vectors and alternate hosts did not play any significant role in the epidemiology of SMV due to adverse environmental conditions. However, SMV transmission through seed played a pivotal role in the epidemiology of SMV in the NWFP. Eighteen out of 30 varieties and 14 out of 20 soybean germplasm lines developed mild to severe mosaic under controlled conditions in screenhouse, revealing highly seed-born infection.

Pak. J. Phytopath., 5(1-2): 21-28,1993.

CONTROL OF SOYBEAN MOSAIC VIRUS THROUGH THERMOTHERAPY

Haque, G., S. Hassan, Akhtar Ali & M. Arif

Thermotherapy of soybean seeds for the control of virus was conducted by hot water and dry heat treatments. Hot water treatment caused a drastic reduction in the seed germination with complete failure of germination at 80°C. The virus was completely eliminated with hot water treatment at 70°C. Dry heat treatment caused reduction in the severity of infection and also adversely affected germination. At 80°C, germination was the lowest with a complete inactivation of the virus. Dry heat treatment was not as effective as the hot water treatment in the eradication of the virus but its effect on the seed germinability was less than the hot water treatment.

Sarhad J. Agric., 9(2): 177-180, 1993.

ASSESSMENT OF YIELD LOSSES IN SOYBEAN DUE TO SOYBEAN MOSAIC VIRUS

Haque, G., M. Arif, S. Hassan, Akhtar Ali & M. Khan

Soybean mosaic virus (SoyMV) infection caused reduction in the growth and yield of soybean plants. Plant height was reduced from 4.04 to 16.9%. Total number of pods per plant were reduced from 8.4 to 33.4%. Infected plants produced shortened and flattened pods with a reduction in pod length from 10.0 to 25.6%. Pods formed on the diseased plants had one or two grains and the reduction in the number of grains per pod was recorded from 6.4 to 15.2%. Loss in

weight of seeds from diseased plants ranged from 6.6 to 17.8%.

Sarhad. J. Agric. 9(3): 227-229, 1993.

SCREENING OF SOYBEAN GERMPLASM FOR THE SOURCES OF RESISTANCE AGAINST SOYBEAN YELLOW MOSAIC VIRUS (SYMV) DISEASE

Ilyas, M.B., A.K. Jaffar, K. Iftikhar & M.A. Ayub

None of the 41 soybean cultivars and test lines evaluated was immune from the disease in field trials under natural infection by the vector, *Bemisia tabaci*. Seven cultivars were resistant on the basis of disease incidence and severity rating while 16 were moderately resistant on one or both of these indicators and the rest were susceptible.

Pak. J. Phytopath. 4(1-2): 1-4, 1992.

BIOLOGICAL CHARACTERIZATION OF SOYBEAN MOSAIC VIRUS

Akhtar Ali & S. Hassan

SoyMV was readily transmissible mechanically by applying through sap of the infected plants to the indicator hosts with 0.01M phosphate buffer pH 7.00. The identity of the virus was confirmed through symptomatology, serology and by electronmicroscopy. SoyMV produced pin point chlorotic local lesions on indicator hosts i.e. *Phaseolus vulgaris* cvs *Bountiful, Top crop*, and *Vigna cinensis* in the family *Leguminosae* while characteristic mosaic pattern of alternate light and dark green patches of leaf tissues and mottling was developed on soybean varieties tested during host range studies. Out of 12 soybean varieties tested only Crowford, Chico, Zane, and 80-B-4007, were evaluated as resistant to SoyMV (Isolate-1), while among 6 exotic germplasm lines, not even a single possessed resistance to the virus.

Sarhad J. Agric., 8(5): 555-561, 1992.

EFFECT OF MUNGBEAN YELLOW MOSAIC VIRUS ON SOME GROWTH COMPONENTS AND

Mungbean yellow mosaic (MBYMV) is a major virus disease of soybean causing stunting of plants, yellowing of foliage and reduction in yield. Losses assessed in 18 cultivars of soybean

YIELD OF SOYBEAN CULTIVARS

Aftab, M., S.M. Mughal & M. Aslam

under disease-induced field conditions revealed, on average basis, a reduction of 14.6% in shoot length and 33% in dry weight of plants. Number of pods, grain production and yield were reduced by 21.5, 29.1, and 36.4%, respectively. These components in susceptible PSC-62, PSC-61, Lakota, PSC-58, Weber and Williams-82 were comparatively less affected.

Sarhad J. Agric., 7(2): 149-152, 1991.

ASSESSMENT OF YIELD LOSSES IN SOYBEAN DUE TO SOYBEAN MOASIC VIRUS AND ITS CONTROL THROUGH THERMOTHERAPY

Haq, G.

Soybean mosaic virus reduced the growth and yield of infected plants, plant height was reduced from 4.04 to 16.9%. Total number of pods per plant were reduced from 8.4 to 33.4%. Infected plants produced shortened and flattened pods with a reduction in pod length from 10.0 to 25.6 percent. Pods formed on the diseased plants had one or two grains instead of the normal three grains and the reduction in the number of grains per pod was from 6.4 to 15.2%. Loss in weight of seeds from diseased plants ranged from 6.6 to 17.8%. Thermotherapy of soybean seeds for the control of virus was accomplished by hot water and dry heat treatments. Hot water treated seeds resulted in a drastic reduction in seed germination with complete failure of germination at 80°C. The virus was completely eliminated at 70°C. Dry heat treatment caused reduction in seed germination and in severity of infection. At 80°C germination was the lowest with a complete inactivation of the virus. Dry heat treatment was not as efficient as the hot water treatment in the eradication of the virus but its effect on seed germinability was less than the hot water treatment.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agri. Univ., Peshawar, 1991.

RESPONSE OF SOYBEAN CULTIVARS TO SOYBEAN MOSAIC VIRUS INFECTION

Jafar, A.K.

A disease screening nursery was planted in the field area of the Department of Plant Pathology, University of Agriculture, Faisalabad for screening of soybean germplasm for the sources of resistance against soybean yellow mosaic virus disease. It included forty one test lines. None of the cultivars evaluated was found to be immune. Only S-39-99 was found to be highly resistant on the basis of severity rating but on the basis of disease incidence it was resistant. Cultivars AP 4321, CORSOY, AP-350, Eriton, Weber, AGS-138 were found resistant both on the basis of disease incidence and disease severity rating. Only Sep-10 was found to be moderately resistant on both the basis. Togyakong and Swat-84 were found moderately resistant of the basis of percent plant infection only while GC-60058, 12-6-6-1-18, Sang, Elgin, Amsoy, AGS-147, LAC 73-13-85, GC 60058-18-6-6-18, M-74-23, FS-85, Chic, Steele, PSC-56 and AGS-20 were found moderately resistant on the basis of severity rating. Remaining 17 cultivars were found moderately to fairly susceptible to soybean mosaic virus infection.

Studies on growth responses of twelve soybean cultivars to soybean mosaic virus infection revealed that the virus may affect some of the vegetative components like number of leaves and size of branches, but the effect on plant height, number of branches and dry stem weight was not significant as a whole. It may be due to the genetic make up of the individual cultivars and their time of infection. However, the decrease in number of leaves ranged from 14.58 to 39.2% while the decrease in size of branch varied from 17.37 to 56.39%.

The effect of SMV on yield components (number of pods, size of pod, seeds per pod, seeds per plant, 100 seed weight and plant yield) was found

to be significant and varied greatly depending upon the cultivar. Various soybean cultivars suffered from 22.45 to 50.24% reduction in pod numbers/ plant, 7.04 to 16.97% reduction in pod size 7.07 to 19.64% reduction in number of seeds/pod, 19.2 to 45.95% reduction in the number of seeds/plant, 6.44 to 18.54% reduction in 100 seed weight and 9.09 to 52.09 percent decrease in plant yield.

M.Sc. Thesis, Dept. Pl. Path., Univ. Agri., Faisalabad, 1990.

FIELD EVALUATION OF SOYBEAN GERMPLASM FOR RESISTANCE TO YELLOW MOSAIC VIRUS

Aftab, M., S.M. Mughal, M. Aslam & M.A. Ayub

Screening of 140 soybean lines and cultivars under disease induced field conditions revealed great genetic variability in their reaction to whitefly transmitted mungbean yellow mosaic virus (MBYMV). Using infected, leaf area affected and yield reduction, the genotypes were classified into six reaction grades. Two accessions were highly resistant, 5 were resistant and 16 were moderately resistant. Resistance of soybean lines in these groups, however, may be confirmed under controlled conditions. About 80% of the germplasm including several high yielding varieties, appeared to be susceptible with varying degrees. The promising and potential material can be further improved through the use of resistant sources identified.

Pak. J. Phytopath., 2(1-2) 52-62, 1990.

BIOLOGICAL CHARACTERIZATION OF SOYBEAN MOSAIC VIRUS IN NWFP

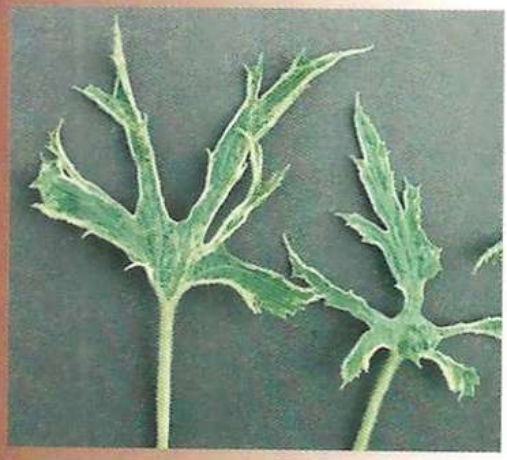
Akhtar Ali

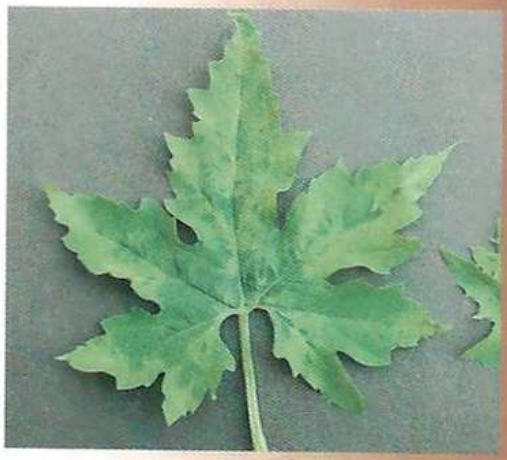
Soybean mosaic is a destructive viral disease of soybean cop caused by soybean mosaic virus (SoyMV). Field infected plants were characteristically stunted, with shortened petioles and internodes, showing predominant mosaic and mottling symptoms on leaves that turn downward at the margins. The incidence of the disease ranged between 7.74-72.14% with an average of 34.40% at 15 different location surveyed.

Eighteen and fourteen out of 30 and 20 soybean varieties and germplasms, respectively, produced mild to severe virus infection through seed in screenhouse experiment. Virus was readily transmissible through seed and mechanically by applying infected sap on indicator hosts with 0.01M phosphate buffer pH 7.0 Crowford, Chico, Zane and 80-B-4007 soybean varieties out of 12 tested were evaluated as resistant to SoyMV (isolate-1). Among 6 exotic germplasms tested, not even a single possessed resistance to the virus.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agri. Univ., Peshawar, 1990.

SQUASH





SQUASH

OCCURRENCE OF ZUCCHINI YELLOW MOSAIC VIRUS IN PAKISTAN

Khalid, S. & I. Ahmad

From naturally infected plants of squash (*Cucurbita pepo*), near Basham (Swat), showing severe mosaic, deformation and shoestring-like formation of leaves, a mechanically transmissible virus was isolated. This isolate produced local lesions in *Chenopodium amaranticolor, C. quinoa, Phaseolus vulgaris* cv. Black turtle soup, *Gumphrena globosa* and systemic infection in *Benincesa cerifera, Citrullus lanatus, Cucumis melo, C. melo* var. Fleruosis, *C. sativa, Cucurbita maxima, C. moschata, C. pepo, Langenaria vulgaris, Luffa acutangula, Memordica charantia* and *Ranuculus sardous*. *Lavatera trimestris* was not infected. Reaction of test plants indicated that our isolate is non-wilting type and is close to the type 'O'. Virus was propagated and purified from zucchini leaves. In isopycnic centrifugation it sedimented as a single band. Purified preparation contained large number of filamentous particles when seen under electronmicroscope. Polyclonal antiserum with a titer of 1:64 in gel diffusion test was produced in rabbit. Our isolate reacted with homologous as well as with other antisera to ZYMV obtained from Drs. Lecoq (France); Gonsalves (USA); Lisa (Italy) and Huang (China). On the basis of symptom produced on test plants, particle morphology and serological tests our isolate was identified as zucchini yellow mosaic virus (ZYMV).

Int. Conf. Integ. Pl. Dis. Manag. for Sustainable Agric., Nov. 10-15, 1997, New Delhi, India, (Abst. PIC-011) p. 225.

SOME PROPERTIES OF A VIRUS ISOLATED FROM NATURALLY

From naturally infected plants of Squash (*Cucurbita pepo*) near Basham, showing severe mosaic, deformation and shoestring-like forma-

INFECTED PLANTS OF *CUCURBITA PEPO*

Khalid, S. &
I. Ahmad

tions of leaves, a mechanically transmissible virus with fairly large host range was isolated. It caused systemic infection in *Benincesa cerifera, Citrullus lanatus, Cucumis melo, C. sativus, Cucurbita maxima, C. moschata, C. pepo, Langenaria vulgaris, Luffa acutangula* and *Mamordiaca charantia.* Reaction on *Chenopodium album, C. amaranticolor, C. quinoa, Gumphrena globosa* and *Phaseolus vulgaris cv.* Black turtle soup was localized. In isopycnic centrifugation it sedimented as single band which contained filamentous virus particles measuring approximately 750 nm. An antiserum, free from host antibodies, with the titre of 1:64 in gel diffusion test was produced in rabbit. On the basis of particle morphology and reaction of test plants the virus was tentatively identified as zucchini yellow mosaic virus (ZYMV).

Workshop on Agroclimat., Pests and Diseases and their Control, Nov. 21-26, 1992, COMSTECH - NIAB, Faisalabad, p. 64.

SUGARBEET

STRATEGIES FOR THE CONTROL OF SUGAR-BEET VIRUSES

Ahmed. M.

Sugarbeet viruses, beet western yellows virus (BWYV), beet curly top virus (BCTV), beet mosaic virus (BtMV), beet yellows virus (BYV) and cucumber mosaic virus (CMV) were controlled through different crop management strategies. Percent infection of these viruses was high in untreated plots and low in treated plots. The best control treatment was elimination of weeds by manual eradication + weedicide application. Percent incidence in manually eradicated + weedicide application plot was 2.47, 2.64, 2.33, 1.58 and 2.38 of BWYV, BCTV, BYV, BtMV and CMV, respectively. Mean root length, diameter, weight, brixb%, pol% and purity% were 29.66 cm, 8.77 cm, 683.77 gm, 14.98%, 14.70% and 83.28% in manually eradicated + weedicide treated plots. In untreated plots, these parameters were 21.52 cm, 5.21 cm, 214.74 gm, 14.86%, 11.27% and 70.81%, respectively. Percent incidence of BWYV was less in plots sown on 30th October and 15thNovember, while incidence of BCTV was low in plots sown on 15th November. BtMV was less in all treatments and varieties. Percent incidence of BYV was less in all treatments and varieties. Percent incidence of BYV was less in plots sown on 15th October and 15th November. CMV infection was minimum in 30th October and 15th November sown plots. A high mean root length was 31.02 cm, and high mean root weight was 754.80 gm in plots sown on 15th November. High mean root diameter 8.52 cm was recorded in 30th October sown plots. High Pol (12.78%) was in 15th November sown plots. High purity percentage was recorded in 30th October sown plots. Cultivars KaweTerma, KaweMera, Mezzanopoly and Seker-861 were screened for

resistance to BWYV. KaweTerma, Kawe Mera and Mezzanopoly became infected and developed symptoms while Seker-861 did not show any symptoms and was apparently resistant.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1993.

EVALUATION OF INDIRECT AND TWO-STEP ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF BEET CURLY TOP, BEET WESTERN YELLOWS AND CUCUMBER MOSAIC VIRUSES IN SUGARBEET

Ali, G.S., Akhtar Ali, M. Arif, M. Shafiq & S. Hassan

Indirect and two-step enzyme-linked immunosorbent assays (ELISA) were evaluated and compared for the detection of beet curly top virus (BCTV), beet western yellows virus (BWYV) and cucumber mosaic virus (CMV) in sugarbeet. In indirect ELISA, lower concentration of tissue extract, antigen-specific antiserum and goat antirabbit immunoglobulin G (IgG) conjugated with alkaline phosphatase. In two-step, higher concentration of tissue extract and lower concentration of both antigen specific antiserum and enzyme-labeled antibody gave better results for the detection of BCTV, BWYV and CMV than indirect ELISA. Indirect ELISA was two times more sensitive than the two-step one, for detecting BCTV and BWYV. In case of CMV, indirect ELISA was more sensitive at lower while two-step at higher tissue extract concentration.

Sarhad J. Agri., 9(6): 591-598, 1993.

THE EFFECT OF BEET WESTERN YELLOWS AND BEET CURLY TOP VIRUSES ON SOME YIELD COMPONENTS IN SUGARBEET ROOT CROP

Arif, M., S. Hassan & M. Shafiq

The effect of beet western yellows virus (BWYV) and beet curly top virus (BCTV) was assessed on sugarbeet root crop in farmers' fields. BWYV and BCTV significantly reduced the sugarbeet yield and sugar recovery. Percent reduction in root length, diameter, weight and pol percentage due to BWYV and BCTV infection was 21.11, 46.40, 81.86, 9.97 and 15.13, 40.00, 75.55 and 10.89, respectively in naturally infected beet roots. In BWYV infected plants root length and diameter

was positively and significantly correlated, while the r-value for root length and diameter with weight was positive and highly significant. In BCTV infected roots, only positive and significant correlation was found between diameter and weight.

Sarhad J. Agric., 7(6): 779-784, 1991.

INCIDENCE AND DISTRIBUTION OF VIRUSES INFECTING SUGARBEET CROP IN NORTH WEST FRONTIER PROVINCE, PAKISTAN

Arif, M., S. Hassan & M. Shafiq

Incidence and distribution of viruses infecting sugarbeet was determined in five sugarbeet growing districts of North West Frontier Province. Beet western yellows virus (BWYV), beet curly top virus (BCTV), beet mosaic virus (BtMV), beet yellows virus (BYV) and cucumber mosaic virus (CMV) were recorded and identified on the basis of biological (Symptomatology, host range and transmission properties) or serological properties or both. The mean incidence of BWYV, BCTV, BtMV, BYV and CMV based on three surveys conducted on the basis of symptomatology was 7.76, 4.36, 1.39, 8.78, 2.98%, respectively. Identity of BWYV, BCTV, BtMV, BYV and CMV was confirmed by symptomatology, host range, and transmission properties. BtMV, BYV and CMV were easily sap transmissible, BWYV was transmitted by green peach aphid *(Myzus persicae* Sulz.) and BCTV by modified mechanical method with little success to their diagnostic hosts. BWYV, BCTV and CMV were also detected by enzyme-linked immunosorbent assay (ELISA)-indirect method with 15.38, 10.84, 4.32% mean incidence.

Sarhad J. Agric., 7(5): 665-673, 1991.

ECOLOGICAL AND EPIDEIMIOLOGICAL STUDIES OF SUGAR-BEET VIRUSES IN NWFP

Shafiq, M.

Occurrence and distribution of sugarbeet viruses were recorded in five major sugarbeet producing districts of the North West Frontier Province (NWFP). Beet western yellows virus (BWYV), beet curly top virus (BCTV), beet mosaic virus (BtMV), beet yellows virus (BYV) and cucumber

mosaic virus (CMV) were identified on the basis of symptomatology, infectivity assay, host range, transmission properties and serological reactions. The mean incidence of BWYV, BCTV, BtMV, BYV and CMV on the basis of symptoms expression was 8.17%, 4.35%, 1.47%, 8.38% and 3.41%, respectively. BWYV, BCTV and CMV were also detected by indirect enzyme-linked immunosorbent assay (ELISA) with an incidence of 16.43, 10.61 and 4.90%, respectively. The vectors of BWYV and BCTV were commonly prevalent in the areas surveyed. Tire-pile and volunteer beet plants served as main source of virus-alternative hosts for beet viruses. BWYV and BCTV significantly reduced the sugarbeet yield and sugar recovery. Percent reduction in root length, diameter, weight and pol percentage due to BWYV and BCTV was 21.11, 46.40, 81.86, 9.97 and 15.13, 40, 75.55 and 10.89, respectively in naturally infected beet roots.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1991.

BIOLOGICAL AND SEROLOGICAL CHARACTERIZATION OF SUGARBEET VIRUSES

Ali, G.S.

Beet western yellows virus (BWYV), beet curly top virus (BCTV), beet mosaic virus (BtMV), beet yellows virus (BYV) and cucumber mosaic virus (CMV) were characterized biologically through host range, symptomatology, transmission properties and serologically through enzyme-linked immunosorbent assay (ELISA) both indirect and two–step methods. In host range studies, BWYV, BtMV, CMV and BYV were reproduced on 8, 10, 15, 5, specific assay hosts out of 34, 35, 36, 37 tested, respectively. BtMV, CMV and BYV were transmitted mechanically, BWYV and BYV by green peach aphid (*Myzus persicae*. Sulzer) and BCTV by modified needle injection method. In serological detection and characterization, ELISA reagents were evaluated and both indirect and

Two-step ELISA methods were compared for the detection of BWYV, BCTV, and CMV. In ELISA-indirect method, lower concentrations of tissue extract, antigen specific antiserum and goat antirabbit immunoglobulin G conjugated with alkaline phosphatase gave better results while in ELISA-Two-step method better results were obtained at higher concentration of tissue extract and lower concentrations of both antigen specific antiserum and specific enzyme-labeled antibodies for all three sugarbeet viruses. ELISA-indirect method was two times more sensitive than Two-step for the detection of BCTV and BWYV. In the detection of CMV ELISA-indirect method showed higher sensitivity than Two-step at lower tissue extract concentration and vice versa at higher extract concentration.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1991.

SUGARCANE

IDENTIFICATION, DISTRIBUTION, AND SYMPTOMATOLOGY OF SUGARCANE MOSAIC VIRUS

Shah, H., S. Hassan & S. Khalid

Sugarcane mosaic, caused by sugarcane mosaic virus (SCMV), is a viral disease affecting sugarcane (*Saccharum officinarum* L.). A study was, therefore , conducted to determine incidence and distribution of the virus in sugarcane growing areas of North West Frontier Province (NWFP), Pakistan. The incidence ranged between 45-86%. All the commercial sugarcane cultivars were susceptible. Symptoms occur on young leaves, and infected plants were stunted with severe mosaic. A number of plants belonging to different families were tested in insect-proof greenhouse and inoculated mechanically for their reaction to SCMV. All the sorghum (*Sorghum bicolor*) and few maize cultivars were infected. Of sorghum cultivar Rio (Exotic) and Pak-SS-II were found best indicators and propagative hosts. None of the plants outside *Poaceae* (*Graminaea*) produced any symptoms. No latent infection was detected upon back inoculation. Flexuous rod shape particles measuring 750 nm in length were observed from infected leaf dip preparation under electronmicroscope.

5th Nat. Conf. Pl. Scientists, Mar. 28-3, 1995, NARC, Islamabad, p. 117

VARIETAL SUSCEPTIBILITY TO YELLOWING MOSAIC OF SUGARCANE

Arif, A.G. & N. Ahmad

Thirty varieties of sugarcane were artificially inoculated with the sap of the diseased canes to study the relative resistance of these varieties. The incidence of yellowing mosaic of sugarcane varied from 0 to 48 %. Varieties No. B.L-19, B.L-4, CoL-89 and CoL-313 were highly susceptible to the disease, varieties No. CoL-71, CoL-77, CoL-48 and CoL-86 were resistant to the disease, whereas varieties No CoL-76 and CoL-77 were immune to the disease.

Proc. 7th Pak. Science Conf. Karachi ,1995, (Abst. part-III) p. A-225.

PURIFICATION AND SOME PROPERTIES OF SUGARCANE MOSAIC VIRUS

Shah, H., S. Hassan & S. Khalid

Mosaic is one of the most prevalent viral disease of sugarcane (*Saccharum officinarum* L.). Sorghum (*Sorghum bicolor*) cv. Pak-SS-II, seedlings were raised in insect-proof greenhouse and mechanically inoculated at 2-3 leaf stages. Systemically infected leaves were harvested after 3 weeks, homogenized in 0.05 M phosphate buffer, pH 7.2, emulsified in 10% chloroform and n-butanol and clarified at 5,000 rpm followed by differential centrifugation. Sucrose density gradient was performed for complete purification. The flexuous rod shape virus particle is approximately 750 nm in length and has a longevity in vitro of 36 h, dilution end point of 10^{-4} and thermal inactivation point of 55°C.

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, pp. 117-118.

SUGARCANE VIRUS DISEASES IN SINDH, PAKISTAN

Haque, S.E., M.J. Zaki, S. Azeemuddin, S.H. Siddiqui & A. Khatri

During a survey of sugarcane cultivated fields in Sindh, Pakistan, five commercial varieties viz., BL-4, PR 1000, L 116, L 113 and L 126 showed 100% infection of sugarcane mosaic virus (SCMV). Infection of mild chlorotic streak virus was also observed on some plants in L 126 and L 113 cultivars in some areas. No symptoms of SCMV were found on *Cynodon dactylon* and *Melilotus officinalis* the common weeds present in all the localities surveyed. In sap transmission test *Sorghum bicolor* cv. Rio appeared more susceptible to SCMV and proved a good indicator plant. In electronmicroscopy virus particles from BL-4, showing symptoms of SCMV were found flexious rods of 750 nm.

Bio. Res. J., 1: 1-5 1994.

INCIDENCE AND SOME PROPERTIES OF SUGARCANE MOSAIC VIRUS (SCMV)

Studies on the biology, incidence, and distribution of sugarcane mosaic disease were conducted in sugarcane growing areas of NWFP. Identification, and characterization of the causal

Shah, H.

agent was done in Plant Virology Laboratory at National Agricultural Research Centre (NARC), Islamabad. Disease incidence ranged from 45 to 85% with a mean incidence of 63%. Host range studies performed on many different cultivars of sorghum, maize, millet, wheat, rice, barley, oat and several graminaceaous grasses, tentatively revealed that the causal agent of this disease was sugarcane mosaic virus, a member of potyvirus group. Host species outside gramineae were not infectible. Physical properties such as thermal inactivation point (TIP), dilution end point (DEP), and longevity in-vitro (LIV) were 55°C, 10^{-4} and 36 hours, respectively. For SCMV purification three different methods were attempted to standardize a purification procedure. Purification schedule was developed in 0.5 M phosphate and borate buffer, pH 7.0 – 8.0, containing EDTA, 2-MCE, PEG, Triton X-100 and chloroform : n-butanol (1:1), gave virus yield of 1.70 mg/ml and 0.56 mg/ml, respectively. In electron microscopy flexuous rods shaped particles of 750 nm modal length were observed.

M.Sc.(Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1994.

PARTICLE MORPHOLOGY AND HOST RANGE OF SUGARCANE MOSAIC VIRUS

Aftab, M., S.M. Iqbal & K.B. Malik

Sugarcane (*Saccharum officinarum* L.) is the major sugar producing crop of Pakistan. None of its presently cultivated varieties has been found free of mosaic virus. Generally occurring strain was identified as sugarcane mosaic virus strain-A (SCMV-A), after confirmation on various test plants of sorghum, maize, millet, wheat, rice, oats, barley and grass species. The virus was mechanically transmissible to all 21 tested sorghum cultivars and 7 out of 19 maize cultivars. None of the 4 grass species were infected. The particle morphology of the partially purified virus was studied under the electronmicroscope, which showed flexious rod

shaped virus particles of model length 750 nm.

Proc. Workshop on Agroclimatol. Pests & Diseases & their Control, Nov. 21-26, 1992, COMSTECH/NIAB, Faisalabad, pp. 411-422.

EFFECT OF SUGARCANE MOSAIC VIRUS ON YIELD AND QUALITY OF CANE

Ahmad, M., R. Ali & M.I. Hassan

Field experiments were conducted to observe the effect of sugarcane mosaic virus on cane yield and quality on sugarcane cultivar Triton. The mosaic reduced 8.12% germination, 28.45% tillers per plant and adversely affected the cane length, number of internodes and cane thickness. The crop raised from mosaic affected seed suffered 9% loss of stripped cane yield and yielded less recoverable sugar (9.5%) as compared with mosaic free crop.

J. Agric. Res., 30 (1): 95-99, 1992.

EFFECT OF SUGARCANE MOSAIC VIRUS ON CANE STAND AND YIELD

Anwar, M.S., M.S. Mirza, M. Ahmad & F. Muhammad

Effect of sugarcane mosaic virus was determined on growth development, sugar contents and cane yield (cv. Triton) for three years (1983-85). The disease decreased on an average 16.46% tillering, 19.59% cane length, 7.58% cane thickness, 18.71% cane yield and 13.10% sugar contents. These losses appear to be very high and need to be checked through healthy seed production.

J. Agric. Res., 30(4): 513-516, 1992.

EFFECT OF SUGARCANE MOSAIC VIRUS ON THE YIELD AND QUALITY OF CANE

Ahmad, M., C.R. Ali & S.D. Fasihi

Seed free from sugarcane mosaic potyvirus infection gave 5-11% more germination and 1.34 more tillers/plant than mosaic infected seed. A disease-free crop raised from mosaic-free seed yielded 48.51 t/ha compared with 44.50 t from infected seed. Mosaic free canes were superior to infected canes in length, number, thickness and length of internodes. Recoverable sugar was 11.8% higher in a mosaic-free crop.

Pak. Sugar J., 4(1): 11-13, 1991.

FERTILIZER REQUIREMENT OF MOSAIC FREE AND INFECTED SUGAR-CANE CROP

Ahmad, M., K.B. Malik, H.A. Tiwana & K. Mehmood

The interaction between mosaic free and infected seed of sugarcane crop at various levels of NPK fertilizer was studied at Sugarcane Research Institute, Faisalabad, during 1985-86 and 1986-87 and the farmers field during 1986-87, to find out the fertilizer requirements of sugarcane seed cleaned from sugarcane mosaic virus. The aim was to reduce the lodging incidence of mosaic free cane causing adverse effects on the yield of crop. The results indicate that mosaic free crop showed significantly higher cane yield of 60.21, 65.01 and 58.67 t ha^{-1} at 168-84-84 (recommended), 84-84-84 (half the recommended N) and 84-42-42 (half the recommended NPK) respectively as compared with the yield of 54.29, 51.08 and 47.81 t ha^{-1} from the mosaic affected crop. It was inferred from the study that mosaic free crop requires less nitrogen as compared with crop infected by mosaic as 27.28% increase in cane yield was obtained at half the nitrogen i.e., 84-84-84 to mosaic free crop when compared with full nitrogen i.e., 168-84-84 kg ha^{-1}

Pak. J. Soil Sci., 5(1-2): 29-31, 1990.

EFFECT OF SUGAR-CANE MOSAIC ON YIELD AND QUALITY OF PLANTED AND RATOON CROP OF SUGARCANE

Munir,A., M.B. Ilyas, M.A.R. Bhatti & M. Ahmad

Infection of cv. Co 975 by sugarcane mosaic potyvirus resulted in 21.39% decrease in tillers/plant and 15.63, 24.63 and 17.45% decreases in cane length, number of internodes and cane thickness, respectively. Loss of yield, was 14.31% in the first year crop and 20.61% in the ratoon. Decreases of 4.28 and 2.06% in juice weight and sucrose content, respectively, occurred in the plant crop and 5.33 and 1.44%, respectively, in the ratoon.

Pak. J. Phytopath., 2(1-2): 63-67, 1990.

ELIMINATION OF SUGARCANE MOSAIC VIRUS FROM DISEASED

An attempt was made to eliminate sugarcane mosaic virus form 19 sugarcane varieties by repeated hot water treatments of bud chips for

SUGARCANE BUDS WITH THERMO-THERAPY

Mirza, M.S., M. Ahmed & M. S. Anwar

four days at 55.0°C, 56.5°C and 57.5°C for 7 and 10 minutes with 24 hours interval between each treatment. Mosaic could only be eliminated in 3 varieties viz., Triton, Co 975 and BF 134. Bud chips which were cured free of mosaic produced healthy plants. Freedom from disease was confirmed by testing the cured plants on indicators.

J. Agric. Res., 24(3): 207-210, 1986.

TOBACCO

TOBACCO

INDUCIBLE EXPRESSION OF DIANTHIN TO ENGINEER RESISTANCE AGAINST TOMATO LEAF CURL VIRUS (TLCV) IN TOBACCO

Tariq, M., S. Mansoor, S.H. Khan, M. Hussain, S. Asad, Z. Mukhtar, Y. Zafar & K.A. Malik

Ribosome-inactivating proteins (RIPs) are naturally occurring plant toxins that are presumed to provide a defense mechanism, against pathogens or predators by disrupting protein synthesis in damaged eukaryotic cells. They have been shown to exhibit antiviral activity against a diverse range of plant and animal viruses. In the present study the action of Dianthin, a potent inhibitor of plant ribosomes isolated from *Dianthus caryophyllus*, has been exploited to engineer resistance to plant DNA virus, Tomato Leaf Curl Virus (TLCV) in transgenic *Nicotiana tabacum*. To achieve this, Dianthin was cloned downstream of the TLCV virion sense promoter (A V1 Pro) that is trans-activated by the product of viral gene AC1. This avoids the need for constitutive expressions of RIP, and so facilitates the production of phenotypically normal plants ensures transgenic expression to be localized to virus-infected cells. When challenged with TLCV, transgenic plants produced typical necrotic lesions on inoculated leaves, the normal progress of infection was disrupted and transgenic plants developed no leaf curl symptoms as compared to control (non-transformed plants).

2nd Nat. Symp. on Pl. Tissue Culture and Genetic Engg. Jun 1-3,1999. Agri. Biotech. Inst., NARC, Islamabad. (Abs.) p. 9.

SEGREGATION OF KANAMYCIN RESISTANCE GENE IN TRANSGENIC TOBACCO PLANTS

Subhani, M.N.,

Seeds of seven transgenic, TMV resistant tobacco (*Nicotiana tabacum* cv. xanthi) lines # 17a, 29a, 30, 32, 286, X-283.3 and 350 were germinated on kanamycin-supplemented MS agar medium, using five concentrations, i.e., 50, 75, 100, 150 and 250 mg/l of the medium. Seedlings were scored on the basis of growth due to antibiotic

I.A. Khan & M.B. Ilyas

resistance. The ratio of kn^r and kn^s was nearly 3 while, in case of non-transformed control tobacco plants (*N. tabacum* cv. Xanthi, *N. tabacum* cv. xanthi-nc and *N. sylvestris*) the plant survival was zero.

Pak. J. Phytopath., 9(2): 127-131, 1997.

SCREENING OF TRANSGENIC TOBACCO PLANTS AGAINST TMV ISOLATES

Jabbar, N.

Screening of plants against virus is an effective method for the detection of resistance. Resistance in viral diseases is of great importance, because the best available method for the control of virus diseases, including tobacco mosaic virus disease of tobacco, is the use of resistant cultivars. The field of resistance breeding has been expanded by the application of biotechnological techniques.

Of the different strategies so far employed for the production of transgenic plants, a recent technique involving a defective TMV replicase gene has exhibited resistance against a broader range of tobamovirus. These replicase-resistant Xanthi tobacco plants were segregated on kanamycin supplemented culture medium and the segregation percentage was found to be 65.94% which is almost similar to that reported by Turpen i.e., 3:1 for kanamycin resistant and kanamycin susceptible plants.

In my studies, screening results of these transgenic lines (No. 4, 7 and 24) showed no plants as immune. These unexpected results were critically observed and it was found that disease development in transformed plants (except # 24) was delayed by six to fifteen days when compared with non transformed plants *(N. tabacum* var . Xanthi, *N. tabacum* var. Xanthi-nc and *N. sylvestris),* which were kept as control hosts with known disease responses. Severity of disease noted on non-transgenic plants which showed distortion of leaves. In # 24, which has been reported as susceptible line, disease

responses were similar to non-transformed Xanthi host.

On transplantation in greenhouse, after twenty days of inoculation, NL-18 inoculated transgenic plants showed distortion of leaves, while only distinct mosaic symptoms in U1-inoculated transgenic lines reveal that NL-type viruses are more severe than U1 strain of TMV.

This disease development cannot be considered as a result of virus mutation, virus mixing or contamination by other viruses because disease was not scattered on few plants.

Virus resistance is attributed to replication inhibition. Plants used in my studies are reported to be resistant due to this mechanism. The development of disease may be considered as inactivity of this replication inhibitor, due to some factor(s) like nutrition, light intensity, temperature and age of the plant.

Now it is to be found out the factors (s) involved in inactivity of replication inhibitor.

M. Sc. (Hons.) Thesis, Dept. Pl. Path., Univ. Agric., Faisalabad., 1994.

RESPONSE OF TRANSGENIC REPLICASE RESISTANT TOBACCO PLANTS AGAINST THE EXTRA VIRULENT STRAIN OF TMV

Nadeem, S.

Viruses are ubiquitous and are causing extensive crop losses. Best control for virus diseases is the use of resistant varieties. Biotechnology is an approach to enhance the scope of resistance breeding. Ultimate application of these techniques will emerge after the selection and confirmation of an appropriate model. TMV-tobacco model has been extensively used in virus genetic studies. A number of biotechnological strategies have been employed to produce transformed virus resistant plants. However, resistance remained restricted to closely related viruses.

Most recently, a technique involving transformation of plants with TMV replicase sequences interrupted at nucleotide 2875 by bacterial insertion element for broader resistance Kanamycin resistance gene were also engineered in these plants as marker Transgenic *Nicotiana tabacum* cv. Xanthi plants, used in the present studies, were transformed by this technique.

Three transformant lines (#2, #8 and #24) of TMV-resistant tobacco plants were tested for disease responses against an extra virulent strain of TMV, NL-18 and wild type TMV variant, U1. NL-type viruses evoke hypersensitive reaction on *Nicotiana sylvestris* while, U1 viruses produce systemic mosaic symptoms on this host.

N. tabacum cv. Xanthi (systemic symptoms by all strains of TMV), *N. tabacum* cv. Xanthi-nc (necrotic lesions by all TMV isolates) and *N. sylvestris* local lesions by NL-18 & systemic symptoms by U1 variant of TMV were kept as non-transformed control hosts.

Disinfested seeds of transgenic plants were segregated on kanamycin-supplemented (50 µg/ml) MS based salt medium and segregation ratio for Kn^r and Kn^s plants was found to be 3:1 and 0:1 for non-transformed plants.

Kanamycin segregated transgenic plants, inoculated with U1 and NL-18 viruses, separately, were tested for resistance along with the responses of non-transformed plants.

Broader resistance against different tobamoviruses, including wild type variant of TMV (U1), has been reported in transgenic TMV-resistant plants. But in the present studies development of systemic symptoms on all transformed lines against both TMV isolates, has opened a new topic for discussion. When

compared with non-transformed plants, it was observed that in transgenic plants (except #24) symptom development was delayed by 7 to 14 days. Severity of disease in #2 & #8 was less than observed on non-transgenic plants.

Line #24 has been reported to be susceptible and the results revealed that disease development and severity in #24 was similar to that of control Xanthi host, which indicated that replicase sequences were biologically active in #24.

After one month, distortion of leaves in transgenic lines, inoculated with NL-18 viruses, was observed. While, only distinct mosaic symptoms were recorded on plants inoculated with U1 strain of TMV. This showed the virulence of NL-type viruses was more than U1 strain.

Disease development on all of the inoculated transgenic plants rule out the possibility of virus mutation, virus mixing and contamination of other viruses.

In viral diseases, resistance or susceptibility is related to virus replication. Virus resistance in plants results from inhibition of replication. Tobacco plants transformed with a defective replicase gene of TMV were reported to be resistant due to this mechanism of reduced multiplication. Lack of resistance in these plants may be attributed to some factor(s), like temperature, light , nutrition and age of the plant etc., which can break or inactivate the replication inhibitor. These results have provided a future line of action to find out the factor (s) responsible for inactivity of replication inhibitor.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., Univ. Agric. Faisalabad, 1994.

SOME PROPERTIES OF CUCUMBER MOSAIC VIRUS ISOLATED FROM TOBACCO IN PAKISTAN

Hameed, S., S. Khalid & I. Ahmed

A spherical virus isolated from tobacco was identified by host range, physical and serological properties as cucumber mosaic virus. Typical isometric particle 28nm in diameter were detected in purified preparations as well as crude extract of infected plants. Purified virus preparations were infectious, opalescent and used to produce high quality antiserum free from host antibodies and non-specific reactions in DAC-ELISA. Anti-serum with titre of 1:32 in gel diffusion test and 1:10,000 in DAC-ELISA was produced. Cucumber mosaic virus encapsidates typical RNA with no Satellite RNA being found. Results indicate that ELISA testing of leaf extracts can be used as methods for the routine testing.

13th Pak. Cong. Zool., Mar. 31 - Apr. 1, 1993. UGC, Islamabad, p. 16.

RESPONSE OF TRANSGENIC TOBACCO PLANTS TO AN EXTRAVIRULENT STRAIN OF TOBACCO MOSAIC VIRUS

Nadeem, S., N. Jabbar, I.A. Khan & S.M. Khan

Five lines of in-vitro grown transgenic *Nicotiana tabacum* cv. Xanthi plants (# 2, 4, 7, 8, and 24), carrying a defective replicase gene of TMV, were tested against U1 and NL-18 strains of TMV. NL-viruses, an isolate of UI strain, are more virulent than UI strain. The transgenic plants have been reported to show broader resistance against different tobamoviruses. When inoculated by UI and NL-18 strains, systemic mosaic symptoms were recorded on all inoculated transgenic plants in our experiments. When compared with non-transformed Xanthi host, 1-2 weeks delay in symptom initiation was noted and disease severity was also less in transgenic plants. Response of line # 24 was similar to that of non-transformed plants. By 30-35 days post inoculation, results revealed that symptoms were more severe on NL-18 inoculated plants than on U1 inoculated plants.

Proc. Int. Symp. Biotech. Sustainable Dev., Dec. 15-20, 1993. NIBGE, Faisalabad, p. 117-121.

PHLOEM TRANSPORT OF TOBACCO MOSAIC VIRUS IN XANTHI NC TOBACCO INDUCED BY POTATO LEAFROLL VIRUS

Ahmed, W. & P.E. Thomas

Before or after aphid inoculation with potato leafroll virus (PLRV) leaves of young *Nicotiana glutinosa* and *N. tabacum* cv. Xanthi-nc, plants were rub inoculated with tobacco mosaic virus (TMV). Lesions developed on inoculated leaves. After 3-4 weeks, systemic necrotic lesions developed on young leaves and stems of plants inoculated with both TMV and PLRV, but not in plants inoculated with either virus individually. TMV was transmitted from the systemic necrotic lesions of dually infected plants, but not from non-necrotic areas, although these areas contained TMV antigen. Stem necrosis caused collapse of some dually infected plants. Regrowth from the base of such plants was non-necrotic but infectious by graft inoculation. TMV could not be transmitted by rub or by graft from noninoculated leaves and stem of plants inoculated with TMV only.

Phytopathology, 8(10): 1167 (Abst.-246), 1991.

CUCUMBER MOSAIC VIRUS AND SOME OTHER VIRUSES ON TOBACCO IN PAKISTAN

Hameed, S., S. Khalid & S.M. Mughal

A tobacco disease in Pakistan, characterized by mosaic, vein clearing, necrosis and reduction in plant height, was found to be caused by a complex of three viruses two of them reported previously as tobacco mosaic virus and potato virus Y. A spherical component was identified by host range, particle morphology and serology as cucumber mosaic virus. This is the first report of the occurrence of CMV in tobacco from Pakistan.

Pak. J. Sci. Ind. Res. 34(4): 137-139, 1991.

CHARACTERIZATION OF COMMON STRAIN OF TOBACCO MOSAIC VIRUS IN PAKISTAN

Hameed, S., S.M. Mughal & S. Khalid

The common stain of TMV was identified and characterized on the basis of its biological, serological and physical properties and electron - microscopy. It sedimented as a single 190 S component, absorbed UV spectrum minimum at 245 nm and maximum at 260 nm and has particle model length of 300 nm. Purified virus preparations were infectious, opalescent and used

to produce high quality anti-serum and ELISA kit free from host antibodies and non specific reaction.

Pak. Tobacco, 15(2): 21-27, 1991.

SCREENING OF TOBACCO CULTIVARS FOR RESISTANCE TO ORDINARY STRAIN OF TOBACCO MOSAIC VIRUS

Hameed, S., S.M. Mughal & B. Ali

Eleven tobacco cultivars alongwith five positive controls and *Nicotiana spp.* were screened for their reaction to ordinary strain of tobacco mosaic virus (TMV) under artificial conditions of inoculation. Based on symptoms expression and quantitative infectivity assays, seven varieties (KHG series 2, 5, 8, 9 and 10, speight G-28 and Coker-48) manifested moderate level of resistance whereas KHG-4, KHG-7, NC-89, Spright-70 and remaining ones were invariably susceptible. There was no co-relation between intensity of symptoms and virus concentration.

Pak. Tobacco, 12(2): 13-16, 1988.

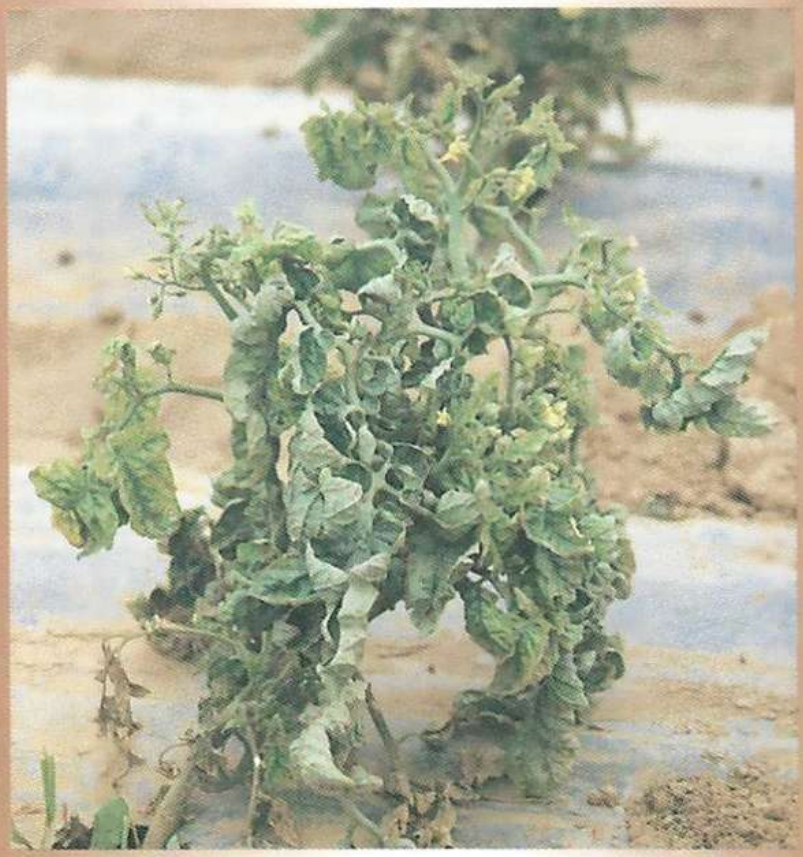
ISOLATION, IDENTIFICATION AND PREVALENCE OF TOBACCO VIRUSES IN PAKISTAN

Mughal, S.M., S. Khalid & Z. Hussain

Tobacco mosaic (TMV), potato virus X (PVX), potato virus Y (PVY) and tobacco leaf curl (TLCV) were the viruses found to infect tobacco crop in Pakistan. These viruses, which ranged between 26-65% in incidence, were isolated and identified on the basis of biological, physical and serological properties. TMV and PVX occurred more frequently in the initial stages followed by PVY (aphid-transmitted) and TLCV (white fly-transmitted) during the season. At the end, multiple infections were common. The problems of proper identification, distribution and control of tobacco viruses are discussed in this paper.

Pak. Tobacco, 10(1-2): 5-9, 1986.

TOMATO



TOMATO

MOLECULAR IDENTIFICATION OF TOMATO LEAF CURL VIRUS (TLCV) - PAKISTAN AND DEVELOPMENT OF TRANSGENE RESISTANCE IN A MODEL SYSTEM

Tariq, M.

Tomato leaf curl is the most important constraint for tomato production in Pakistan and causes 30-40% yield losses. The disease is caused by a bi-partite whitefly transmitted geminivirus. To identify tomato leaf curl virus (TLCV) prevalent in Pakistan, virus specific primers were designed from TLCV-India (Padidam *et al.* 1995). These primers amplified expected 448 bp coat protein promoter region between start of AC1 (replication associated protein) and start codon of AV1 (coat protein) including intergenic region. The sequence analyses of intergenic region present in amplified 448 bp coat protein promoter (AV1 Pro) revealed that it was 98-99% homologous to intergenic region of TLCV India. On the basis of sequence homology it was concluded that TLCV-Pk is a strain of TLCV-India. PCR based diagnosis of infected samples of tomato showed that TLCV-Pk, identified to be a strain of TLCV-India, is most dominant and prevalent strain in Pakistan as 448 bp AV1Pro was amplified from 30 out of 36 (83 %) samples collected from different tomato growing regions.

In the present study, a ribosome inactivating protein (RIP), Dianthin, a potent inhibitor of plant ribosomes isolated from *Dianthus caryophyllus* was used to engineer resistance against TLCV-Pk in transgenic *Nicotiana tabacum*. To achieve this, dianthin was cloned downstream of the TLCV virion-sense promoter (AV1-Pro) that is transactivated by the product of viral gene AC2. This avoids the need for constitutive expression of RIP, and so facilitates the production of phenotypically normal plants and ensures transgene expression to be localised to virus infected cells. When challenged with TLCV-Pk and TLCV-India infectious clones, transgenic plants produced typical necrotic lesions on

inoculated leaves. The normal progress of infection was disrupted and plants developed no leaf curl symptoms as compared to non-transformed plants.

M.Phill. Thesis, Dept. Biol. Sci., Qauid-i-Azam Univ., Islamabad, 1999.

OCCURRENCE, DISTRIBUTION, HOST RANGE, SYMPTOMATOLOGY AND PURIFICATION OF ToMV ON TOMATO

Khan, I. A.

Tomato growing areas in Malakand Agency and Peshawar Division were surveyed to record the incidence to ToMV virus infecting tomato. ToMV was tentatively identified on the basis of symptoms developed in the infected plants. An average incidence of 29.79 and 25.49% of ToMV was recorded in tomato leaves and seeds, respectively. ToMV was transmitted to indicator host by rub inoculation and was purified in 0.2 M phosphate pH 7.0, successfully. Identification of ToMV was confirmed through different serological techniques and electronmicroscopic studies. ToMV reacted with homologous antisera in gel diffusion tests.

Pak. J. Zool., 29(4): 385-389, 1997.

EVIDENCE FOR THE ASSOCIATION OF A BIPARTITE GEMINI-VIRUS WITH TOMATO LEAF CURL DISEASE IN PAKISTAN

Mansoor, S., S.H. Khan, M. Saeed, A. Bashir, Y. Zafar & K.A. Malik

Tomato leaf curl disease is the most important constraint on tomato production in Pakistan, where it is found throughout the country. The disease, which occurs in high incidence in Punjab and Sindh provinces, causes 30 to 40% yield losses in the spring crop and uneconomically high losses when grown as an autumn crop. The symptoms of disease include upward or downward leaf curling, vein thickening, and stunting of the plant. The disease is transmitted by *Bemisia tabaci* whiteflies (non-B, biotype K) and is suspected to be caused by a geminivirus. For the detection of geminivirus, total DNA was extracted from infected plants, fractionated in an agarose gel, transferred to a nylon membrane, and Southern

blotted. A full-length clone of DNA-A of cotton leaf curl virus from Pakistan was labeled with [^{32}P]dCTP by the oligo-labeling method and hybridized at medium stringency. Geminivirus DNA forms that are normally found in infected plants were detected in plants with tomato leaf curl disease but not in healthy plants. To further confirm the presence of a whitefly-transmitted geminivirus, universal primers for dicot-infecting geminiviruses were used in polymerase chain reaction (PCR) and a product of expected size (approximately 2.7 kb) was detected. The 2.7 kb PCR-amplified DNA from diseased tomato plants was labeled with [^{32}P]dCTP and used as probe in Southern hybridization. This probe also detected geminivirus DNA forms at medium stringency. Both monopartite and bipartite geminiviruses transmitted by whiteflies have been reported to cause leaf curl symptoms on tomato from the Eastern Hemisphere. Degenerate primers (PBLv 2040 and PCRcl), which amplify B component DNA, were used to determine if tomato leaf curl was monopartite or bipartite. A product of expected size (0.65 kb) was amplified, suggesting this virus to be bipartite. DNA-B PCR product obtained from diseased tomato plants was hybridized as described above and detected geminivirus DNA forms at medium stringency. Samples of diseased tomato plants were collected from tomato fields throughout Punjab. DNA-A was detected in all 20 samples whereas DNA-B was detected in 17 samples when hybridized by dot blot method at medium stringency. Our data show that tomato leaf curl virus from Pakistan is a bipartite geminivirus. This is the first evidence for a bipartite geminivirus in tomato plants from Pakistan.

Pl. Disease, 81(8): 958, 1997.

SCREENING OF TOMATO LINES/ VARIETIES AGAINST TOMATO LEAF CURL VIRUS

Hameed, S. & S. Khalid

Viral diseases of tomato have almost reached to epidemic proportion during the past few years. Among them tomato leaf curl virus (TLCV) is very important The high incidence of TLCV was found all over the country. Drastic yield and quality losses have resulted due to tomato leaf curl virus. In this study resistant/tolerant germplasm reported was collected through AVRDC and screened against tomato leaf curl virus under controlled conditions using whitefly as vector. The presence of virus was further confirmed by TAS-ELISA using monoclonal antibodies of indian cassava mosaic virus (SCR-60 specific to whitefly transmitted geminiviruses) and polyclonal antibodies to african cassava mosaic virus (ex SCRI). Only one hybrid Ty-King and *L. hirsutum* (LA-1777) & *L. chilense* (LA-1969) shows satisfactory level of resistance, while the remaining varieties and lines showed different levels of tolerance and susceptibility.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agric. Univ. Peshawar, (Abst.) p. 39

A DISEASE DIAGNOSTIC SURVEY OF TOMATO CROP IN KATHA SUGHRAL AREA, DISTRICT KHUSHAB, PUNJAB

I. Ahmad, S. Hameed, S. Khalid, L.A. Hijazi, M. Tayyab & A. Tareen

Because of specific location of the Katha Sughral area next to Potohar hills, a frost free environment has provided optimum climate for the production of tomato which has emerged as the major crop of the area. Monoculture of tomato over the last 30 years has lead to establishment of a host of pathogens which are badly affecting the productivity. However, no systematic survey has been made to provide a basis for integrated management of the diseases and pests. The present diagnostic survey was done with the support of National Rural Support Program, Islamabad. Tomato leaf curl virus and *orobanche*, a parasitic weed are the major problems. Bacterial wilt, late and early blight and root knot nematode are the emerging problems. Among insects pests green aphid was found to be widely prevalent. Other insect pests although not encountered, are

known to be present in the area according to farmers and agricultural field staff. Farmers are well aware of the need for crop protection measures and regularly apply insecticides and fungicides. However, the use of these chemicals is non-judicial. Our conclusion is that systematic disease and pest monitoring of the area is essential for an effective management approach.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agric. Univ., Peshawar, (Abst.) p. 40.

TRANSMISSION OF TOMATO LEAF CURL GEMINIVIRUSES BY BEMISIA TABACI: EFFECTS OF VIRUS ISOLATE AND VECTOR BIOTYPE

McGrath, P.F. & B.D. Harrison

Cultures of *B. tabaci* from Ivory Coast (IC), Pakistan (PK) and the USA (USA B-type) were compared for the frequency with which they transmitted 3 tomato virus isolates: Indian tomato leaf curl virus from Bangalore (ITmLCV) and tomato yellow leaf curl bigeminiviruses from Nigeria (TYLCV-Nig) and Senegal (TYLCV-Sen). The frequency of transmission from tomato to tomato depended both on the whitefly culture and the virus isolate. USA B-type and IC whiteflies transmitted TYLCV-Sen more frequently than ITmLCV whereas PK whiteflies transmitted ITmLCV more frequently than TYLCV-Sen. USA B-type whiteflies transmitted both viruses 4 to 9 times more frequently than IC whiteflies. TYLCV-Nig was transmitted rarely by USA B-type and not at all by IC whiteflies. Previous work indicated that the geminivirus coat protein controls vector transmissibility. The differential adaptation of TYLCV-Sen to transmission by USA B-type whiteflies and of ITmLCV to PK whiteflies was associated with a large difference in epitope profile of the coat proteins of the 2 viruses. Also, the readily transmissible TYLCV-Sen differed appreciably in epitope profile from the poorly transmissible TYLCV-Nig, which reached a consistently greater concern in source tissues but lacked epitope 18.

However, the lack of epitope 18 in ITmLCV did not prevent its transmission by USA B-type whiteflies. Differences in the frequency and specificity of geminivirus transmission by whitefly cultures from different countries were associated with differences among epitope profiles of the coat proteins of the viruses, but it is concluded that the structural features of the proteins that control transmission remain to be determined.

Annals-of-Applied-Biology. 126(2): 307-316, 1995.

SOME PROPERTIES OF TOMATO LEAF CURL VIRUS FROM PAKISTAN

Hameed. S. & S. Khalid

Tomato leaf curl is one of the major viral diseases, effecting tomato production in Pakistan. Although the disease was known to occur, but for the first time its geminiviral etiology was confirmed by using standard virological and molecular biology techniques. Symptoms were reproduced from naturally infected tomato plants, collected from the province of Balochistan, NWFP, Punjab and Sindh, through whitefly inoculation. Symptoms developed after 20-25 days on viruliferous whitefly fed indicator plants of *Datura stramonium*, *Nicotiana tabacum* cvs. samsun, xanthi and white barley, *N. benthimiana*, *Lycopersicon esculentum* cvs. Money Maker, Roma and Rutger. Geminate particles along with monomers were observed under electronmicroscope . from partially purified preparations. Serologically its identity was confirmed by TAS-ELISA using panels of monoclonal antibodies (Mabs), raised against other whitefly transmitted geminiviruses and polyclonal antibodies to african cassava mosaic virus (ACMV). The DNA hybridization test with a mixture of P^{32} labeled probes also confirmed the presence of geminiviruses. On the basis of symptoms produced, particle morphology, serology and positive DNA hybridization tests it was concluded that the leaf curl disease in tomato is caused by a whitefly

transmitted tomato leaf curl geminivirus.

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, pp. 115-116.

INVESTIGATIONS ON VIRUS DISEASES OF TOMATO IN MALAKAND, PAKISTAN

Hassan, S.

Studies were conducted to investigate incidence, etiology and epidemiology of viruses infecting winter tomatoes in Malakand Agency. Viruses identified on the basis of serology and biology were ToMV, PVY, PVX, TYTV, TYLCV, TRSP, TSWV and PLRV. Mosaic, mottle, severe stunting, fern leaf and shoe string leaf lamina, leaf curling and rolling, ring spots, and asymmetrical fruits were the characteristic symptoms of viral infection of tomatoes. Both single and mix virus infections were prevalent in tomato crop. ToMV virus was detected in all commercial seed lots, and ToMV, PVX and PVY were found in tomato nurseries. Whitefly, aphids and leaf hoppers, vectors of many tomato viruses were predominantly present in tomato crops. Chillies, okra, squashes, cucumber and several wild *solanaceous* and *cucurbitaceous* spp. served as alternate and reservoir hosts for tomato viruses and insect vectors.

Sarhad J. Agric., 11(1): 89-96, 1995.

DISTRIBUTION AND INCIDENCE OF TOMATO VIRUSES IN PAKISTAN ON THE BASIS OF DAC-ELISA

Hameed, S., H. Shah, S. Khalid & L. Ray

Major tomato growing areas of all the four provinces of Pakistan were surveyed for the prevalence of viral diseases. Percent incidence and distribution of viruses were determined by testing randomly collected field samples through DAC-ELISA. During 1993, 351 samples were tested. An average infection of tomato bushy stunt virus (TBSV), tomato aspermy virus (TAV), cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), potato virus Y (PVY), tomato yellow top virus (TYTV), potato leaf roll virus (PLRV), and potato virus X (PVX) was 46.29%, 32.47%, 26.07%, 21.08%, 2.26%,

8.26%, 4.55% and 2.84%, respectively. In 1994 three districts of Sindh were surveyed and incidence of viruses detected was TBSV (93.98%), TAV (78.90%), CMV (66.68%), PVY (6.8%), PVX (30.68%), TMV (2.27%) and TYTV (7.76%).

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995, NARC, Islamabad, pp 127-128.

VIRAL DISEASES OF TOMATO IN MALAKAND AGENCY AND THEIR CONTROL

Hassan, S., A. Ali & G. Haq

Malakand Agency a frost-free zone, is the major tomato growing area in winter. Intensified and continuous tomato cultivation with no rotation, has build up viral disease epiphytotics with concomitant drastic reduction in yield, fruit quality and heavy economic losses to the growers. The most destructive and highly prevalent viruses in the area are tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV), cucumber mosaic virus (CMV), tomato yellow top virus (TYTV) and potato virus Y (PVY).

Various management strategies were tried to control the above viral diseases. Encouraging results were obtained with a significant decrease in virus incidence, disease severity and increase in yield and yield components of tomato with better quality fruits in our preliminary studies (Second Annual Report).

To ascertain and verify results of the preliminary studies, verification trials were conducted on farmer fields in Heroshah, Malakand Agency. Viral management strategies adopted and tested were as below:

1. Eradication of ToMV from seed by chemo and thermotherapy.
2. Control of virus vectors by insecticidal sprays.

3. Screening and selection of tomato cultivars against tomato viruses.

4. Control of tomato viral diseases through cultural practices.
5. Effect of mix cropping vs. monoculture of tomato on viral diseases of tomato.
6. Effect of direct sowing vs. transplant sowing on viral diseases.

On the basis of these studies conclusions are drawn and results are given.

Final Report. 1994. Dept. Pl. Path., NWFP Agric. Univ., Peshawar.

TOLERANCE, RESISTANCE TO MULTIPLICATION AND IMMUNITY TO TOMATO YELLOW TOP VIRUS AND POTATO LEAFROLL VIRUS IN *LYCOPERSICON PERUVIANUM* AND OF ITS TOMATO HYBRID PROGENIES

Hassan, S.

Plants in populations of *L. peruvianum* and 2 F4 hybrid progenies of *L. peruvianum* X *L. esculentum* were graft inoculated with isolates of tomato yellow top luteovirus (YT) and potato leafroll luteovirus. The viruses were recovered from some but not all plants of each population 9 weeks later. Some but not all plants from which virus was recovered failed to sustain recoverable virus levels when they were grown as cuttings independently of the infected scion. Those that did sustain the virus were tolerant, and virus concentration was very low in them. Plants that were not invaded by one virus isolate were invaded by some other isolates, and plants that did not sustain one isolate independently of the virus source did sustain others. While some isolates invaded and were sustained in more plants than others, the resistance of plants to virus invasion and sustainment appeared to be isolate specific. Some plants were found apparently immune to invasion by most isolates and very resistant to isolates that did invade; when grown independently of the infected scions used for

inoculation, they would not sustain any isolates that invaded them. Some YT isolates were more invasive than other, but immunity to each isolate was found among both hybrid and parental plants.

Durability of Disease Resistance, Current Pl. Sc. Biotech. Agric. Netherlands Acad. Pub., 18: 347, 1993.

PRELIMINARY STUDIES ON VIRAL DISEASES OF TOMATO IN MALAKAND AGENCY OF NORTH WEST FRONTIER PROVINCE, PAKISTAN

Hassan, S., M. Arif & T. Defoer

Intensive cultivation and monocroping of winter tomato in Malakand Agency (North West Frontier Province, Pakistan) have resulted in the occurrence of many destructive viral diseases which have caused tremendous losses both in yield and quality. Detailed and systematic work was conducted to determine the incidence, distribution, and losses due to viral diseases in Malakand Agency. An average seedling infection of 30%, 15% and 20% of tomato mosaic virus (ToMV), potato virus X (PVX) and potato virus Y (PVY) was recorded in tomato nurseries. Mean incidence of ToMV, cucumber mosaic virus (CMV), tomato yellow leaf curl virus (TYLCV), tomato yellow top virus (TYTV), tomato bushy stunt virus (TBSV), tomato aspermy virus (TAV), potato leaf roll virus (PLRV) and tomato ring spot virus (ToRSV) on the basis of symptom expression and serodiagnosis, was 34.38, 12.92, 15.06, 8.26, 4.05, 1.48, 3.90 and 3.70%, respectively. The predominant symptoms observed were mosaic, mottling, curling, bushy growth, shoe string, fern leaf, chlorosis, shoot proliferation and a variety of mixed symptoms. A reduction of 22.24% in fruit weight, 15.38% to 78.56% in fruit number and 25.77% in plant height was recorded, resulting in significant decrease in total yield.

Sarhad J. Agric., 9(2): 181-187, 1993.

ETIOLOGICAL STUDIES OF VIRAL DISEASES OF TOMATO IN MALAKAND AGENCY OF NORTH WEST FRONTIER PROVINCE OF PAKISTAN

Hassan, S., M. Arif & T. Defoer

Mosaic, mottle, rolling of leaves, leaf chlorosis, leaf malformations, fern leaf and shoe string, curling, bush growth and ring spot symptoms were predominantly recorded in winter tomato plants in Malakand Agency of North West Frontier Province (NWFP) Pakistan. Viruses tentatively diagnosed on the basis of these characteristics and typical symptoms were tomato mosaic virus, cucumber mosaic virus, tomato yellow leaf curl virus, tomato yellow top virus, potato leaf roll virus, potato virus Y and X, tomato bushy stunt virus, tomato aspermy virus and tomato ring spot virus. Both single virus and mixed virus infections were found. These viral infections were confirmed and verified through serological (Indirect enzyme-linked immunosorbent assay (ELISA) and double diffusion test) and biological (host range, transmission to indicator hosts and transmission properties) infectivity assay. Tomato mosaic virus was the most destructive and widely prevalent virus in the area.

Sarhad J. Agric., 9(1): 27-36, 1993.

EPIDEMIOLOGICAL STUDIES OF TOMATO VIRUSES IN MALAKAND AGENCY OF NORTH WEST FRONTIER PROVINCE OF PAKISTAN

Hassan, S., M. Arif & T. Defoer

The major epidemiological studies showed that nursery plants were the initial and primary source of tomato mosaic virus (ToMV) infection due to high percentage of the infected seed. Whitefly (*Bemisia tabaci* Gennadius), aphids (*Myzus persicae* Sulzer, *Macrosiphum euphorbiae* Thomas, *Acyrthosiphon solani*) and different species of leaf hopper as vectors of several tomato viruses were predominantly present in tomato fields. Symptoms of viruses were recorded on volunteer scientific and cucurbitaceous plants, *Nicotiana tabacum*, *Capsicum*, *Amaranthus* spp., black night shade, (*Solanum nigrum*), (*Solanum melongena* annum) and many *solanaceous* and seed plants. These wild and volunteer plants served as alternate and reservoir

hosts for vectors and viruses of tomato.

Sarhad J. Agric., 9(1): 37-44, 1993.

SCREENING FOR TOBACCO MOSAIC VIRUS RESISTANCE IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

Hameed, S., M.A. Khan & S. Khalid

Fifteen tomato lines were screened for their resistance to tobacco mosaic virus under artificial conditions of inoculation. On the basis of symptom expression, biological assay and ELISA, line 942 and 838 appeared to be moderately resistant and line 943 was tolerant. Rest of the lines were invariably susceptible. TMV symptoms were not indicative of virus titre in tomato lines.

Proc. Nat. Symp. Status of Pl. Path. in Pakistan. Dec. 3-5, 1992, Univ. Karachi, pp.315-319.

OCCURRENCE AND IDENTIFICATION OF VIRUSES ON TOMATO PLANTS IN SINDH, PAKISTAN

Rana, N.H., S.A. Rao, M.A. Qureshi & M.B. Jilani

Field survey of different localities of district Hyderabad revealed that 68% of the tomato plants were infected with different viruses. After assaying the plant samples, five viruses, viz. TMV, TYLCV, TRSV, TBRV and CMV were the most frequently found associated with tomato plants.

The assessment was based on host reactions, physical properties and double diffusion serological tests.

Pakphyton, 4: 57-63, 1992.

EFFECT OF SINGLE AND DOUBLE INFECTION OF MECHANICALLY TRANSMISSIBLE VIRUSES ON TOMATO YIELD AND YIELD COMPONENTS

Khan, T.M.

Tomato viruses, tomato mosaic viruses (ToMV), potato virus X (PVX), potato virus Y (PVY) and cucumber mosaic virus (CMV) reduced yield and growth component significantly. The yield losses were higher when the viruses infected tomato plant in combinations. In double infection the effect of ToMV + PVX on fruit size, number of fruits, weight of fruit, dry weight of plant and plant height, was higher (61.22, 61.85, 76.44, 80.26, 51.59%, respectively) than other combina-

tion and significantly reduced the yield and growth component of tomato. In other combination the infection of ToMV + PVY, ToMV + CMV, PVX + PVY, PVX + CMV and PVY + CMV caused reduction in fruit size, number of fruits, weight of fruit, dry weight of plant and plant height ranged from 35.11-61.22, 19.74-61.85, 23.63-76.44, 54.07-80.26, and 25.88-51.59%, respectively. In single infection of ToMV, PVX, PVY and CMV reduction in fruit size, number of fruit, weight of fruit, dry weight of plant and plant height, ranged from 19.98-45.48, 9.88-37.03, 21.78-43.07, 24.98-73.92 and 12.15-38%, respectively.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1992.

CHEMOTHERAPY AND THERMOTHERAPY O F TOMATO MOSAIC VIRUS

Khan, H.

Tomato mosaic virus (ToMV) is a destructive and widely prevalent seed-borne virus in tomato. In chemotherapy tomato seeds were treated with sodium orthophosphate at concentrations of 50, 100, 200 and 250 g/l for 1,2, 4 and 6 hours, while in thermotherapy the tomato seeds were treated at 60, 70, 80 and 90°C for 24, 48, 72 and 96 hours. In enzyme linked immunosorbent assay (ELISA) of treated seeds and in infectivity assay on *Nicotiana glutinosa* indicated that ToMV inactivated at 250 g/l of Na_3PO_4 and also at 80°C and 90°C but these temperatures adversely affect germination. The germination at 90°C was probably zero but at 80°C it was about 62-70%. Percent ToMV infection on the basis of symptoms of the plants raised from the seeds treated at 250g/l of Na_3PO_4 and at 80°C and 90°C were significantly less than control. The data on yield indicated that the yield of the Na_3PO_4 treated seeds at 250g/L and at 80°C temperature were significantly greater than the rest of the treatments.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1992.

STUDIES ON THE OCCURRENCE AND IDENTIFICATION OF VIRUSES ON TOMATO PLANTS IN YDERABAD DISTRICT

Nahiyoon, N.H.

In a survey of tomato crop conducted in Hyderabad district during 1989-90, 68% of the plants indicated the presence of viruses. However, tomato mosaic viruses, tomato ring spot virus, tomato yellow leaf curl virus, tomato bushy stunt virus and cucumber mosaic virus were frequently isolated from the fields located in Hyderabad district. The identification was based on host/reactions and physical properties. The viruses produced local as well as systemic symptoms on different indicator plants.

TMV and TBSV being very stable, CMV moderately stable and TRSV, TYLCV were found more sensitive to heat, dilution, longevity and pH.

MSc. (Hons.) Thesis, Dept. Pl. Path., Sindh Agric. Univ., Tando jam, 1991.

EVALUATION OF TOBACCO MOSAIC VIRUS RESISTANCE IN TOMATO (*LYCOPERISCON ESCULENTUM* MILL)

Hameed, S., M.A. Khan, S.M. Mughal & S. Khalid

Eleven tomato cultivar/hybrids/lines were screened for their resistance to tobacco mosaic virus under artificial conditions of inoculation in the greenhouse. Based on symptom expression, infectivity test and ELISA, Portanto and Sarras appeared to be highly resistant and also be used to study mechanism of resistance to TMV. Carmello was resistant and the remaining lines were invariably susceptible. TMV symptoms in tomato lines were not indicative of virus titre in the plants. Problems in resistance breeding and effective screening technique for quantitative assessment of virus contents in the plants are discussed.

Status of Pl. Path. in Pakistan, Univ. Karachi, 3-5 Dec. 1991. (Abst.) p. 93.

ETIOLOGY AND EPIDEMIOLOGY OF VIRAL DISEASES OF

Occurrence, prevalence and distribution of viruses infecting tomato was determined through extensive surveys, symptomatology and enzyme-

TOMATO IN MALAKAND AGENCY

Irshad, M.

linked immunosorbent assay (ELISA) basis in major tomato growing areas of the Malakand Division (Herushah and Palai). Tomato mosaic virus (ToMV), tomato yellow top virus (TYTV), cucumber mosaic virus (CMV), tomato yellow leaf curl virus (TYLCV), tomato bushy stunt virus (TBSV), potato leaf roll virus (PLRV) and tomato ringspot virus (TRSV) were found to infect tomato crop in the areas surveyed. Incidence of ToMV, CMV, TYLCV, TYTV, PLRV, TBSV and TAV were recorded 36.67, 12.94, 16.04, 7.72, 4.07, 5.065 and 1.81%, respectively. ToMV, PVX, PVY, TYTV and CMV were successfully detected serologically by using ELISA-indirect method with percent mean incidence 33.01, 10.3, 9.8, 9.39 and 6.28%. ToMV was the most prevalent and destructive virus, transmitted through infected seeds and contact. TYLCV was much prevalent but can not be detected serologically. Identity of both mechanical and vector transmissible viruses (ToMV, PVY, PVX, CMV) was confirmed in the screen house, however, pathogenic nature of TYTV and TYLCV confirmed by transferring from their original hosts to suitable diagnostic hosts. Effect of virus in naturally infected tomato fields was assessed. Viral infection in tomato significantly reduced yield and growth components i.e. number of fruits, fruit size, height of the plant, fruit weight and weight of the 100-seeds.

M.Sc. (Hons.) Thesis, Dept. Pl. Path., NWFP Agric. Univ., Peshawar, 1991.

EFFECT OF POTATO VIRUS X (PVX) ON LEAF CHLOROPHYLL AND SOME GROWTH COMPONENTS IN TOMATO

Effect of PVX infection on leaf chlorophyll level, plant height, and fresh and dry weight of shoot and root in five tomato cultivars was studied in glasshouse under artificial conditions of inoculation. Reduction in leaf chlorophyll, and fresh and dry weight of shoot and root of virus infected plants was noticeable, but it was insignificant

Rashid, F., S. Khalid, I. Ahmed & S.M. Mughal

statistically. Significant reduction in main shoot length of infected plants was recorded in cultivars Tobol and Parana.

Pak. J. Phytopath., 2(1-2): 43-51, 1990.

SEPARATION OF TOMATO YELLOW TOP VIRUS AND BEET WESTERN YELLOWS VIRUS FROM DOUBLY INFECTED PLANTS

Hassan, S. & P.E. Thomas

The efficacy of double antibody sandwich (DAS) form of ELISA and the differences in transmission behavior of two closely related persistent phloem restricted circulatively aphid transmitted viruses, tomato yellow top virus and beet western yellows virus was evaluated for the separation of single virion form mixed infection. Both viruses were successfully separated from doubly infected plants on account of extreme specificity, complete absence of heterologous reactivity and sensitivity of this technique.

Pak. J. Bot., 21(2): 323-330, 1989.

PURIFICATION AND SOME PROPERTIES OF TOMATO YELLOW TOP VIRUS

Hassan, S. & P.E. Thomas

A method was developed to purify tomato yellow top virus (TYTV) from infected leaves of *Datura tatula*. The method yielded as high as 1,670 µg of highly pure, infectious virus/kg of tissue, based on an estimated A260 extinction coefficient = 8.6. Some TYTV isolates yielded much more virus than others. The method involved enzymatic digestion of triturated tissue, clarification by partitioning in a chloroform-n-butanol mixture, precipitation with polyethylene glycol, and two cycles of differential centrifugation. The pH and content of buffers and processing temperature were critical to yield and purity of virus achieved. A unique method was developed to separate host contamination from the partially purified virus which involved precipitation of virus at low pH and selective redissolution of the virus. The virus sedimented with an S_{20w} value of 114 in a single U.V. light-absorbing, infectious zone in rate zonal sucrose density gradients that contained uniform spherical particles about 24.3

nm in diameter. The virus had a buoyant density of 1.350 g/cm^3 when centrifuged to equilibrium in CsCl, a A260/A280 ratio of 1.8, and an isoelectric point about pH 5.2. The amino acid content of its coat protein is given. The virus was highly immunogenic and antisera with no reaction against healthy plant juice in agar gel double diffusion or ELISA tests were prepared against several TYTV isolates. The antisera reacted with potato leaf roll virus (PLRV) in ELISA tests.

Sarhad J. Agric. Pak., 5(5): 507-519, 1989.

POTATO VIRUS X (PVX) RESISTANCE IN TOMATO CULTIVARS

Rashid, F., S. Khalid, I. Ahmad & S.M. Mughal

The response to infection with potato virus X of tomato cultivars Robin, Jacinto, Tobol, Turquesa and Parana, was studied in a greenhouse trial. Cultivars Robin and Jacinto developed severe mosaic symptoms giving very sensitive and sensitive reactions, respectively and they had a high virus contents as assessed by local lesion assay. Tobol, Turquesa and Parana were moderately sensitive and had low virus contents.

Trop. Pest Manag., 35(4): 357-358, 1989.

VIRAL DISEASES OF TOMATO AND THEIR CONTROL

Mughal, S.M.

Symptoms of the major viral diseases (TMV, TLCV) are described while minor, (CMV, PVX and PVY) are mentioned. Possible control measures such as soaking of tomato seed solution of Na_3PO_4, sanitation, early rouging and spraying with suitable insecticides against whitefly is suggested.

Progressive Farming, 5(2): 20-23, 1985.

ATTENUATION AND SYMPTOMS AMELIORATION OF TOMATO YELLOW TOP VIRUS ISOLATES

Hassan, S. & P.E. Thomas

Attenuation, symptoms amelioration and an increase in virulence of tomato yellow top virus (TYTV) isolates was observed when continuously propagated for several generations in glass or fiber glass house in the same or different hosts. These changes were permanent and irreversible.

Sarhad J. Agri., 1(2): 243-249, 1985.

URDBEAN

URDBEAN LEAF CRINKLE VIRUS (ULCV) AND ITS EFFECT ON YIELD AND GROWTH COMPONENTS OF GENOTYPES

Atab, M., S.M. Iqbal, S. Khalid & M. Bashir

A seed transmissible urdbean leaf crinkle virus (ULCV) causing darkening, thickening and downward curling of trifoliate, proliferation of florescence and giving bushy appearance of infected plants, was purified from natural infected leaves of urdbean (*Vigna mungo* L.). In partially purified preparations spherical virus particles were observed under the electronmicroscope. Losses assessed in ten urdbean genotypes under field conditions revealed reduction in plant height (23.77 to 53%). Number of pods and seeds per plant were decreased upto 88% when compared to healthy plants. Maximum reduction in pod length (46.5%) and 100 seed weight (32.6%) was observed in genotype NM 6-48, whereas maximum loss in yield was 88.3% in genotype NM 33-40.

4th Nat. Conf. Pl. Scientists, Feb. 16-18, 1993. AARI, Faisalabad, pp.

STUDIES ON THE RESPONSES OF GROWTH COMPONENTS OF URDBEAN AGAINST LEAF CRINKLE VIRUS INFECTION

Ilyas, M.B., M.A. Haq & K. Iftikhar

The growth responses of ten urdbean genotypes to leaf crinkle virus revealed that, depending upon the genotype infected, the virus may affect both the vegetative and the yield parameters. Invariably the affected urdbean susceptible exhibited 19.69 to 39.68% reduction in plant height, 34.44 to 49.08% reduction in branches, 31.92 to 53.09% reduction in number of leaves per plant, 36.56 to 59.48% reduction in dry stem weight. Leaf crinkle virus also affected the reproductive components. Different cultivars displayed 45.0 to 67.26% decrease in number of pods per plant, 20.62 to 47.44% decrease in pod size, 19.51 to 50.24% decrease in number of seeds per pod, 33.26 to 56.43% decrease in 100 seed weight and 35.01 to 77.40% decrease in yield per plant.

Pakphyton., 4:51-56, 1992.

SCREENING OF URDBEAN GERM-PLASM AGAINST LEAF CRINKLE AND YELLOW MOSAIC VIRUSES AND QUANTITATIVE DETERMINATION OF GROWTH RESPONSES OF URDBEAN CULTIVARS AGAINST VIRUSES INFECTION

Haq, A.

Two disease screening nurseries of urdbean, one against mungbean yellow mosaic virus (MBYMV) and the other against urdbean leaf crinkle virus (ULCV) were planted in the field area of Department of Plant Pathology, University of Agriculture, Faisalabad, for finding the resistant, sources against the respective diseases. Of the 49 germplasms screened against MBYMV in disease screening nurseries No.1 none of the cultivar was found to be immune or resistant. However, five cultivars (AARI M-13, AARI M-26, AARI M-27, AARI M-196 & AARI M-202) were found to be moderately resistant. The remaining cultivars were moderately to highly susceptible. Among the same test lines screened against ULCV in disease screening nursery No.2 cultivar AARI M-1 and AARI M-35 were proved to be immune and highly resistant respectively. Ten cultivars were resistant, thirty one moderately resistant, four moderately susceptible and two susceptible.

Studies on growth responses of ten genetically different urdbean cultivars to MBYMV and ULCV revealed that both the vegetative and reproductive components were reduced on infection to both viruses. However, the affected cultivars varied in their response to viruses infection and were suffered to various extends probably due to their different genotype. Incase of MBYMV the cultivars exhibited 21.62 to 39.81% decrease in plant height, 25.81 to 55.63% decrease in number of branches, 21.85 to 59.70% decrease in number of leaves, 45.52 to 84.12% decrease in dry stem weight.

The response of MBYMV on reproductive components also varied and depending upon the cultivars, there was 43.08 to 82.5% decrease in number of pods per plant, 25.32 to 37.00% decrease in pod size, 22.73 to 47.67% decrease in number of seeds per pod, 30.55 to 57.40%

decrease in 100 seed weight and 29.29 to 77.56% decrease in plant yield.

Similarly the vegetative components of all the cultivars were affected on infection by UBLCV. Invariably the affected cultivars exhibited 19.69 to 39.68% reduction in plant height, 34.44 to 49.08% reduction in number of branches, 31.92 to 53.09% reduction in number of leaves per plant, 36.56 to 59.48% reduction in dry stem weight. ULCV also affected the reproductive components. Different cultivars showed 45 to 67.26% decrease in number of pods per plant 20.62 to 47.44% decrease in pod size, 19.51 to 50.24% decrease in number of seeds per pod 33.26 to 56.43% decrease in 100 seed weight 35.01 to 77.40% decrease in yield per plant of urdbean.

M.Sc.(Hons.) Thesis, Dept. Pl. Path., Univ. Agri., Faisalabad, 1991.

REACTION OF URDBEAN CULTIVARS AGAINST LEAF CRINKLE VIRUS DISEASE

Iqbal, S.M., A. Ghafoor, M. Zubair & B.A. Malik

Nineteen genotypes/cultivars of mash selected from local races were screened against leaf-crinkle disease for two consecutive years (1988 & 1989) under natural infection conditions. Genotypes varied greatly in their reaction against disease. The disease intensity was high during second year which might be due to seed transmission nature of virus. Four genotypes *viz.* S-210, MM 5-60, S-250 and Mash Sialkot were found resistant while other showed average reaction to leaf crinkle virus disease.

J. Agri. Res., 29(3): 411-415, 1991.

EFFECT OF URDBEAN LEAF CRINKLE VIRUS ON URDBEAN PLANTS

Ilyas, M.B. & A. Haq

The effect of Urdbean leaf crinkle virus on plants of ten urdbean cultivars revealed that the virus affects both the vegetative and yield components. However, the extent of effects varied with the cultivar infected and probably depended on its genetic make up. Depending on the cultivar, the

virus decreased 13-40% plant height, 8-49% number of branches, 8-53% number of leaves/ plant and 37-59% dry stem weight. Similarly, the virus caused 45-67% decrease in number of pods/plant, 21-47% decrease in pod size, 20-50% decrease in number of seed/plant, 33-56% decrease in 100 seed weight and 35-77% decrease in plant yield.

Status Pl. Path. Pak., Karachi Univ., Karachi Dec. 3-5, 1991, (Abst.) p. 94.

ASSESSMENT OF YIELD LOSSES DUE TO LEAF CRINKLE VIRUS IN URDBEAN, *VIGNA MUNGO* (L.) HEPPER

Bashir, M., S.M. Mughal & B.A. Malik.

Losses inflicted by leaf crinkle virus (LCV) were determined by comparing components of infected and healthy plants of urdbean (*Vigna mungo*) cv. M-133. The disease reduced plant height by 8%, number of pods (90.8%), pod length (18.4%), seed weight (26.5%) and yield by 81%. LCV affected all the yield contributing components of plants except number of branches and leaves.

Pak. J. Bot., 23(1): 140-142, 1991.

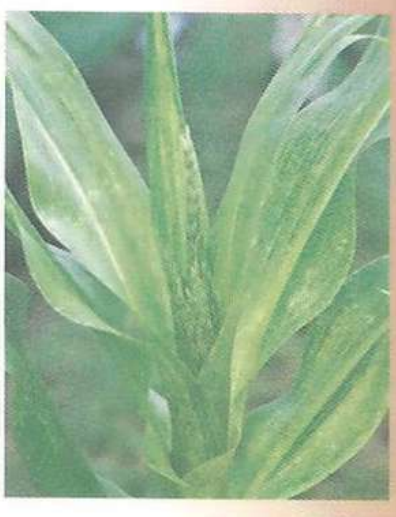
STUDIES ON A WHITEFLY-TRANSMITTED YELLOW MOSAIC OF URDBEAN (*PHASEOLUS MUNGO*)

Mushtaq A. & R.F. Harwood

Yellow mosaic (YM) of urdbean (*Phaseolus mungo)* has characteristics of a viral disease, but transmission experiments did not preclude a pathogen with similar characteristics. No transmission occurred by mechanical means, or by dodder (*Cuscuta*), but grafting and whitefly (*Bemisia tabaci*) form infected mung field yielded symptoms; no transovarian carryover of pathogen by whiteflies was demonstrated. The same pathogen also affects mungbean (*P. aureus*), moth bean *(P. aconitifolius*), and soybean (*Glycine max)*. Partial resistance to YM was found in urdbean lines that could be incorporated into susceptible varieties with otherwise desirable characteristics.

Pl. Dis. Reporter. 57(9): 800-802, 1973.

MISCELLANEOUS



MISCELLANEOUS

DETECTION OF A WHITEFLY (*BEMISIA TABACI*) TRANSMITTED GEMINIVIRUS IN *CESTRUM NOCTURNUM* (L) IN PAKISTAN

Khalid, S., H. Shah & S. Hameed

Raat ke Rani (*Cestrum nocturnum* L.) is grown as an ornamental shrub for its delicate fragrance and oil for perfumery. Recently few plants of *C. nocturum* L. showing virus-like symptoms of leaf distortion, rugosity and epinesity were observed in sector G-10 and National Agricultural Research Center (NARC), Islamabad. These plants were also heavily infested with whitefly (*Bemisia tabaci*). Two samples from Islamabad and one from NARC were tested against a panel of 14 monoclonal antibodies (MAbs-SCRI 18, 20, 23, 52, 53, 55, 56, 58, 60, 62, 66, 102, 104 and 106), raised against other whitefly transmitted Geminiviruses (WTGs) i.e african cassava mosaic virus (ACMV), indian cassava mosaic virus (ICMV) and okra leaf curl virus (OLCV). Polyclonal antiserum of ACMV was used for trapping antibodies. Triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) was applied as detection technique. All three samples showed reaction against WTGs, however, their epitope profile was different. Samples from sector G-10 gave positive result against MAbs 18, 20, 52, 53, 55, 56, 60, 66, and 102 whereas NARC sample reacted only with MAbs 18, 52 and 60, indicting that variation does exist among isolates.

6th Nat. Conf. Pl. Scientists, Oct. 20-22, 1998, Dept. Bot., Univ. Peshawar, (Abst.) p. 55.

EVALUATION OF LEGUMES GERMPLASM FOR SEED-BORNE VIRUSES

Ahmad, Z., M. Bashir &

Chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), mung bean (*Vigna radiata*) mash bean (*Vigna mungo*), peas (*Pisum sativum*), cowpea (*Vigna unguiculata*), moth bean (*Vigna aconitifolia*), pegeon pea (*Cajanas cajan*), faba bean (*Vicia faba*) are stored in the genebank of

T. Mitsueda

Plant Genetic Resources Institute (PGRI), Islamabad. Conservation and seed distribution are major functions of the genebank. More than 200 seed-borne viruses have been reported to be seed transmitted. Food legumes are notorious for hosting most of the seed-borne viruses. Due to seed contamination, several viruses have been introduced into many geographical regions. Contaminated seeds deteriorate more rapidly than healthy seeds even at low temperatures. In order to keep and distribute healthy seeds from the genebank, the information on health status of germplasm is important and the germplasm of various legumes was screened for seed-borne viruses and to find resistant sources against these viruses.

Proc. 8th SABRAO Gen. Cong. and the Ann. Meeting of the Korean Breeding Society, Sep. 24-28, 1997, Seoul Olympic Parktel, Seoul Korea, pp. 117-118.

SCREENING SESAME FOR RESISTANCE TO MYCOPLASMA PHYLLODY DISEASE

Mirza, M.S. & M. Aslam

Thirty five sesame accessions were screened for their resistance to Phyllody caused by Mycoplasma-like organism (MLO) transmitted by the leafhopper vector (order Homoptera) *Orosius albicinctus* Dist., under natural field conditions at the National Agricultural Research Center, Islamabad. Only 6 accessions, K-5, K-206, Pechequet-50, Reg-Canasto, P-35-40, and V-49/205 were found resistant (0.66 to 1.98% plants affected), 11 intermediate, 5 susceptible and 4 highly susceptible.

2nd Int. Cong. Entomol. Sc., Mar. 19-21, 1996 (Abst. PM-10), pp. 47-48.

ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF

Aphids and whitefly are the vectors of many important virus diseases. Long term and repeated use of insecticides has resulted in the

INSECT VECTORS OF PLANT VIRUSES

Fahmeed, F., I. Ahmad, S. Khalid & J.I. Mirza

development of resistance posing problems in pest management as well as negative environmental impacts. Alternative strategies are therefore being sought. Entomopathogenic fungi offer one such strategy, which is largely unexplored. We have used *Paecilomyces fumosoroseus* collected and isolated from whitefly during a natural epizootic in Multan region by an USDA team. Five different isolates namely PF92114, PF92118, PF92115, PF92117 and PF92111 were tested against turnip aphid and whitefly using in vitro bioassay. All the five cultures were highly affective and killed the insects within 24-48 hrs. at 18°C. Further work on characterization, pathogenicity and developing technologies for their use in an integrated management program are in progress at CDRI, NARC.

Crop Prot. Conf., Apr. 20-22, 1996, NWFP Agric. Univ., Peshawar, (Abst.) p. 37.

CHARACTERIZATION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES FROM PAKISTAN

Haider, M.S.

Five different wild (natural) host plants were found to be infected with whitefly transmitted geminiviruses in and around cotton field in Pakistan. Viruses from all the five original host plants could be transmitted by the whitefly *Bemisia tabaci* (Gennadius) back to their natural hosts and to some other alternative host plants. Comparative transmission study by different whitefly (*B. tabaci)* biotypes was also carried out. A significant difference in transmission was noticed under certain conditions (e.g., temperature, geographic impact, "B" and " non-B" biotypes). Viruses were named with reference to their respective natural host as follows, *Zinnia* leaf curl virus (ZLCV), *Eclipta prostrata* yellow vein virus (EPYVV), *Solanum* yellow leaf curl virus (SYLCV), pepper leaf curl virus (PLCV) and

Ageratum yellow vein virus (AgYVV) infecting *Zinnia elegans, Eclipta prostrata, Solanum nigrum, Capsicum annum* and *Ageratum conyzoides*, respectively.

Only SYLCV was mechanically transmissible but all the five were graft transmissible. Typical geminivirus particles were seen either in plant sap or in purified virus preparations. All the five viruses were found to be serologically related to african cassava mosaic virus (ACMV) in western blot, to cotton leaf curl virus (CLCuV) and to each others in enzyme-linked immunosorbent assay (ELISA); using the polyclonal antisera raised against ACMV, CLCuV, ZLCV, EPYVV, SYLCV, PLCV and AgYVV. Nucleic acid hybridization was used to compare homologies between four different probes of four of the whitefly–transmitted geminiviruses (WTGs) namely ACMV, watermelon chlorotic stunt virus (WCSV), CLCuV and SYLCV [DNA-A]. Relative levels of cross hybridization were analyzed under similar optimized conditions for each test. A full-length DNA-A genome was PCR amplified for all Pakistan viruses except EPYVV and amplification was confirmed to be of viral origin either by southern blot or partial nucleotide sequence.

The coat protein gene was PCR amplified, cloned and sequenced of ZLCV, SYLCV and AgYVV. Sequences were compared with previous known sequences of the WTGs by multiple sequence alignment and a phylogenetic tree was obtained. The viruses from Pakistan were found to be closely related to Indian subcontinent viruses i.e., TLCV-Ind, ICMV and CLCuV but probably new and different viruses

(strains) [SYLCV was found to be a mechanically transmissible strain of TLCV-Ind and ZLCV was found the strain of AgYVV-P]. Host range of the Pakistan viruses was also found to vary from one virus to another. Although some common alternative hosts (*N. benthamiana*, tomato, *Zinnia*) were found for all the viruses with the exception of one or two (e.g., SYLCV did not infect *Zinnia* and EPYVV did not infect tomato), there were different symptoms and incubation periods for the development of typical symptoms of the relevant virus.

On the basis of these observations and results it is suggested that the five virus isolates are new members (strains) of the whitefly-transmitted geminiviruses, subgroup III, but for precise differentiation complete nucleotide sequence would be required from each virus.

Ph.D. thesis, University of London, 1996.

SURVEY OF GROUNDNUT VIRUS DISEASES IN PAKISTAN

Bashir, M., S.N. Malik & A.S. Reddy

In July 1995, survey of virus diseases was conducted in the major groundnut-producing areas in Pakistan, including Attock, Chakwal and Rawalpindi districts. On the basis of this survey, we conclude that two virus diseases: peanut bud necrosis virus (PBNV) and at least two known serotypes of peanut clump virus (PCV) occur in farmers' fields in Pakistan. The overall crop condition was very good and other virus diseases (e.g., peanut stripe, peanut mottle and cowpea mild mottle viruses) were not observed.

Int. Arachis Newsletter, 15: 51-53, 1995.

SEED-BORNE VIRUSES OF LEGUME CROPS

Most of the viruses reported in legumes have the potential to be seed transmitted in one or other

(SYMPTOMOLOGY, EPIDEMIOLOGY, DETECTION AND CONTROL)

Bashir, M.

host. Presently, enzyme-linked immunosorbent assay (ELISA) is the most widely used serological method. Several important viruses such as pea seed-borne mosaic, bean common mosaic, soybean mosaic, black eye cowpea mosaic, bean yellow mosaic, cowpea aphid-borne mosaic, peanut stripe, peanut mottle and broad bean stain viruses seem to perpetuate in infected crop seeds. The seed health concepts particularly seed certification programs must be initiated in the country to reduce the risk of introducing new viruses in different localities. Important seed-borne viruses of legume crops have been discussed and future needs and strategies suggested.

Proc. Training Course on Legume Seed Health Testing, Mar. 14-20, 1995, ICARDA/FSCD, Islamabad, Pakistan, pp. 31-47.

FIRST REPORT OF GEMINIVIRUS IN SUNFLOWER

Hameed, S., S. Khalid, I. Ahmed & H. Shah

Virus-like symptoms on sunflower were first observed in 1993 at Sahiwal and Khanewal. Pakistan. Symptoms included distortion, thickening of veins and stunting. These symptoms resembled those caused by cotton leaf curl virus (CLCuV). In December, 1994 near Pakpattan such plants were again noticed where its incidence ranged from 27-30% in commercial hybrids of sunflower. However, in some fields incidence was up to 100%. Evidence that sunflower disease is caused by geminivirus, was obtained through serology. Triple antibody sandwich ELISA (TAS-ELISA) was performed using monoclonal antibodies (Mabs) i.e. african cassava mosaic virus (ACMV) and indian cassava mosaic virus (ICMV), to whitefly transmitted geminiviruses. Polyclonal antibodies to ACMV were used for coating plates. All tested samples, in 1993 and 1994, gave positive reaction and high absorbence value at 405 nm in TAS-ELISA, confirming the geminivirus etiology of

the disease (antibodies were provided by Prof. B.D. Harrison , SCRI, Dundee, UK).

5th Nat. Conf. Pl. Scientists, Mar. 28-30, 1995. NARC, Islamabad, p. 129.

WITCHES' BROOM - A NEW DISEASE ON POTATO IN PUNJAB

Ahmed, W. & A. Farooq

This disease, caused by a mycoplasma-like organism, was found for the first time in Pakistan during Jan. 1994. The cv. Patrones was highly susceptible while Cardinal and Desiree showed less infection. Witches' broom was transmitted by grafting and by sap inoculation from potato to tomato but not to Capsicum. The disease is tuber perpetuated. Dormancy of infected tubers did not break completely; when they were planted without treatments, germination began after 26 d, but when treated with 2% thiourea for 1 h and 5 ppm. gibberellic acid for 5 min, infected tubers started to germinate 6-7 d after planting.

Pak. J. Phytopath., 6(1): 67-68,1994.

VIRAL DISEASES OF GRAIN LEGUMES (PULSES) IN PAKISTAN

Bashir, M. & B.A. Malik

Virus diseases are widely distributed and become serious under favorable conditions and are the major cause of yield reduction in legumes. Major and minor diseases of legumes are described which include: chickpea stunt virus, chickpea chlorotic dwarf virus, chickpea luteovirus, cucumber mosaic virus, lentil strain of pea seed-borne mosaic virus, lentil strain of cucumber mosaic virus, mungbean yellow mosaic virus, urdbean leaf crinkle virus and cowpea viruses (black eye cowpea mosaic virus, cowpea aphid-borne mosaic, cowpea mottle virus, cowpea severe mosaic virus and southern bean mosaic virus.

In-Legume Seed Technology, (Edt: Ahmad, S.I.) "Train-the-Trainers Course on Legume Seed Production", Apr. 5-14, 1994, FSCD ICARDA, Sahiwal, pp. 133-136.

OCCURRENCE OF VIRUS VECTOR NEMATODES IN PAKISTAN

Nasira, K. & M.A. Maqbool

A survey of vegetable, fruit, cereal and other crops in Pakistan revealed the presence of *Xiphinema americanum*, *X. brevicolle*, *X. index*, *X. rivesi*, *Longidorus elongatus* and *Paratrichodorus minor*. The distribution and host association of these nematodes are listed.

Pak. J. Nematol., 12(1): 79-85, 1994.

EFFECT OF MUNGBEAN YELLOW MOSAIC VIRUS ON THE YIELD AND GROWTH COMPONENTS OF ASPARAGUS BEAN

Aftab, M., S. Asad, K.M. Khokhar, M.A. Ayub & T.B. Butt

Mungbean yellow mosaic virus disease is the most common disease of many food legumes in Pakistan. The crop of asparagus bean was found infected with this disease in the field area of National Agricultural Research Centre, Islamabad, during 1990. The disease symptoms on the leaves were pale to yellow spots mixed with green areas. The disease spread rapidly with increase in whitefly population and consequently resulted in reduced plant growth and yield. Percent decrease over control in respect of plant height (m), number of pods, seeds and yield plant^{-1} (g) and 100-grain weight (g) was 10.3, 50.5, 44.7, 49.2 and 4.4, respectively. Effect on nodulation was non-significant.

Pak. J. Phytopath., 5 (1-2): 58-61, 1993.

NATURAL OCCURRENCE AND SOME PROPERTIES OF TURNIP MOSAIC VIRUS IN PAKISTAN

Aftab, M., M. Bashir & S. Khalid

Turnip (*Brassica rapa*) and mustard raya (*Brassica juncia*) are the important autumn vegetable and oilseed crops in Pakistan. During winter season of 1992, a few plants of turnip and raya mustard planted in the experimental fields of National Agricultural Research Center, Islamabad, were observed with virus-like symptoms. Infected turnip cvs. Golden ball and Purple top were showing typical symptoms of turnip mosaic virus (TuMV), i.e. vein clearing, mottling, dark green patches, blistering, chlorosis and stunting of plants. Symptoms in mustard cvs. BARD-1, S-9, KS-51, KS-53, KS-

56, RL-18, VCD-3/4, and P-269 were similar to that of turnips but more severe. Some cultivars of *Brassica rapa*, DGL, Shiralle, Westar, R-D-10, Maluka, and Ganyou-5, were tested in the green house by sap inoculation, which showed less severe local and systemic reactions. Both the isolates were readily transmissible through mechanical inoculation to various test plants of the families *Chenopodiaceae*, *Brassicaceae* and *Solanaceae*.

Virus was partially purified in 0.1 M phosphate buffer, pH 7.0 and emulsified with 10% butanol. Further purification was completed by two differential cycles of low and high speed, followed by sucrose density gradient centrifugation. In purified preparation a large number of virus particles measuring about 750 nm were observed under the electron-microscope. Leaf samples collected from the field-grown virus-infected plants and inoculated plant when tested by indirect enzyme-linked immunosorbent assay (DAC-ELISA), gave positive ELISA values 405 nm ranging from 0.98 to 2.00 against the polyclonal antiserum to TuMV. On the basis of symptomatology, test plants reactions, particle morphology and serology, the virus was identified as turnip mosaic potyvirus. This is believed to be the first report of TuMV naturally occurring in turnip in Pakistan.

2nd Pak. Sci. Conf., Lahore, Pakistan, Dec. 26-30, 1993.

VIRUS DETECTION METHODS IN POTATO

Khalid, S. & A. Hussain

This training course was attended by participants from Bangladesh, India, Nepal, Sri Lanka and Pakistan. The first part of this manual contains papers on the economic importance of plant virus diseases, potato production and development in Pakistan, symptoms of virus diseases in plants,

transmission of potato viruses with special reference to *Myzus persicae*, some important virus diseases of potato and the serology of plant viruses. The second part includes practical laboratory exercises on mechanical inoculation of plant viruses, serological techniques and electron microscopy.

SAARC Training course on virus detection methods in potato, April 11-15, 1992.NARC, Islamabad

BREEDING FOR RESISTANCE AGAINST PLANT VIRUSES IN PAKISTAN - A REVIEW

Khalid, S. & M. Aftab

Plant Virology is a recently established discipline in Pakistan. The work done was generally based on visual observations until recently, when trained plant virologists became available and full-fledged and well-equipped plant virus research laboratory at the National Agricultural Research Center, Islamabad was established. Since no plant virus was well characterized, very little importance was given to the work on screening crop varieties to identify sources of resistance against economically important viruses. Whatever little work has been done so far in this direction was based on field observations under natural conditions. Recently, Plant Virology Program and Vegetable Program, NARC, Islamabad started work to identify sources of resistance in local as well as in exotic germplasm. Some success has been made in identifying resistant material against tomato mosaic virus (TMV) in tomato and chillies. Work on other crops is in progress. The paper describes the work done so far in this direction, the problems faced and the future strategies for breeding for resistance to plant viruses in Pakistan.

Int. Symp. on New Genetical Approaches to Crop Improvement, Feb. 15-20, 1992. AEARC, Tandojam. (Abst. E-12).

STATUS OF PLANT VIROLOGICAL RESEARCH IN

Plant Virus research was started in the Plant Virology Section at the Ayub Agriculture Research Institute, Faisalabad in 1973. In this

PAKISTAN

Khalid, S. & M. Aftab

paper the history, achievements, current status, difficulties and future prospects of plant virology research in Pakistan is described. Currently, only Plant Virology Laboratory at National Agricultural Research Center, Islamabad is well-equipped to conduct basic plant virus research whereas at other places research is mostly being done on the applied aspects. The main objectives of the Plant Virology Laboratory at the NARC is to conduct basic as well as applied plant virus research, to coordinate plant virus research in the country and to train manpower in the field of plant virology. The program's main contribution and achievements include: characterization of major viruses of potato, tomato and tobacco and identification of source of resistance in tomato and chillies against TMV, in tobacco against PVX and in maize and millet against MDMV and SCMV. Research work on other crops is also in progress.

Status of Pl. Path. in Pakistan, Univ. Karachi, Dec. 3-5, 1991, (Abst.) p. 95.

OCCURRENCE OF MAIZE DWARF MOSAIC VIRUS IN MAIZE

Aftab, M., S. Khalid & S.M. Mughal

From naturally infected maize hybrid (YH 202) showing mosaic symptoms, maize dwarf mosaic virus was isolated. It was mechanically transmissible only to hosts belonging to *Gramineae*. Partially purified preparation showed large number of flexous rods measuring about 750-800 nm in length. On the basis of host range and particle morphology the virus was tentatively identified as maize dwarf mosaic virus (MDMV). This is the first report of the occurrence of MDMV in maize from Pakistan.

Pak. J. Bot., 22(2): 129-135, 1990.

DISEASES OF MAJOR PULSE CROPS IN PAKISTAN-A REVIEW

Bashir, M. &

The major pulse crops of Pakistan are chickpea, lentil, mungbean, and urdbean. Diseases are a constant threat and often a limiting factor in the cultivation of pulse crops. Chickpea is attacked mainly by blight, wilt and root rot complex. The

B.A. Malik

main diseases of mungbean and urdbean are yellow mosaic virus (YMV), leaf crinkle virus(LCV), *cercospora* leaf spot, bacterial blight and charcoal rot. Lentil is attacked by rust, wilt and blight. Details are given of the symptoms, distribution and control of the diseases of economic importance.

Trop. Pest Manag., 34 (3): 309-314, 1988.

INTERACTION OF ZINNIA MOSAIC VIRUS WITH ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON *ZINNIA ELEGANS*

Jabri, M.R.A., T.A. Khan, S.I. Husain & K. Mahmood

Virus infected Zinnia plants were more susceptible and better host for *Meloidogyne incognita* than healthy plants. Leaf extracts from healthy plants were twice as nematicidal as those from virus infected plants. The two pathogens together caused greater damage than any one of them separately. The length and fresh weight of plants was reduced to half of the control plants in case of simultaneous inoculations indicating synergistic relationship of the pathogens.

Pak. J. Nematol., 3(1): 17-21, 1985.

IDENTIFICATION OF SOME VIRUSES INFECTING *SOLANACEOUS* HOSTS IN SINDH

Solangi, G.R., S.M. Mughal & S.D. Khanzada

Virus diseases identified on the basis of symptomatology, host range, serology and transmission were tobacco mosaic virus (TMV) in tomato, tobacco and chillies, X, Y and leaf roll virus (LRV) in potato and leaf curl virus (LCV) in tomato, tobacco and chillies. Leaf curl virus group transmitted by whitefly was predominantly present in Sindh.

Pak. J. Bot., 15(1): 19-24, 1983.

STUDIES WITH A WHITEFLY-TRANSMITTED YELLOW VEIN MOSAIC OF *DIGERA ALTERNIFOLIUS*

A yellow vein mosaic disease, presumed to be caused by a virus, showing the symptoms of bright yellow coloration in the leaf veins was recorded on *Digera alternifolius* Linn. It was only transmitted by grafting and the whitefly, *Bemisia tabaci* Genn.; no transmission occurred by

Ahmad, M.

mechanical means, dodder, seed and soil or nematodes. There was no transovarian passage of the pathogen in the whitefly.

Phytopath. Z., 96: 21-24, 1979.

A WHITEFLY-VECTORED YELLOW MOSAIC OF JUTE

Ahmad, M.

A yellow mosaic disease of jute (*Corchorus capsularis*), exhibiting the characteristics of a virus, was recorded. It was not possible to transmit it by mechanical means, dodder, seed, soil or nematodes. Transmission was only effected through grafting and the whitefly, *Bemisia tabaci* Genn. The transovarial passage of the pathogen to whitefly offspring was negative, suggesting that the virus survived off-season in some alternate host plants. The disease was found innocuous to another specie of cultivated jute, *C. olitorius*, and a wild jute *C. trilocularis*.

FAO Pl. Prot. Bull. 26(4): 169-171, 1978.

COLOR PREFERENCE AS A POPULATION INDEXING TECHNIQUE IN THE WHITEFLY, *BEMISIA TABACI* GENN. (ALEYRODIDAE; HOMOPTERA)

Ahmad M. & R.F. Harwood

The present studies investigated color preferences of *Bemisia tabaci* so as to devise a population indexing technique. Sticky stakes with yellow colored papers were found to be a very good technique for indexing whitefly populations. This color preference has also suggested the behavior of *B. tabaci* underlying its effective transmission of YMV to host plants. It further indicates a basis for exploitation of host color characteristics in *B. tabaci*-vectored plant viruses by avoiding yellow in varietal improvement programs.

Pak. J. Agri. Sci., 10 (1-4): 19-24, 1973.

THE HEALTHY JUICE EFFECT IN VIRUS TRANSMISSION

Azeemuddin, S. & C.E. Yarwood

Inoculum of tomato ringspot virus (TRSV) was taken from systemically-*infected Erigeron glaucus*, and assayed on primary leaves of Pinto bean. Inoculum of cucumber mosaic virus was taken from locally-infected cucumber cotyledons. The healthy tissues used were primary cowpea leaves, cucumber cotyledons or

secondary beet leaves. Inoculum was applied by dipping cotton swabs (Q-tips) into the inoculum and rubbing them over the surface of the indicator plants.

The addition of healthy tissue increased virus transmission in 13 out of 14 cases. The greatest increase, 750-fold, was for healthy sugar beet tissue added to CMV and assayed on cowpea. The average increase due to healthy tissue was 50-fold.

Pak. J. Sci. Indust. Res., 10: 227-228, 1967.

AUTHOR INDEX

Born on 4th June 1955 in Gujranwala, Pakistan, Dr. Saif Khalid completed his secondary education in the city. He did M.Sc. in Plant Pathology in 1978 from Higher Agricultural Institute "Vassil Kolarov", Plovdiv, Bulgaria and received Ph. D. in Plant Virology from Plant Protection Institute Kostinbrod, Sofia, Bulgaria, in 1983. In March 1984 he joined Pakistan Agricultural Research Council (PARC), Islamabad, and was among the pioneers of plant virology research in the country. Dr. Khalid was instrumental in the establishment of a well equipped Virology Laboratory, which is now rated as one of the best, not only in the country but has also acquired an international standard and repute.

He has developed scientific collaboration with renowned laboratories around the world and was instrumental in generating funds from European Commission (EC), Asian Development Bank (ADB) and other donor agencies. In 1993-94 he received EC Post Doctorate Fellowship to work on cotton leaf curl virus (CLCuV) with Professor B.D. Harrison at Scottish Crop Research Institute, Dundee, UK. Since 1991 he is heading Plant Virology Programme at National Agricultural Research Center (NARC), Islamabad. During his 15 years of service he worked on viruses of tomato, tobacco, potato, banana, cotton and chillies. Dr. Khalid's current interests are on banana bunchy top virus (BBTV) and whitefly transmitted Begomoviruses.

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